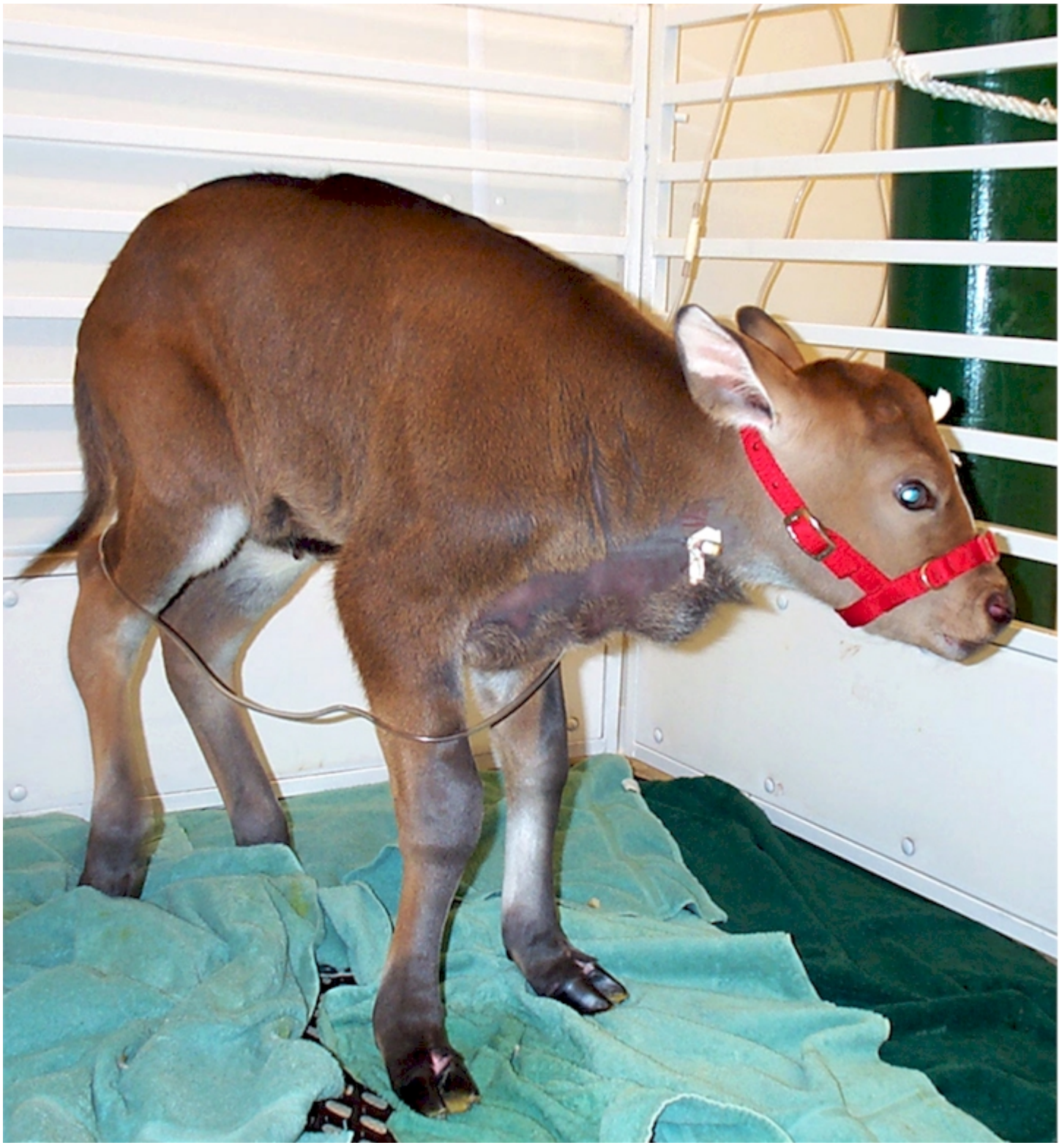

Genetically Modified and Cloned Animals. All in a Good Cause?

A Report by GeneWatch UK

Genetically Modified and Cloned Animals. All in a Good Cause?

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Cover photograph:

Gaur Standing, © Advanced Cell Technology, Inc., Worcester, Massachusetts.

The gaur calf, 'Noah', born in January 2001, was cloned from an adult gaur with a cow as a surrogate mother. The calf died 48 hours after birth from an infection. The gaur is an endangered species and the calf was cloned as part of an attempt to preserve the species.

CONTENTS

LIST OF TABLES

EXECUTIVE SUMMARY

In 2000, nearly 582,000 scientific procedures were performed on genetically modified (GM) animals in the UK. The first genetically modified mammals - mice - were produced in the mid 1970s and since then transgenes (genetic material from different species) have been inserted into fish, rats, guinea pigs, rabbits, sheep, goats, pigs, cows, chickens and quail. However, GM animals - other than laboratory animals - are not yet commercially available.

Genetic modification and cloning are presented by scientists and biotechnology companies as merely an extension to selective breeding that allows us to introduce new characteristics more quickly and accurately. This is misleading - no matter how skilled or diligent the breeder, cows do not cross with mice or bacteria or humans. Genetic modification of animals represents a watershed in our relationship to the natural world and a significant further step towards seeing animals only as commodities to be created for our convenience. Genetic modification can also cause pain and distress to the animals involved.

GM and cloning techniques are highly inefficient and many animals are subjected to surgical procedures or killed in order to produce a GM founder (the GM animal which is used to breed a transgenic line). Approximately 60 sheep or 80 cows will undergo reproductive interventions to produce each transgenic lamb or calf. A large number of the resultant offspring are killed - either because they have not integrated or do not express the transgene - with the result that more than100 animals may be used for each GM founder produced.

The random nature of genetic modification means that unintended mutations will inevitably result. The expression of the transgene may itself cause ill effects and has in some instances caused death. For example, transgene expression of erythropoietin (EPO) in rabbits, intended to be expressed exclusively in milk, produced low levels in other tissues and resulted in greatly elevated numbers of red blood cells. Most animals died prematurely and were infertile. Damaging mutations may not surface for many generations. The unpredictability of the techniques means that it is extremely difficult, or impossible, to reliably anticipate ill effects and be able to ameliorate them.

Animals are being genetically modified and cloned for:

- use in biological and medical research;
- safety testing;
- drug production (so-called ‘pharming’);
- use in intensive agriculture.

Biological and medical research

This is by far the most common reason for genetically modifying animals (over 99% of UK procedures on GM animals). It includes both the modification of mice as ‘disease models’ to mimic conditions which affect humans and disruption of the mouse genes to identify gene function. Mice are most frequently used for this type of research because they are cheap, their breeding time is short and they have been extensively studied. Transgenic lines are even available by mail order. The purpose of disease models is ultimately to discover therapies which will be effective in humans. However, there is considerable controversy over the utility of animal models – even transgenic ones - to detect useful treatments for humans. The progress of cancer, for instance, varies enormously between species and substances found to be therapeutically effective in mice are frequently toxic and/or ineffective in humans. Advances may be more likely through research in human cell culture, followed by toxicity testing and human trials, than through animal experiments.

Safety testing

New chemicals and drugs go through a testing regime for toxicity and carcinogenicity which depends heavily on animals. Carcinogenicity testing has traditionally required two-year tests in two different species but mice have now been genetically modified to increase their propensity to develop cancer in order to carry out short (six month) tests. Non-animal alternatives - primarily cell and tissue cultures - may offer greater accuracy in predicting human response. However, it is likely that current opportunities to reduce animal testing will be lost in the rush for transgenic development and the misapprehension that genetic modification will 'fix' the problems inherent in using different species to assess human safety.

Pharmaceutical production – 'pharming'

Animals have been genetically modified by the insertion of human genes coding for therapeutic proteins, which are generally produced in the animal's milk. There are also attempts to modify chickens to produce drugs in eggs. The annual world market for just one potential transgenic product - blood coagulating factor VIII - is estimated at $880 million, which could theoretically be produced by just one transgenic cow. There are alternative production systems which may offer more reliable products with less associated risks: bacterial and mammalian cell cultures, transgenic plants, and transgenic plant cell cultures. At present, by far the most important deciding factor in which system will be developed is the potential profits for the companies concerned. There is a need for a systematic appraisal of alternatives which takes into account the technical, social and ethical aspects of how we are to meet the need for drugs.

Agriculture

Increasing agricultural production has been the aim of much of the work on larger transgenic animals: increasing growth, the proportion of lean meat to fat, raising milk or wool production, or altering milk composition. In addition, Canadian scientists have genetically modified pigs so they do not excrete phosphorous (which can pollute waterways) – the so-called 'Enviro-Pig'. A US company, Nexia Biotechnologies, has engineered goats to produce spider's silk in their milk. The protein is one of the strongest materials in the world and has been called 'BioSteel'. However, the same company can produce BioSteel in transgenic plants or in cell culture so the need to use GM animals is questionable.

The requirement to increase food production to feed a growing world population is frequently put forward as a justification for genetic modification. However, although the GM applications being developed could increase productivity in the breeds used in high input intensive agriculture and significantly increase profits in subsections of the food production industry in the developed world, they are highly unlikely to impact on areas of the world currently experiencing food shortages. Modifications aimed at changing complex physiological processes such as growth are in any case likely to severely compromise the animal's health and welfare.

Xenotransplantation

Demand for human donor organs currently exceeds supply (the 'organ gap') and there are proposals that organs from pigs could be used instead. By genetically modifying pigs, the aim is to produce 'humanised' organs which will not be rejected. However, apart from animal welfare and ethical issues, there are clinical and safety problems. It may be impossible to remove the risks of transferring diseases between species which could threaten not only the patient but the wider population, or to overcome the incompatible physiological differences between pigs and humans. There are alternatives to xenotransplantation, some of which could address the organ

gap problem immediately, such as improvements to the provision of NHS services and encouraging donation. Other areas of science, such as the regeneration of tissues from stem cells, may offer solutions for the future.

Cloning

Using the process of 'nuclear transfer', this has been put forward as the holy grail of transgenic technology – the technique that will enable targeted genetic modification in large mammals and make it possible to reproduce transgenic lines quickly and cheaply. Australian companies have already begun to market cloned calves of high value bulls in China. However, the technology is fraught with problems. Cloned embryos tend to have severe abnormalities, resulting in an extremely high abortion rate, and the majority of those that are born alive seem to have some form of health defect. The reasons are poorly understood but the problems have appeared in all species which have been cloned. The domestic cat has now been cloned and efforts are being made to clone dogs to meet owners' desires to replace pets during which large numbers of cats and dogs will have to be sacrificed. There have been a number of attempts to clone extinct and endangered animals, including the Asian gaur (an endangered wild ox), the mouflon lamb (a rare breed of sheep), the woolly mammoth and the panda. Only the gaur and the mouflon were born live and only the mouflon survived for more than a few days. There are also plans to clone the Indian cheetah, which became extinct 50 years ago. Using cloning to 'rescue' endangered species is a bizarre strategy as the major factor in extinctions is habitat loss. The considerable resources used for cloning would be better spent contributing to more effective habitat management and preservation.

Conclusions

Transgenic work is seductive, fashionable – and expensive. It is frequently linked to drug development, which is generally concentrated on those diseases for which there will be adequate financial returns. There is a danger that the glamour associated with genetic modification, the technological possibilities and the potential profits in pharmaceuticals will drive development rather than medical or social need. Genetic modification of mammals other than mice is an expensive business. The cost of one transgenic calf, for example, is estimated as $300,000. It is not surprising, therefore, that the main area of development in larger animals is pharmaceutical production, where there are potentially very large profits to be made.

The profit driven manner in which the technology is being applied has led to sustained overstatement of the achievements of genetic modification in order to maintain investor confidence. For example, in December 2000 a chicken called 'Britney' was characterised as the transgenic chicken helping to fight cancer even though it had merely been announced that scientists intended to *try and produce* a transgenic chicken. None of the agricultural applications – increased growth, altered milk composition, improved wool growth - are approaching the stage where they would actually be applied to production animals.

With the exception of laboratory mice, we have not yet reached a point where genetic modification of species via artificial gene transfer is routine. Although products derived from such modification do not yet contribute to either medicine or agriculture, there are high expectations that they will. There seem to be no limits on how animals may be used. In the USA, for instance, scientists are proposing to genetically modify cats to make them less allergenic. Xenotransplantation research continues despite the poor prospects and very real risks involved and drug production in the milk of animals is well advanced despite the alternative systems available. It is important that society as a whole is engaged in the debate about what is acceptable and desirable before the technology progresses to a point where transgenic animals become a normal part of production processes and the relationship between humans and animals is changed irrevocably.

Genetic modification of animals and allied technologies such as cloning have been seized upon by scientists and the agricultural and pharmaceutical industries as if they raised no special ethical issues. However, as this report shows, the ability to fundamentally change the genome of other species does raise new ethical issues which are not being adequately addressed in the current regulatory system. Existing legislation does not encompass the ethical appraisal that is needed and neither does the system in place ask sufficient questions about the justifications for experimentation. This demonstrates a serious mismatch between public opinion and the operation of the regulatory system. Research indicates that the public are uneasy about the production of GM animals and believe that genetic modification should only be allowed under exceptional circumstances. For the majority of people, the moral acceptability of genetically modifying animals and xenotransplantation – the question 'is it right or wrong?' - has been found to outweigh considerations of the potential benefits.

GeneWatch does not consider that any of the current applications, with the possible exception of some medical uses, justify the genetic modification of animals. Genetic modification other than for direct medical benefit should be stopped immediately and any applications for medical uses should undergo the most rigorous scrutiny. Our treatment of other species in this way reflects on human dignity and diminishes human society.

Recommendations

To meet the public's expectations that animals should be treated with respect, that animal welfare is prioritised, and that the grave ethical concerns about genetic modification are addressed, the Government should take the following steps:

1. **Introduce a requirement that broad ethical issues (including the use of genetic modification, its justification and the existence of alternatives) form an explicit part of the assessment of experimentation involving GM animals.**

2. **Establish boundaries for the genetic modification of animals and a framework for their evaluation including, as a minimum, that:**
   - **the genetic modification or cloning of companion animals (including dogs, cats and horses) is not allowed;**
   - **the genetic modification or cloning of farm animals (including for drug production) is not allowed;**
   - **experiments intended to reduce the sentience of any species are not allowed;**
   - **explicit consideration of alternatives is included in each application, with the onus on the applicant to demonstrate that other approaches could not achieve broadly similar goals.**

3. **The Animal Procedures Committee or the Home Secretary should commission a detailed independent evaluation of the way the use of genetically modified animals has been justified under Animals (Scientific Procedures) Act and the need to 'Reduce, Replace and Refine' the use of animals in experimentation. Xenotransplantation and GM animal disease models should be included in the scope of this study.**

4. **Provide public information about the nature of, and justification for, animal experimentation using GM and allied technologies.**

5. **Increase public debate about the use of genetic technologies on animals and involve the public in forming public policy and practice in this area.**

1. INTRODUCTION

The first genetically modified (GM) mammals - mice - were produced in the mid 1970s[1]. In 1982, a mouse was genetically modified to produce a foreign protein, rat growth hormone, which caused it to grow visibly oversize. A 'transgene' had been introduced into its genetic material, which was passed on to its offspring[2]. By the year 2000, UK laboratories contained more than 575,000 transgenic mice[3] and mice had undergone many hundreds of different genetic modifications [e.g.4]. Transgenes (genetic material from different species) have been inserted into fish, rats, guinea pigs, rabbits, sheep, goats, pigs, cows, chickens and quail[5]. The ability to alter the genetic makeup of species has been hailed as the answer to many human problems, from the need for lifesaving drugs to the solution for potential world food shortages [e.g.6,7].

Modification of animals and plants via selective breeding is the basis of modern agriculture and has been going on for thousands of years. Modern farm animals bear little resemblance to their wild ancestors and selective breeding has enabled agricultural systems to support expanding human populations. Genetic modification is often presented as merely an extension to selective breeding[8,9,10,11] which will allow us to introduce new characteristics more quickly and accurately.

However, genetic modification by the insertion of foreign genes into an animal's genome is not simply a further step along the path of selective breeding. No matter how skilled or diligent the breeder, cows do not cross with mice or bacteria or humans. Genetic modification of animals represents a watershed in our relationship to the natural world, bringing the potential to mix genes from species in a way that could not happen otherwise. It represents a significant further step towards seeing animals purely as commodities to be created for our convenience.

There are also physical consequences for the animals involved. In selective breeding, it takes generations to dramatically alter a particular trait and possible combinations are limited by evolution. Deeply damaging cocktails of genes are unlikely to survive and be used for breeding – although some have (e.g. broiler chickens which grow so quickly that their legs cannot support their weight[12]). With genetic modification, it is perfectly possible to disrupt a balance of beneficial effects reached over hundreds of years or to introduce a damaging trait in the space of one generation.

Selective breeding has already produced some very damaging results. A breed of cattle, the Belgian Blue, has been selected in the last thirty years by such traditional breeding methods to produce approximately 30% more 'meat' than normal on the same feed intake. The cattle are 'double muscled' and their rumps are so big that they have difficulty walking and calves have to be delivered by Caesarian section[13]. Dogs have been inbred over hundreds of years to the extent that several breeds have serious genetic defects[14]. How much faster could such damaging effects be produced by random integration of DNA from other species in the pursuit of traits that appear useful or attractive to humans?

Genes do not act in isolation and deletion or addition of genes with apparently minor significance can have major effects. For example, there are two species of fish, the platyfish (*Xiphophorous maculatus*) and swordtail (*Xiphophorus helleri*), which do not interbreed naturally but can be induced to do so in captivity. The platyfish has a pattern of intensely black, completely harmless spots on its side. When crossed with the swordtail, the black spots of the offspring develop into malignant melanomas which are usually lethal. It is thought that a promoter gene in the platyfish is transferred to the progeny but the necessary suppressor is not so that formation of black spots has no end mechanism[15,16]. Thus, a gene benign in one species can be lethal even in a closely related species and – as with the platyfish - it may take many years to unravel the causes.

The speed with which fundamental change can be achieved and the ability to introduce 'foreign' genes make transgenics very attractive both scientifically and commercially. It is precisely these factors which bring such potential for damaging and unpredictable effects on animal welfare.

1.1 Scope of the report

This report is concerned with the genetic modification of mammals and birds. It gives a short description of the main genetic techniques, an overview of the actual modifications that have been carried out in species other than mice and looks at the major applications of the technology. It then discusses the ethical and welfare implications of the genetic modification of animals and examines how this is being regulated in the UK.

There are two major areas which are not covered by the report - the genetic modification of fish and of insects. While some of the ethical questions raised are the same, there are major environmental issues which are not comparable. Farm animals and laboratory mice are generally contained and farm animals do not have large wild populations - although there may be instances of escape and cross breeding. By contrast, escapes from fish farms are well documented as are the potential effects on wild populations[17]. The *objective* of some schemes for the genetic modification of insects is to replace wild populations by enabling the modified genes to cross out - in malaria control programmes for example[18]. These two areas have been left for subsequent reports.

1.2 GeneWatch UK's position

There are serious concerns about animal welfare in both the production of GM animals and the effects of the modification, particularly because of the unpredictability inherent in the technology. There is also an ethical question about changing the genetic makeup of species and particularly mixing genes from different species.

GeneWatch UK takes the position that fundamental alteration of the genetic code of other species should not be undertaken lightly and that there should be a presumption against such modification unless there are compelling arguments to do it. These should include both the necessity of the application and a lack of acceptable alternative methods to achieve the same end.

From 1991 to 2000, the number of scientific procedures on GM animals in the UK increased by more than 800% from 62,445 to 581,740. The vast majority (more than 98%) of these procedures were performed on GM mice. Procedures on species other than mice increased from 639 to 6,580[3,19].

With the exception of laboratory mice, we have not yet reached a point where genetic modification of species via artificial gene transfer is routine. Products derived from such modification do not yet contribute to either medicine or agriculture although there are high expectations that they will.

It is important that society as a whole is engaged in the debate about what is acceptable and desirable before the technology progresses to a point where transgenic animals become a normal part of production processes and the relationship between humans and animals is changed irrevocably.

2. OVERVIEW OF THE TECHNOLOGY

Genetically modified (GM) animals are being created for use in medical research, for safety testing, for drug production (so called 'pharming'), and for use in intensive agriculture. Medical research is the only field in which their use has become established and the other applications are almost all at very early stages. This section summarises the actual modifications that have taken place in species other than mice and rats and gives a brief explanation of the techniques being used. Sections 5-9 look at each of the applications in more detail.

2.1 What modifications have taken place?

Hundreds of genes have been inserted into mice. A review of mouse disease models listed 93 genetic modifications[20] and just one project to investigate gene function generated 60 GM mouse lines[21]. Each genetic modification of a larger species is generally tried in mice first, frequently using several different combinations of promoter and transgene.

Genetic modification of mammals other than mice is an expensive business. The cost of one transgenic calf, for example, is estimated as $300,000[22]. It is not surprising, therefore, that the main area of development in larger animals is pharmaceutical production, where there are potentially very large profits to be made. This is reflected in the massive amount of investment in this area. In 1998, drug discovery accounted for $286 million dollars or nearly 30% of all biotechnology venture capital[23].

Table 1 shows the genetic modifications to date in birds and mammals, excluding mice and rats. It lists the functional gene inserted and the purpose although many of the modifications have had the primary aim of developing or validating genetic techniques.

There are 78 genetic modifications listed, nearly half of which (36) have been to develop the production of pharmaceutical proteins from transgenic animals. Most of the remaining modifications are for agricultural applications. 18 of these are additions of genes coding for growth hormones or growth releasing factors in the quest for GM farm animals with increased productivity. 9 relate to disease resistance although the majority of these were primarily to develop transgenic strategies and techniques. The remaining agricultural applications are increased wool growth, altering the protein content of milk and the production of 'BioSteel'.

2.2 Genetic modification techniques

The main methods used for genetic modification are microinjection (also called pronuclear injection), viral transfection, and manipulation of embryo stem cells. There are also some researchers who have had success with sperm mediated techniques. Cloning, or nuclear transfer, is not strictly speaking a method for genetic modification as the purpose is to create a genetically identical animal. However, if the problems that currently beset nuclear transfer are resolved, the present techniques for genetic manipulation would be revolutionised.

2.2.1 How does it work?

All cells in living organisms contain genetic material made of the very complex molecule, deoxyribose nucleic acid (DNA). Sequences of this molecule form genes, which 'code' for particular proteins (i.e. contain instructions for how to produce them). The proteins made within organisms - to grow, digest food, regulate bodily functions - are coded for by genes. Most cells in the body contain a complete copy of the entire genetic code of the organism. Germ cells (the egg

and sperm) only contain half the genetic material contained in other cells so that when they fuse together the new organism has a complete set of genetic material known as its 'genome'.

In order to genetically modify an organism, the new gene construct (known as a transgene) must be inserted into the DNA of the host cell. If the new gene codes for a particular protein, then this protein may be expressed in the animal - this depends on the site at which the DNA integrates and whether the gene is 'switched on'.

The transgene, or gene construct, consists of the gene coding for the protein of interest plus a promoter sequence which is intended to regulate the expression of the gene product by 'switching on' and 'switching off' the gene at the appropriate time. Sometimes the purpose of modification is to 'knock out' a gene, or disrupt its function, rather than to add a new gene.

Providing the integration happens early enough in an organism's development, preferably when it is at the single cell stage, the new DNA will be copied along with the original DNA as cells divide and every cell of the organism should contain the new gene. Approximately half of the germ cells should contain the new gene so that the genetic modification will be passed to some offspring. This is called 'germ line transmission'. It is unlikely that all germ cells will contain the new DNA as there is a non-replicative division when sperm or eggs are formed.

Table 1: Summary of animal genetic modifications (excluding rats and mice) (Page 1 of 2)

PURPOSE	ANIMAL	NO. OF GENETIC MODIFICATIONS	TRANSGENE(S) : *SOURCE*	REF
AGRICULTURAL APPLICATIONS				
Faster growth/ Leaner meat/ Development of techniques	Cattle Pig Rabbit Sheep	18	Growth hormones/ factors: *Human, Bovine, Porcine, Rat, Chicken*	227, 28, 229, 230, 200, 231, 232,202, 174, 201, 233, 226
Altered milk composition (higher protein)	Cattle	2	Extra copies casein genes; disruption of lactoglobulin gene: *Cow*	161
'BioSteel' production in milk	Goat	1	Spider gene	223
Reduce phosphorus in pig faeces	Pig	1	Phytase gene: *Bacteria*	219
Increased wool growth	Sheep	4	Cysteine synthesis gene: *Bacteria* Growth factor: *Sheep*	235, 90
Disease Resistance	Pig Sheep Rabbit	9	Monoclonal anitibodies: *Mouse* Viral envelope genes	213, 29, 211, 212
PHARMACEUTICAL PRODUCTION				
Treatment of multiple sclerosis, blood disorders	Cattle	2	Human serum albumin: *Human* Myelin basic protein: *Human*	160, 161
Improved infant formula	Cattle	1	Lactoferrin: *Human*	162
Treatment of hepatitis, some cancers	Chicken	At least 5	Antibodies/ human growth factor: *Human*	163, 164

Table 1: Summary of animal genetic modifications (excluding rats and mice) (Page 2 of 2)

PURPOSE	ANIMAL	NO. OF GENETIC MODIFICATIONS	TRANSGENE(S) : *SOURCE*	REF
Treatment of cystic fibrosis, thrombosis, hepatitis, blood disorders	Goat	4	α-proteinase inhibitor: *Human* Antithrombin III: *Human* Hepatitis B antigen: *Human* Tissue plasminogen activator: *Human*	93, 166, 167
Treatment various diseases	Goat	7	7 different monoclonal antibodies	160
Blood disorders, tissue sealant, growth disorders	Pig	4	Factor VIII: *Human* Haemoglobin: *Human* Protein C: *Human* Fibrinogen: *Human* Growth hormones: *Human*	169, 155, 89, 62, 213
Treatment of Pompe's disease, osteoporosis, Paget's disease, anaemia, cancer, blood disorders	Rabbit	8	α-glucosidase: *Human* Calcitonin: *Salmon* Erythropoieten (EPO): *Human* Extracellular superoxide dismutase: *Human* Insulin-like growth factor: *Human* Interleukin-2: *Human* Tissue plasminogen activator: *Human*	171, 172, 97, 173, 92, 175, 176, 177
Treatment of cystic fibrosis, blood disorders	Sheep	3	Alpha-1-antitrypsin: *Human* Factor IX, Factor VIII: *Human* Fibrinogen: *Human*	178,179, 180
XENOTRANSPLANTATION				
To 'humanise' organs for transplantation	Pig	4	CD55 (DAF-decay activating factor): *Human* CD59: *Human* CD59 and CD55: *Human* GnT-III gene: *Human*	246, 24, 245, 244, 243
OTHER APPLICATIONS				
Disease model - human retinitis pigmentosa	Pig	1	Mutated pig gene	122
Disease models for AIDS, atherosclerosis, lipid metabolism and diabetes	Rabbit	3	Various : *Human, Bovine*	121
Develop techniques	Quail	1	Marker gene: *Bacteria*	186
Develop techniques	Monkey	1	*Jelly fish*	25

2.2.2 Microinjection

Microinjection – also called pronuclear injection – was until recently the only successful method for producing GM livestock. DNA is injected into the nucleus of a single cell embryo using a very

fine needle. Typically 200 – 500 copies of the gene construct are injected into each embryo. The injected DNA is incorporated randomly into the genome of some of the embryos.

In animals other than mice, the embryo is then cultured *in vitro* (in a laboratory in a test tube or similar) for 24 hours, after which it is implanted into a 'pseudopregnant' surrogate mother - an animal which has been chemically synchronised with the egg donors by the administration of hormones. In mice, pseudopregnancy is achieved by mating with sterile males.

If injection takes place after the single cell stage, the transgene may still be integrated but results in a 'mosaic', where only some cells of the animal carry the transgene. Depending on which cells integrate the transgene, this may or may not result in transgenic germ cells (egg and sperm). This in turn determines whether offspring will inherit the transgene.

Microinjection techniques are not suitable for poultry because it is extremely difficult to gain access to the fertilised egg when it is still at the single cell stage, which is necessary for any genetic modification to be integrated into all the cells of the developing embryo. Most success with GM poultry has been achieved via viral transfection (see below). However, the Roslin Institute has reported producing transgenic birds containing one of two marker genes by microinjection of zygotes (single cell embryos)[26].

2.2.3 Viral transfection

Viruses - particularly retroviruses - are often used as 'vectors' to introduce new genetic material into cells because they are naturally well equipped to infiltrate them. Retroviruses are a type of virus which replicates by integrating itself into the host DNA and is then copied with the host genetic material as the cell divides. Several retroviruses have been modified to contain a transgene which is then integrated into the target animal's genome as part of the normal viral lifecycle. Retroviral infection was the first method used to produce transgenic mice in 1976[27]. The main use of viral transfection methods is in attempts to modify poultry because of the unsuitability of microinjection.

Viruses have been used which can repeatedly infect different cells (called 'replication competent') or defective viruses can be used which should only be able to infect cells once. When replication competent viruses are used, the transgene will be reinserted many times – in the founder, in the transgenic offspring, or even in other animals which come into contact with them.

Mammalian and avian retroviruses have been used to transfer genetic material into sheep, pigs and chickens[28,29,30]. These include Moloney Leukaemia Virus, which causes lymphoid leukaemia in mice, rats, and hamsters; Rous Sarcoma Virus, which is associated with cancer formation in humans; and Avian Leukosis Virus, which has many strains and is widespread in commercial poultry flocks.

There are serious safety concerns about viral vectors, which are especially pronounced with replication competent viruses[31]. Viruses can often infect several hosts and have related wild viruses. There is the danger that the retrovirus could recombine with wild viruses and form entirely new pathogens (disease-causing agents).

There are also practical drawbacks to viral transfer of genetic material. There is a size limit on the amount of DNA which can be inserted in the virus, making it unsuitable for larger gene constructs. Viral vectors cannot replicate in early embryo cells[32] so all the GM animals produced are chimeras, in which the transgene only appears in some of their cells. The percentage of offspring which inherit the transgene is therefore even lower than normal.

2.2.4 Embryonic stem cell modification

Embryonic stem (ES) cell culture and modification allows a much more targeted approach to genetic modification. However, despite many attempts to obtain ES cells from rats and farm animals, ES cells have so far only ever been isolated from some strains of mice.

ES cells are derived from very early embryos known as blastocysts. They have the potential to develop into any cell in the organism but can also be maintained in culture conditions that allow the cells to replicate without developing into the different cell types which make up the organism. The importance of the technique is that the ability to culture the ES cells allows for much more selective modification techniques with some control over the integration site. For example, modification can be targeted so that a transgene replaces the equivalent native gene or so that genes are 'knocked out' - made ineffective by removal or disruption.

If the ES cells are injected into another developing embryo, they will sometimes be integrated into the embryo, which will develop as a 'chimera' of the two cell lines. The resulting animal will have a mixture of modified and unmodified cells and, providing modified cells have differentiated into germ cells, a proportion of the offspring will be transgenic.

2.2.5 Sperm mediated transfer

Using genetically modified sperm as a vector for introducing foreign DNA into the egg has obvious attractions as artificial insemination of livestock and poultry is routine. Genetic modification of mice using sperm as vectors followed by *in vitro* fertilisation was reported in 1989[33] but other researchers have had little success in replicating the results. A large scale experiment reported in 1998 obtained an average of 7.4% transgenic mice using sperm which had been incubated with plasmid DNA. However, results were extremely variable – transgenic offspring were only obtained in 13 of 75 trials[34].

Higher success rates have been obtained by injecting modified sperm directly into the egg (intracytoplasmic sperm injection – ICSI) and plasmid DNA into unfertilised eggs[35]. However, although ICSI is a standard reproductive technique, it is much more complicated than artificial insemination and has low efficiency in livestock. The requirement to use ICSI therefore removes some of the attraction of sperm mediated modification[32]. In poultry, although researchers have reported progress in uptake of DNA into sperm, integration of the transgene into the germ line has not been achieved[36,37].

2.2.6 Poultry

The reproductive system in chickens and other birds makes the genetic modification techniques used in mammals unsuitable. Hens ovulate daily and eggs are fertilised almost immediately in the oviduct. By the time the egg is laid, the embryo consists of approximately 60,000 cells and microinjection is very unlikely to result in either germ line transgenic birds or integration into the target cells of interest.

Retroviral transfection (see Section 2.2.3) is the most common method of producing GM chickens. Transgenes were first introduced using the replication competent viruses Avian Leukosis Virus (ALV) and Reticuloendotheliosis Virus (REV) to infect laid egg embryos via holes made in the shell[38]. A small proportion of eggs produced chimeric males and a small proportion of their offspring were transgenic. However, the risks associated with using replication competent viruses have meant that this technique is generally not being pursued. Apart from the risk of new recombinant viruses[37,39], chronic viral infection with ALV may increase birds' propensity to develop cancer[40]. Viruses unable to replicate have also been used[30,41]. The rate of transgenesis is

extremely low. For example, in one study, 2,599 eggs were injected, only 33 chicks carried the vector in sperm and only 2-8% of their offspring were transgenic[41].

Culture and modification of primordial germ cells (PGCs) - the cells that will later form into sperm and ova - has become the focus of work on transgenic poultry as it potentially allows easier and more successful genetic modification. The PGCs in avian embryos develop semi separately from the embryo itself and isolation is relatively easy even at the laid egg stage. If they are subsequently reintroduced to embryos, some will integrate. At present, the most common method for transfection of PGCs is the use of retroviruses, although a group in Korea has reported producing transgenic chicks using liposomes[42]. Should attempts at culturing PGCs prove successful, the use of transgenic chickens could become a reality[43,44].

2.2.7 Cloning

A clone is a genetically identical individual grown from a single donor cell. Mammals (mice and sheep) were successfully cloned from embryonic cells in the 1980s and frogs decades earlier[45].The first report of a clone from an adult somatic (non-reproductive) cell was in 1997 – Dolly the sheep - produced by nuclear transfer from an adult sheep cell at the Roslin institute in Edinburgh[46]. Since then, pigs, cows, mice and cats have been cloned from either cultured foetal cells or adult somatic cells[47,48,49,50,51,292].

Cloning has been achieved by nuclear transfer, where the nucleus of the cell to be cloned is inserted into an egg from which the nucleus has been removed (enucleated). An electric current is used to fuse the donor nucleus with the recipient cell and to start embryonic development. The resultant organism is a clone of the animal from which the donor cell was taken although it does contain a small amount of DNA from the mitochondria in the original egg cytoplasm so it does have a small amount of genetic material which is not present in the donor nucleus.

Nuclear transfer itself is not genetic modification but is a technique which has the potential to make transgenesis both cheaper and more accurate. Targeted genetic modification – which is not currently possible with embryos - theoretically becomes possible if cells can be modified and cultured before being transferred to enucleated eggs as the techniques for targeted modification utilise cell division processes. Cells could also be screened and those which are not transgenic, or which have integrated at the incorrect site, can be rejected, although this is not current practice. The first targeted modification using nuclear transfer has been reported by PPL[278].

Cloning has been put forward as the holy grail of transgenic technology – the technique that will enable targeted genetic modification in large mammals and make it possible to reproduce transgenic lines quickly and cheaply. However, there are fundamental problems to be resolved before the technique is used outside a research context and many would argue that it should not be used at all. Cloned embryos tend to have severe abnormalities, resulting in an extremely high abortion rate[52], and the majority of those that are born alive seem to have some form of health defect[53,54]. For the moment, nuclear transfer techniques have a long way to go before they transform transgenics.

2.2.8 Localised gene transfer

Localised gene transfer, also known as somatic gene therapy or *in vivo* transfection, aims to induce cells in an organism to produce a protein either therapeutically, or for production purposes (e.g. pharmaceutical proteins in milk). In contrast to the other means of genetic modification, there is no intention for the introduced gene to be integrated into the germ line and expression of the transgenic protein is usually only temporary. The introduction of the new genetic material may be achieved using a viral vector, where a modified virus is used to ‘infect’ the cells with the gene

construct, although there are always safety issues associated with viral systems. Transfection may also be by particle bombardment of plasmids containing the new gene, often coated onto gold beads. While there are certainly some ethical and safety questions about localised gene transfer, it is not the subject of this report as it does not represent the same fundamental alteration to an animal's genome.

2.3 What does genetic modification involve for the animal?

Microinjection, the most common procedure for genetic modification of animals, requires many animals to be subjected to surgical procedures.

In the production of transgenic sheep or pigs, large numbers of eggs are produced by superovulation. In sheep, this is achieved by donor ewes having a hormone impregnated sponge inserted in the vagina for 14-20 days prior to insemination and receiving twice daily hormone injections for 3 days before surgical insemination. Insemination involves the insertion of an endoscope through the abdominal wall under general anaesthetic. Following insemination, the fertilised eggs are surgically removed and genetically modified. The cultured microinjected embryos are then surgically implanted into surrogate mothers which have been 'synchronised' with the donor ewes by a similar programme of hormone injections. Assuming 8 eggs per superovulated sheep[55], approximately 60 sheep will undergo surgical procedures to produce one transgenic lamb[56]. In pigs, the procedure is similar except that the donor pigs may be slaughtered to recover their eggs.

In cattle, eggs were originally obtained by superovulation followed by slaughter or surgical removal of the oviducts. More recently, eggs have been obtained from slaughterhouse carcasses, reducing both the cost of surgical procedures on cows and the animal suffering involved. There may be an intermediate surrogate mother (a sheep or rabbit) to screen out non-viable embryos and reduce the number of cattle used. Embryos are then non-surgically implanted in synchronised cows.

2.4 How successful is genetic modification?

Genetic modification of animals - particularly larger farm animals - is difficult, inefficient, unpredictable and very expensive.

Table 2: Average efficiency of producing transgenic animals by microinjection (pronuclear injection)

	Percentage of transgenic animals successfully produced per microinjected embryo
Pig	0.9%
Sheep	0.9%
Cattle	0.7%
Rat	4.4%
Mouse	3%
Goat	1%

Table adapted from Wall (1996)[57] and Pinkert and Murray (1999)[153].

Microinjection is the only successful method for genetic modification of mammals other than mice. However, the overall efficiency of even this technique is low (as shown in Table 2) and a lot of animals will be killed, miscarry or be subjected to surgery in order to produce each transgenic founder (the GM animal which is used to breed a transgenic line). Overall, only about 10% of injected embryos survive to birth - the rest die either because of the microinjection process or abort during pregnancy. Only about 10% of the offspring will be transgenic so the overall rate of successfully modified offspring per injected embryo is 1%, rising to 3% in mice[57,153].

Estimates of average costs for a transgenic founder are $120 for a mouse, $25,000 for a pig, and $300,000 for a cow (assuming that eggs are obtained from the slaughterhouse rather than from live cows)[58,22]. Once the founder animal is obtained, the transgenic line can in theory be bred normally. According to standard rules of inheritance, 50% of offspring will be transgenic.

Integration of the transgene is random and may occur at any site in the animal's genome. Multiple copies are often incorporated. The random integration means that transgenic animals may not express the transgenic protein at all, as happens in about half of transgenic lines[57], or it may not be expressed as expected. In about 7% of cases, the transgene integrates within one of the animal's own genes, disrupting its function (insertional mutation)[59]. The effects on transgenic progeny are also unpredictable as the transgene itself or insertional mutations may initially be masked but may surface in subsequent generations, causing the expression of formerly unexpressed proteins or damaging insertional mutations[60,61,62].

2.5 What stage is the technology at?

> ***"Research in transgenic farm animals has a unique character. Thousands of person years of effort, much of it from the private sector, have been expended without yielding any product."***
>
> G.E. Seidel (1999)[7]

There has been sustained overstatement of the achievements of transgenic technology as press reports frequently imply that transgenic livestock are already in the fields and drugs from GM animals are about to stock the shelves of every chemist. An example is the report that appeared in December 2000 about 'Britney', characterised as the transgenic chicken helping to fight cancer – after Roslin merely announced a collaboration with Viragen to *try and produce* a transgenic chicken[63,64].

None of the agricultural applications – increased growth, altered milk composition, improved wool growth - are approaching the stage where they would actually be applied to production animals. This is both because of the high cost of transgenic livestock (between $25,000 and $300,000) and because the applications themselves have not been successful. It is possible that the advent of cloning could change this situation both by reducing costs and by making the genetic modification more accurate – but cloning is also beset with problems. There would also be a long regulatory procedure required before a GM animal could become part of the food chain or be released from a trial situation.

Three pharmaceutical products produced by GM animals are currently in clinical trials:

- alpha-1-antitrypsin (AAT) produced in the milk of transgenic sheep has completed Phase II clinical trials;
- antithrombin III from transgenic goats is in Phase III clinical trials;
- alpha-glucosidase in the milk of transgenic rabbits was in Phase II/III trials until the company responsible, Pharming, went into receivership in August 2001.

PPL, one of the major companies developing pharmaceutical applications and responsible for AAT, is also experiencing major difficulties attracting sufficient investment to scale up production.

There are many other transgenic pharmaceuticals in development and this is the arena in which GM animals are likely to be an increasing reality.

3. ETHICS

"There is a striking mismatch between the traditional concern of regulators with issues of risk and safety, and that of the public, which centres on questions of moral acceptability."

Biotechnology and the European Public Concerted Action Group[65].

The genetic modification of animals arouses grave ethical concerns about species integrity as well as all the questions normally associated with animal experimentation. A recent Eurobarometer survey showed that the public is primarily concerned with the question of whether animals *should* be genetically modified rather than questions of usefulness or risk. There was effectively a 'moral veto' on the pursuit of this type of biotechnology[65].

Consideration of GM animals cannot be undertaken in isolation from the use of animals in general. 2.2 million cattle, 19 million sheep or lambs, 14.7 million pigs[66] and 803 million chickens and turkeys[67] were slaughtered in the UK in 1999. Most UK farm animals are raised in intensive systems in conditions which allow them little if any quality of life or opportunity for normal behaviour[68]. Harmful genetic effects have been bred into farm animals[13], dogs[14] and laboratory mice using traditional breeding methods. Intensive agricultural practices cause more animal suffering, at least in terms of numbers, than genetic modification. However, existing treatment which denies animals a reasonable quality of life does not justify the creation of a new arena where animals potentially or actually suffer.

The creation of GM animals also represents a significant alteration in our relationship to other species and represents a further step towards seeing them purely as commodities without regard for their inherent worth as sentient beings. This is at odds with current trends in society, which increasingly see animals as having rights[69]. For example, the European Directive of 1986 on animal experimentation forbids the use of an animal if another scientifically acceptable method exists[70]. The 'normalisation' of transgenic animals in laboratories is in opposition to this trend and could indirectly impact on wider attitudes towards animals.

Any use of animals arouses strong moral feelings, characterised by extreme and often mutually exclusive positions. On the one hand, there are those who consider themselves superior and intrinsically different to animals and that any use of animals is justified providing it is of benefit to humans. On the other, are those taking the position that humans and animals are morally equivalent and that any usage which would be judged unethical in humans is unethical in animals.

This section examines the specific ethical issues raised by genetic modification and does not attempt to address the ethical debate surrounding the use of animals in general except in so far as to say that existing misuse does not justify the further abuses of genetic modification.

The impacts of genetic modification on animal welfare are also relevant to the ethical debate as, for many people, welfare is the key factor in judging animal uses. The effects of genetic modification on animal welfare are discussed in Section 4.

3.1 Species integrity and crossing species barriers

The concept of 'telos' originated with Aristotle, who contended that every creature had a goal in life which he designated its telos. It has since been described as the 'dignity and integrity' or 'inherent worth' of a being[69]. Few would dispute that every human has telos and, crucially, many people take for granted that it is also possessed by animals.

Many ways in which animals are treated may be seen as assaults on their integrity. For example, the ability to live a normal life is denied by many intensive agricultural systems. This does not, however, justify other infringements.

GeneWatch UK takes as a self evident truth that animals have telos and that assaults on the integrity of animals are therefore unacceptable.

Germ line genetic modification is a fundamental alteration of the genome, one of the most basic attributes of both individual and species. It continues beyond the individual lifetime, reaching into future generations of animals. Certainly, genetic codes change over evolutionary time and to a very much lesser degree as a result of breeding programmes. However, the direct, deliberate alteration possible with genetic modification is qualitatively different. The ability to 'engineer' genes unconstrained by species boundaries and the haphazard nature of genetic recombination are entirely new.

There are fears that these developments could herald a eugenic future with humans also undergoing genetic modification[71,72]. These fears are not unfounded since techniques used on people are generally first used on animals and there is already a group in Italy which has publicly stated its intention to clone human beings[73].

All this has provoked strong reactions, leading to accusations of scientists 'playing god'[74] and references to 'Frankenstein's farmyard'[75]. These reactions are not facile. They stem from a deep unease that genetic modification, especially when it involves crossing species boundaries, is an assault on the sanctity of life and represents a seismic shift in our relationship to the natural world.

3.2 Utilitarianism

Utilitarianism is a moral philosophy which aims to judge all actions by their consequences with the objective of producing the greatest good for the greatest number[76]. According to this framework, genetic modification should be assessed solely by an analysis of costs and benefits without reference to moral arguments. In most utilitarian assessment, the costs and benefits to animals are given much less weight than costs and benefits to humans.

Many things could be justified from a utilitarian point of view that are intuitively unacceptable today. For example, sacrificial medical experiments on humans - even if they are insensate – are not considered acceptable even though they might be very useful scientifically.

An extreme example of the utilitarian approach is how it justifies the use of genetic modification to reduce sentience. There have already been attempts to selectively breed pigs and chickens to make them more tolerant of intensive farming conditions and there are further proposals to use genetic modification with the same aim[77]. This raises the prospect of 'animal vegetables' unable to feel pain or without the desire to move around[77].

It has been argued that reducing, for example, antisocial or fearful behaviour in poultry would bring about an improvement in animal welfare (as well as an increase in productivity)[78]. Such behavioural and productivity improvements have been demonstrated using traditional breeding methods. However, the same productivity improvements can be achieved by such simple methods as improving the cage environment for young birds or increasing the number of times they see humans so that poultry become familiarised[78]. There is a fundamental issue of whether the animal should be altered to fit the environment or whether an environment should be created which meets the animal's needs.

> ***"..the usual response to the suggestion that we deliberately breed [insentient animals] is very strong disapproval."***
>
> Animal Procedures Committee, 2001[79].

The Farm Animal Welfare Committee lists modification for insentience as an 'intrinsically objectionable' act[80]. The Animal Procedures Committee also recommended that licences should not be given for genetic modification with the intention of rendering animals insentient[79].

The welfare impacts of genetic modification can be assessed within a utilitarian framework and this can certainly bring some useful insights although the relative weighting of animal and human benefits needs to be made explicit. However, the fundamental moral issues cannot be addressed precisely because utilitarianism aims to ignore moral constraints.

3.3 The human-animal relationship

The advent of transgenic technology has added an entirely new dimension to this relationship as we now have the power to alter animals, mix species and create a different animal if the existing one does not conform to our requirements. This treats animals as objects for our convenience and is a significant further step towards seeing animals as mere commodities. The intensification of farming has already pushed our relationship with animals in this direction as demonstrated even by the language we use to describe it. 'Factory farming' does not only reflect the conditions in which animals are kept but also their objectification. In the United States, intensively reared animals are now referred to as 'animal units'. An animal unit may equal 1,000 cattle or 100,000 chickens.

The concept of '*ubuntu'* is described by Desmond Tutu in his book about the Truth and Reconciliation process in South Africa following the end of apartheid[81]. *Ubuntu* roughly translates as the essence of being human. It is about the communality of life and maintains that anything which attacks another's humanity not only subverts one's own but also damages the community. *Ubuntu* means that the perpetrator and the victim are inextricably linked.

Although Tutu did not extend the concept of *ubuntu* beyond the human community, there are many parallels with the human-animal relationship. It could be argued that as a society we are deeply affected by our treatment of other species. The lack of respect inherent in our current practices has serious implications for our collective well-being.

3.4 Conclusion

GeneWatch UK considers that genetic modification of animals is an assault on the integrity of living beings and rejects a utilitarian approach to its assessment. Genetic modification should not be undertaken without extremely compelling reasons and the presumption in every case should be against such interventions. Genetic modification of animals changes our relationship with the natural world and contributes to the commoditisation of animals. Our treatment of other species in this way reflects on human dignity and diminishes human society.

4. ANIMAL WELFARE

> ***"…examples are accumulating of transgene instability and unexpected patterns of gene expression in transgenic animals. In many cases, the insertional mutation is recessive and is not expressed until subsequent generations"***
>
> Royal Society of Canada, 2001[61].

Genetic modification has created new challenges to animal welfare as mutations and transgene expression can have entirely unforeseen results. Present regulatory structures may be insufficient to either recognise the suffering caused or to ameliorate it effectively. The welfare of GM animals may be compromised by five types of effects:

- reproductive and other interventions;
- mutations in endogenous genes caused by the transgenic process and/or further mutations in the transgene;
- expression of the transgene;
- 'wastage' of animals;
- associated housing or husbandry effects to suit requirements of the application.

The production process for transgenic founder animals (the GM animals which are used to breed a transgenic line) results in many thousands of surgical procedures, interrupted pregnancies and many hundreds of deaths. Theoretically, this is a 'one off' effect although it will in fact stretch over many years. Qualitatively different are effects on the transgenic line, which can be expected to persist through many generations.

The moral framework that has been adopted in the European and UK legislation[70,82] is that animals should not be used when there is a reasonable alternative; that whatever use there is should be minimised; that any use requires justification; and that suffering should be eliminated or reduced to very low levels. This is effectively the '3 Rs' first put forward by Russell and Burch in 1959[83], which now underpin humanitarian practice in animal research:

- ***Replace*** the use of conscious, living vertebrates by non-sentient alternatives;
- ***Reduce*** the number of animals needed to obtain information;
- ***Refine*** procedures to reduce to a minimum the incidence or severity of suffering experienced by those animals which have to be used.

The '3Rs' are the basis for animal welfare considerations and are enshrined in the Animals (Scientific Procedures) Act 1986 (ASPA).

4.1 Reproductive and other medical interventions

Genetic modification requires reproductive interventions on a large scale because the process is so inefficient. An analysis of experiments during ten breeding cycles showed that the production of one transgenic sheep involved superovulation of 17 ewes, microinjection of 139 zygotes (one cell embryos), transfer into 41 recipient ewes, followed by 25 pregnancies and 39 live births[84]. Approximately 60 sheep[85] or 80 cows[86] must undergo surgery to produce one GM founder.

Female 'donor' animals are induced to superovulate (produce more eggs than normal) by drug administration and the eggs are then harvested, which may be surgical or may – in the case of mice, for example – be achieved by killing the animals. Fertilisation occurs *in vitro* and is followed by embryo implantation in surrogate mothers. The creation of 'pseudo pregnancies' in the surrogate mothers is also achieved with drugs and, in the case of mice, by mating with vasectomised or sterile males. Laparotomy or laparoscopy may be required both for egg

extraction and/or embryo implantation. All of these procedures are likely to cause stress and/or pain to the animal. The donor females may also be mated when extremely young.

A very high proportion (87-95%) of implanted embryos in animals other than mice and rats are not carried to term[57]. This means that a large number of animals go through miscarriages or stillbirths. Many offspring die soon after birth. It is hard to know how severely this affects each species but certainly larger farm animals are known to suffer distress at miscarriages[87]. A high proportion of surrogate mothers may suffer ill effects: in one study of nuclear transfer where pregnancy was confirmed in 18 cows, 6 aborted and a further 4 died as a result of the pregnancy[54].

There have been proposals to produce transgenic pharmaceuticals in urine. Harvesting would require constant catheterisation and is likely to mean the animal is forced to remain upright[88]. This would deny the animal any semblance of a normal life and make it subject to frequent painful interventions.

4.2 Mutations

> ***"The injection procedure involves breakage of chromosomes, so that in the process of their spontaneous self repair the transgene may become incorporated, randomly, into the genome. As a result, mutations, chromosome deletions, translocations, and inversions are common occurrences."***
>
> B. Mepham, 2000[69].

Transgene integration is a chance process with multiple copies of the transgene inserted at random in the genome. In one study, the transgene had integrated into at least three different chromosomes in a transgenic pig[89], with the potential for genetic damage at each location. The process frequently damages the animal's own genes[59,69].

Damaged genes may cause many types of abnormalities. There are examples in mice of deformities including fusion or absence of the radius and ulna, tibia and fibula (bones of the lower front and hind limbs), and bones in the feet fused or missing[60]. Other insertional mutations have caused sterility[60].

Many mutations will remain undetected because they cause the embryo to die, or because the effect is internal or subtle. Subtle mutational changes may go undetected until an animal's welfare is grossly compromised as animals may appear normal. Such changes include, for example, altered cellular respiratory function which can lead to chronic low-grade pain, gradual heart failure or changes in pain or stress thresholds[86].

The impact of the transgene will be crucially affected by the position at which it is inserted, and the genes that surround it. This will determine, for example, whether and at what level the transgene is expressed, which varies widely between transgenic siblings and lines[90]. It may also have a myriad of other effects on the animal, which may compromise its health or well-being. Effects on gene function or phenotype may surface only after successive generations[62].

Genetic modification programmes have a higher than normal level of perinatal deaths[e.g.174,91]. Many of these deaths may be the result of unintended mutations which do not prove lethal until birth and result in obvious distress to the neonate.

4.3 Expression of the transgene and unexpected effects

Transgene expression, even when it occurs in the intended tissue, can severely compromise animal welfare. There is also usually some 'leaking' or ectopic expression of the transgene so that

gene products intended, for example, to be produced only in milk, appear at low levels in the general circulation or in non-target organs[92,93].

There are many documented ill effects from transgene expression. The expression of exotic growth hormone in mice has caused severe organ damage in liver, kidneys and heart as well as early death[94,95]. The damage to the Beltsville pigs has been well documented[96] (see Section 7.1). Sterility has also been a common outcome[94].

Transgene expression of erythropoietin (EPO) in rabbits, intended to be expressed exclusively in milk, produced low levels in other tissues and resulted in severe polycythemia (abnormal increase in the number of red blood cells). Most animals died prematurely and were infertile[97].

Promoters have had unexpected effects in different species. The mouse promoter, Whey Acidic Protein (WAP), inhibited mammary gland development and lactation in three lines of transgenic pigs. The sows were virtually unable to produce milk, despite piglets suckling normally, and mammary gland tissues showed a mixture of immaturity and inflammation. It was thought that the transgenic promoter interfered in normal mammary development[98].

The effect of transgenes may vary widely because of species differences. GM mice have developed unexpected types of cancer following the insertion of various 'oncogenes' [e.g.99,100]. Quite apart from the suffering associated with developing cancer at all, this may mean that tumours go undetected for a significant period until they reach a very late stage[101].

Many failures and unexpected effects of genetic modification go unrecorded in the scientific literature so those that are recorded probably represent the tip of the iceberg.

4.4 Wastage

The very low efficiency of microinjection means that many animals are produced which do not carry the transgene and are therefore 'surplus to requirements'. Only 5% of cattle and 7% of goats born alive from microinjected embryos are transgenic. Even in mice the proportion reaches only 30% at best. Thus, a minimum 70-95% of animals produced in transgenics programmes undergo surgical procedures to determine whether they are carrying the transgene and, if not, they are killed. Even animals that do carry the transgene may not express it and these are also killed.

4.5 Housing and husbandry effects

As well as the direct effects of genetic modification, housing and husbandry requirements for transgenic animals may also compromise their well-being. Pigs raised for organ donation or cattle for pharmaceutical production may be isolated, deprived of proper bedding, rooting material or sufficient exercise space for reasons of hygiene. On the other hand, if the needs of the animals are properly considered, their health and welfare may be better than non-GM agricultural animals of the same species[102].

4.6 Conclusion

Genetic modification compromises animal welfare because of the high level of surgical intervention required and the unpredictable effects of the modification itself. Many unintended mutations have already occurred with damaging effects, as witnessed by the high proportion of embryo and perinatal death. These mutations may surface many generations later and, if effects

are subtle, may cause suffering which goes undetected.

The effect of the transgene itself has also caused ill effects of varying severity (including death). As expression levels vary within a line, it is unclear whether a particular expression level can ever be regarded as stable. While extreme effects are likely to be noticed, more subtle changes may not.

The inefficiency of the techniques means that very high numbers of animals are required to undergo reproductive interventions to produce GM animals and that the vast majority are killed either because they have not integrated the transgene or do not express it. Allowing for reproductive interventions and killing of 'surplus' animals, this may mean that more than 100 animals are killed or undergo surgery for each transgenic founder.

GeneWatch UK considers that genetic modification is in opposition to the welfare principles of *Reduce* and *Refine*. Very large numbers of animals are used in order to genetically modify very few and it is likely that continued genetic modification will lead to an overall increase in the number of animals undergoing surgical procedures. The unpredictability of the effects of genetic modification means that it is extremely difficult or impossible to anticipate ill effects and therefore be able to ameliorate them.

5. LABORATORY ANIMALS

This section looks at developments in the genetic modification of laboratory animals and the ways in which they are being used. It does not address the question of whether experimentation on animals is right or wrong although the introduction of transgenic animals should prompt us to reflect on our use of laboratory animals in general and particularly the relationship we have with the laboratory mouse. The section focuses particularly on mice - as they are by far the most numerous laboratory animal - and gives an overview of the development of disease and toxicity models in the mouse.

When considering the use of transgenic animals in the laboratory, there are some fundamental questions that should be borne in mind:

- Does transgenesis represent a significant assault on species integrity?
- Is it a radical change in our relationship to laboratory animals?
- Do transgenic models work and do they offer the best means to study disease and develop treatments?

Most potential therapeutic agents identified through screening on mouse models of disease (particularly cancers) have proved ineffective in humans for two main reasons. Firstly, genetic models of disease ignore environmental factors that can grossly affect manifestation of the disease, even in diseases considered to be the result of single gene disorders. Secondly, models of genetic disease resulting from interpretation of a gene acting within the genome of a different species may have little in common with the disease picture in humans.

Our relationship with laboratory animals is different from our relationship with agricultural animals. While the use of animals for food inevitably involves death, the animal may, in theory, have a reasonable quality of life and a painless death. In practice, of course, most agricultural animals are reared in intensive farming systems with varying degrees of unpleasant conditions and - at least in the UK - undergo traumatic journeys *en route* to slaughter[68].

With laboratory animals, however, suffering is almost inevitable since this is often a prerequisite for their very existence. In medical experiments, the *raison d'être* of the animal is generally to develop a disease in order to cure it, study it or determine which genes are influential. In toxicity testing, substances are administered at increasing doses to determine when harmful effects occur. On the one hand, it is easy to see such deliberately induced suffering as morally unjustifiable. On the other, it is argued that many people would suffer without the advances that animal research brings to medicine. Some authors estimate that human life expectancy has been extended by 20 years as a result of medical advances resulting from animal experimentation[103], while others assert that significant advances do not derive from animal experimentation but from a combination of astute clinical observation, epidemiology and luck[104,105]. Whatever the contribution of animal experimentation, however, improvements in diet and sanitation are likely to have been the most significant factor in improving life expectancy, health and well-being[106].

2.1 million scientific procedures were carried out on mice and rats in the UK in 2000 - 91% of all the procedures on mammals. Just under 579,000 of these were on GM mice or rats - over 99% of all procedures involving GM animals. Another 250,000 involved animals which had been deliberately bred to have a harmful genetic defect[3]. While the total number of procedures has decreased by nearly 10% since 1991, procedures on animals with harmful genetic defects and transgenic animals have increased dramatically - by 50% and 800% respectively[19,3].

Whilst mice and rats are the most commonly used animals in laboratory experiments, a significant number of guinea pigs and rabbits are also used (97,000) as well as some cats, dogs, poultry, ferrets and a sizeable number of agricultural animals. With the exception of some rabbits and livestock, the animals used are not transgenic.

The '3Rs' - Replace, Reduce, Refine (see Section 4) - are taken to be a minimum standard in the following discussion and are assumed to apply to all laboratory uses of animals, transgenic or otherwise. The first priority should be replacement – the use of non-sentient altternatives. It is only if replacement is impossible and the experiment is sufficiently important that the use of animals should be considered. The framework adopted for consideration of alternatives and judgement of 'sufficiently important' is crucial and should be informed by a wide range of opinion.

5.1 Species integrity and our relationship to laboratory mice

Since mice are so widely used in laboratory experiments, it is especially important to question whether the creation of transgenic mice is a significant assault on the species integrity and if it represents a radical change in our relationship to them.

Since the early 1900s - when mice which had developed tumours were used to breed a line particularly susceptible to cancer - mice have been deliberately inbred to develop genetic defects. Mice have also been subjected to regimes (radiation and chemicals) which trigger germ line mutations. This arose from research into radiation and toxicity risk assessment where it was observed that the programme caused mutants. These mice were subsequently selected and used to produce inbred lines[107]. Now there are deliberate mutagenesis programmes where mice are injected with proven mutagens to generate random mutations. Some of these programmes are formally linked to the Human Genome Project [e.g.108] and some are part of the resultant drive to identify the maximum number of genes[109].

The development of transgenic techniques has led to an explosion of mouse disease models as researchers attempt to insert genes to make mice susceptible to human diseases or display symptoms that mimic human diseases. Transgenic mice are also used extensively in basic biological study as genes are selectively knocked out or disrupted to observe the effect this has on phenotype or function[107].

Selective breeding for harmful genetic defects is a phenomenon restricted to laboratory animals. In no other situation do debilitating or even fatal conditions form the basis for selection. In the case of current mutagenesis programmes and transgenic mice, the initial defect is also deliberately induced. There are currently a large number of 'mutant' strains of mice and rats available through mail order via the Internet. These include both inbred strains and transgenic lines [e.g 110].

The crossing of species barriers in mice is certainly an assault on species integrity. The insertion of genes from other species – as has happened countless times to mice – is a fundamental alteration of an animal's genome which could not be achieved except by using GM techniques. However, deliberate mutation by means of chemicals or radiation is also an assault on species integrity although qualitatively different. It could be argued that systematic breeding for harmful defects resulting from spontaneous mutation is likewise an assault.

In answer to the question, 'Does genetic modification radically change our relationship with mice?', the insertion of genes from different species is certainly different from what has been done before. However, even though they do not involve the introduction of foreign genes, mutagenesis programmes also set out to deliberately alter the mouse genome on a massive scale. The alteration was not to be achieved by breeding for desired traits, even if harmful, but by deliberate gene mutation. Mutagenesis programmes have therefore already raised similar ethical issues to those arising from genetic modification.

While the distinction between deliberate mutagenesis and opportunistic breeding for observed mutation may be small for the mouse, the ethical distinction for humans is important. In previous selection, mutation has either been spontaneous or the result of screening work which was

thought to be important for human safety. In the mutagenesis programmes, genetically abnormal mice are deliberately created with the full knowledge that they are likely to suffer (as is also the case in transgenic disease models).

There is also an issue of scale, which is certainly relevant to questions of welfare. Literally thousands of mice are being used in the mutagenesis programme - 40,000 were screened in just two studies[108,109].

It might be claimed that mice have already been subjected to so much interference that species integrity is no longer a valid argument and that concerns should centre on welfare and the three 'Rs'. It has also been argued that the use of transgenic mice could reduce the use of other species and promote animal welfare in that way. Do mice for some reason no longer deserve the attention afforded to other species?

Abandoning the laboratory mouse as a species worthy of consideration probably has more to do with mice being small, cheap and easy to work with compared to other species rather than any ethical considerations. Scientists - and to a lesser extent, society - have become habituated to experiments on mice for these practical reasons. However, insults to a species in the past do not morally justify genetic modification as an additional insult. Because the interests of mice can so easily be overridden on grounds of convenience, there should be even more careful examination of the justification for transgressing their species integrity as well as their individual welfare.

The watershed in the human relationship with the mouse arguably arose at the start of the deliberate mutagenesis programme. Perhaps the advent of transgenesis, with the ethical issues it raises, should be the trigger to reassess that programme.

5.2 Basic biological research

Laboratory mice have been used for several hundred years to investigate basic biological processes. This use exploded at the start of the 20th Century with the development of the science of genetics. The extension of Mendelian hereditary theories to animals made use of the 'fancy mice' that had been bred in China, Japan, Europe and then America for several thousand years[111].

Genes usually occur in pairs, one inherited from each parent. If both genes governing a particular trait are the same, the organism is *homozygous* for that gene but if the genes are different, the animal is *heterozygous.* Only one of a gene pair governing a particular trait can be active so only the dominant form of the gene will have effect while the animal is heterozygous. Any genes for which the organism is homozygous will therefore be passed on to any offspring automatically, while for any heterozygous gene it is unpredictable which form will be inherited.

Inbred lines are defined as the result of brother/sister matings for at least 20 generations, by which time they are 98% homozygous. In practice, many lines have been inbred for more than 150 generations and are therefore completely homozygous and effectively genetically identical. Many of the 'inbred lines' used today were developed between 1900 and 1930. Animals were selected for specific traits from spontaneous mutations - for example, susceptibility to cancer, increased rate of ageing or increased preference for alcohol or narcotics[112,111].

The ongoing mutagenesis programmes have taken this significantly further. In one large-scale programme, male mice were injected with ethyl nitrosourea, the most potent mouse mutagen, and 14,000 offspring (up to 100 per male mouse injected) were screened. Mice displaying abnormalities - including limb deformities, cataracts, immune deficiency, hypersensitivity and deafness - were selected for breeding in order to generate new mutant lines[108].

The development of transgenic techniques has added yet another dimension. It is now possible to 'knock out' or disrupt genes to identify gene function. In one programme, referred to as 'gene-trapping', marker genes are randomly integrated in embryonic stem (ES) cells. ES cells which contain unknown 'tagged' genes are then selected to generate transgenic mutant lines. In one study, 500 cell lines in which unknown genes had been disrupted were obtained and 60 selected for development. 20 caused fatal defects pre- or peri-natally. 7 lines had postnatal defects (2 lethal) including severe lung pathology, tremor and abnormal gait[21]. The effect of a gene knockout may also vary between strains of mice and some effects are not detected except under certain environmental conditions[113], making interpretation and extrapolation to humans extremely problematic.

The mutagenesis programmes – both chemical and transgenic – are bound by their nature to generate unexpected effects and suffering, which is likely to be prolonged by preferential selection and further breeding. Transgenic - as opposed to chemical - methods could potentially lead to fewer mice being used as some selection can occur at ES cell stage.

It is hard to see how detailed information about genes could be obtained quickly without work on animals. However, is knowing the function of each human gene so vital at a time when cures have not been affected for even the known single gene disorders? Certainly, the 'necessity' criteria are difficult to argue as there is no immediate medical benefit deriving from most of the genes identified. Both transgenic and chemical mutagenesis programmes are bound to use many thousands of animals. It is questionable whether such a 'fishing expedition' would be condoned if the 3Rs were systematically applied. These approaches may also lead to far too simplistic or even false assumptions about gene function because interactions between genes and between genes and the environment are not addressed.

5.3 Disease models

> ***"With the exception of basic genetic mechanisms, the mouse is a relatively poor model for the human"***
>
> Petters and Sommer, 2000[114].

The use of inbred mice to study genetic factors in cancer started in the early 1900s with strains inbred for susceptibility to tumours[111]. The development of mouse disease models continued using spontaneous and induced mutants. The advent of transgenic mice and 'knockout mice' (in which a specific gene has been disrupted or replaced) brought the hope that faithful mimics for human diseases could be created and could be used to develop and test gene therapy techniques[103].

A 1997 review listed mouse disease models for 110 different human conditions. 158 mutated genes were listed. 91 resulted from transgenic techniques, 16 from chemical, 12 from radiation and 39 were originally from spontaneous mutations[20]. Transgenic mice have been created to act as disease models for neuro-psychiatric, hearing and vision, cardiovascular, pulmonary, inflammatory, immunological and metabolic disorders, and for many types of cancer[102,20]. Taconic Farms Inc offers 31 transgenic mice strains for research into cancer or carcinogenicity testing (11), immunological disease (15), inflammatory disease (4) and endocrinology (1)[110].

The simplest disorders to detect, model and treat are those caused by disruption of a single gene - for example, cystic fibrosis or Huntington's disease. Yet genetic environment may still have a crucial effect: as the same mutation can show no symptoms at all or trigger a severe condition even in different individuals of the same species. One of the best researched single gene disorders is ß-thalassaemia, where red blood cell production is impaired and patients are anaemic. People carrying the gene may be completely healthy, mildly affected or severely anaemic[115].

Cystic fibrosis (CF) is the most common Caucasian severe single gene disorder, affecting one in two thousand births. Sufferers usually die in their mid twenties. Several transgenic mouse models have been created by insertional mutation and display similar molecular changes to CF patients. However, the disease progress is extremely different. Serious lung disease is the cause of death in 95% of human patients but CF mice develop lung disease infrequently and mildly. CF mice generally die peri-natally from severe intestinal obstruction while only a minority of human CF patients develop serious intestinal problems[103,20].

Lesch Nyan syndrome is another single gene disorder, characterised by mental retardation and distressing behavioural abnormalities such as compulsive self-mutilation. Several mouse models were created with the same genetic defect but the mice did not display abnormal behaviour[20].

The situation is much more complex in multifactorial conditions such as cancer. In breast and ovarian cancer, 5-10% of cases are associated with defects in the BRCA1 and BRCA2 genes. However, possessing these mutations does not mean that cancer will develop - estimates range from 36-85% for breast cancer and 10-44% for ovarian cancer - and the age of onset varies widely. These variations are associated with many other factors including environment, other genetic effects and random processes which are not understood[116].

If even single gene disorders cannot be mimicked in mice, what hope is there for other diseases where the genetic component of susceptibility to disease is much less? In many conditions, environment - not genetic susceptibility - is the determining factor. Not only are mice suffering as a result of rather simplistic genetic determinism but the focus of health care becomes diverted from prevention to cure.

> ***"The fundamental problem in drug discovery for cancer is that the model systems are not predictive at all."***
>
> Alan Oliff, Executive Director Cancer Research, Merck Research Laboratories[117].

> ***"The history of cancer research has been a history of curing cancer in the mouse. We have cured mice of cancer for decades - and it simply didn't work in humans."***
>
> Dr Richard Klausner, Director of the National Cancer Institute, USA[118].

The purpose of disease models is ultimately to discover therapies which will be effective in humans. However, there is considerable controversy over the utility of animal models – even transgenic ones - to detect useful treatments for humans. The progress of cancer is extremely different from species to species[119,120] and substances found to be effective therapeutically in mice are frequently toxic and/or ineffective in humans[117,118]. Advances may be more likely to be made through research in human cell culture, followed by toxicity testing and human trials.

As well as mice, large animals - including rabbits, pigs, and sheep - have also been genetically modified as disease models. Transgenic rabbits have been produced to model atherosclerosis and lipid metabolism, AIDS, acromegaly and diabetes, lymphocytic leukaemia, lymphoid and non-lymphoid tumours, papillomas and skin cancer. The single transgenic rabbit produced to model acromegaly was sterile – which meant it could not be used to breed further rabbits[121]. Sterility is not a symptom of human acromegaly. A transgenic pig has been produced as a model for human retinitis pigmentosa[122], which causes progressive loss of vision followed by blindness, even though there are naturally occurring hereditary retinal degenerations in dogs, cats, chickens, and rodents.

All the question marks over applicability of data from mice to humans are amplified in other animals. Transgenic techniques in large animals are far less targeted than in mice, leading to random integration and multiple copy insertion, so additional genetic changes are almost bound to appear alongside the intended insertions. The mouse genome is much better researched and understood than any other animal's. Even with these advantages, mice can make unexpectedly

poor models of human disease, even of single gene disorders such as cystic fibrosis. It is likely that attempts to use other species will be even less successful as their genomes are so much less known and techniques for modification are so much poorer.

The use of transgenic techniques is resulting in an explosion in the development of new animal disease models despite concerns over whether mice can in fact accurately model human diseases. Human genes can be inserted into mice but they are still operating in a mouse genetic background and physiology. Gene interactions will be different to those which take place in humans. There is the worrying prospect that increasing numbers of transgenic animals will be generated, which are bound to suffer and whose impact on human diseases may be marginal[86].

GeneWatch UK does not consider that the potential advantages to be gained by current proliferation of transgenic disease models fully justifies the further suffering involved or the crossing of species boundaries in this way. Rather, there should be much closer scrutiny of the scientific justification for each proposed animal disease model and a much broader questioning of whether the focus on genes is justifiable in terms of health care provision and prevention of disease.

5.4 Safety testing

Thousands of new chemicals are developed every year. Before release, new substances require toxicity testing whether they are food products, cosmetics, drugs or industrial fluids. Testing has traditionally been carried out using animals. Toxicity testing – particularly of cosmetics – was the subject of extensive public campaigns in the 1980s which were instrumental in changing test regimes to use fewer animals and inflict less pain[123].

Traditional regulatory safety tests have often included long term (two year) animal studies in two different species to identify potential carcinogens. These are expensive, involve a lot of animals (and potentially animal suffering) and cannot hope to keep up with the constant flow of new substances requiring testing. Approximately 400-500 mice or rats are used per compound[102]. These tests have increasingly come under critical scrutiny for accuracy and reproducibility[124] and for their ability to predict effects in humans, particularly at the high doses used in animal tests[125,126].

One study compared results for 121 diverse chemicals from the Carcinogenic Potency Database[127]. The database holds two data sets - results from the National Cancer Institute/ National Toxicology Program and results taken from the general literature which meet defined quality criteria. The overall agreement between the data sets was found to be only 57%. Even substances classified as two species carcinogens did not come out substantially better[124]. With so little agreement between different laboratories' animal test results, how accurately can these tests predict human toxicity?

Another report concluded that animal tests producing tumours at multiple sites had little predictive worth for humans because such tumours were a 'side effect' of the testing method[125].

> ***"Uncritical reliance on the results of animal tests can be dangerously misleading, and has cost the health and lives of tens of thousands of humans."***
>
> J.C.W. Salen, 1994[128].

There are many instances where disastrous effects in humans have followed animal toxicity tests which indicated that products were safe. For example, Clioquinol (an anti-diarrheal) was withdrawn from sale in Japan in 1970 after more than 8,500 people had been affected, in some cases permanently, by neurotoxic symptoms including loss of motor control and blindness[129].

There is a risk that reliance on animal tests leads to clinical trials that are too short and an inadequate reporting system when a drug is actually released – which, after all, is the first time it is tested in a large variety of human subjects and conditions[104].

The fact that a product may be harmless to an animal but harmful to humans is not the only reason that safety testing on animals may be misleading. The reverse effect may also be true. Drugs which are effective and relatively benign in humans, for instance, may be noxious in other species[128] so that animal tests may lead to the abandonment of promising human therapies[104]. Penicillin, for example, is fatal in guinea pigs and aspirin is teratogenic (causes embryo or foetus malformations) in cats, dogs, guinea pigs, rats and mice[128].

> ***"The current reliance on chronic tests in laboratory animals is of limited value, because of species differences, general economic and logistical considerations, and the high cost of mechanistic studies."***
>
> Pfaller *et al*, 2001[130].

An extensive review of regulatory carcinogenicity testing found little or no evidence that results from the second species animal test influenced regulatory decisions. In 1997, the Safety Expert Working Group of the International Conference on Harmonisation of Technical Guidelines for Registration of Pharmaceuticals published new guidelines. After considering reducing the requirement to a single test – which would have halved the number of animals involved in safety testing - the working group disappointingly decided only to allow for substitution of the second long term test with a short or medium term test. Transgenic mouse models or new born mouse tests were cited as alternatives to traditional long term rodent tests[131].

Table 3: Transgenic mice proposed for carcinogenicity testing

	DESCRIPTION	ORIGIN/COMMENT	REF
TG.AC	Contains mutated viral tumour gene v-ha-ras.	Developed accidentally at Harvard Medical School, USA. Intended for study of basic developmental processes, researchers noticed that mice developed tumours after minor skin abrasions. They went on to breed a line of TG.AC mice to evaluate for carcinogenicity testing. Available from Taconic Farms Inc.	99
rasH2	Human tumour initiator gene H-ras inserted and over-expressed	Developed in Japan at Central Institute for Experimental Animals. Approximately 50% of mice will spontaneously develop tumours by 18 months of age. Available from Taconic Farms Inc.	137
p53 +/-	Heterozygous knockout of p53 gene, a tumour suppressor. Mutated p53 genes are found in up to 50% of human tumours.	Developed at Baylor College of Medicine, Texas. Available from Taconic Farms Inc. as homozygous, heterozygous, and with the Big Blue marker gene. Homozygous mice spontaneously develop widespread tumours by 6 months, the most common being malignant melanoma.	138
XPA	Homozygous knockout of a gene responsible for DNA repair. Particularly associated with UV-B effects.	Developed at University of Utrecht, the Netherlands. If the XPA mouse is adopted for tests it will require medium term (1 year) tests. Embryos display growth retardation and liver abnormalities. 50% of embryos die of severe anaemia.	139

The driving force behind the use of transgenic models is quicker, cheaper tests in order to mass screen drugs and environmental chemicals for toxicity and carcinogenicity[132,133]. GM mice with

increased susceptibility to cancer have been proposed to enable the shorter six month testing regime[134].

Four transgenic mouse models have been developed for carcinogenicity testing so far and are summarised in Table 3. Carcinogenicity tests using transgenic mice have been compared to traditional rodent tests in a two-year study co-ordinated by the International Life Sciences Institute in Washington. Results are ambiguous and the study has not endorsed the replacement of traditional tests with tests on transgenic animals. Rather, it advised that the transgenic animal tests could provide additional information and could be a component of an assessment which included the traditional testing on rats[135]. There was no consensus that transgenic models offered any particular advantage over traditional testing on mice apart from the reduced time and expense required[136].

In an entirely different programme, transgenic mice have been developed for testing polio vaccine. Only primates are susceptible to polio and 100 monkeys are currently used to test every batch of polio vaccine for safety and effectiveness. Validation studies are currently underway to determine whether transgenic mice could replace monkeys[140].

Safety testing regimes are under pressure and their relevance is under scrutiny. The chief argument for transgenic models is to save on the expense and time of long-term bio-assays and therefore enable many more chemicals to be tested. If transgenic mouse models, rather than non-animal alternatives, are developed and become the norm for toxicity testing, it will lead to a sizeable increase in the numbers of animals used.

Whilst some toxicity testing on animals appears inevitable in the short term, GeneWatch believes that more stringent justification should be required for test programmes and the following questions addressed:

- Are there alternatives that have already been tested?
- Is the new product essential?
- Is the test method proposed the only one possible?

Rather than developing transgenic animal models for testing, the research effort should be concentrated on improving alternative models such as human cell culture which have the potential to be more accurate predictors of human response.

5.5 Alternatives

> ***"An experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practicably available."***
>
> Directive 86/609/EEC

The alternatives to animal experiments include better use and dissemination of information; computer-based systems; physico-chemical techniques; microarrays; cell, tissue and organ cultures; human studies; and lower organisms and embryos[141]. In some situations, these may reduce the number of animals used for experiments or reduce the stress which animals are subjected to rather than replace the experiments altogether.

- ***Effective use of information:*** Good dissemination of results can reduce the numbers of duplicate experiments which are carried out. The integration of historical information and information gathered from human subjects into risk assessment, and the use of analytical tools such as decision networks or 'parallellogram approaches' could both improve the accuracy of predictive systems[142] and reduce the number of animals used.

- ***Computer-based systems:*** Developments in computer modelling have revolutionised drug development. For example, protease inhibitors used as part of the AIDS triple therapy were developed through computer modelling, which is one of the techniques being used to develop further AIDS therapies[141].

- ***Physico-chemical techniques:*** The first chemical substitution of an animal toxicity test was announced in March 2000[143]. Corrositex, a chemical mixture on a collagen matrix, serves as a synthetic skin. Chemicals are placed on this rather than on a rabbit. Once the chemical penetrates the barrier, it causes a colour change. The time taken results in the corrosivity classification. If a 'non corrosive' result is forthcoming, an animal test follows. Many cosmetics companies now use a proprietary reagent to predict the potential of chemicals to irritate the eyes[141].

- ***Microarrays:*** The development of microarrays - plates coated with a variety of genes linked to marker systems which indicate when they are activated - to determine whether genes are activated are showing potential in drug screening for toxicity[144].

- ***Cell, tissue and organ cultures:*** Cell cultures could be used extensively for toxicity testing (see below)[130]. There are also instances where *in vitro* systems can be used as disease models. It is important that human cell lines and tissues should be used to avoid the problems of extrapolating from one species to another which undermine the usefulness of animal models. The Dr Hadwen Trust is funding research into cell culture research on various diseases including meningitis, liver disease, breast cancer and cataracts[146]. Since 1990, the USA National Cancer Institute has screened 60,000 potential cancer drugs on 60 human cell lines[145] after a twenty-five year animal screening programme failed to yield a single human drug[128].

- ***Human studies:*** Human studies offer the best information on human disease. These include a wide variety of methods - clinical studies of patients, autopsy, epidemiological studies, drug testing using healthy volunteers and volunteer patients, and rigorous reporting on initial release. New techniques for scanning and imaging allow non-invasive methods for gaining accurate information.

- ***Lower organisms and embryo stages:*** Bacteria, plants, and insects can all be used in some instances, particularly for toxicity testing. The Ames Test for genotoxicity, which has been validated and accepted for screening, uses *Salmonella* bacteria. Hydra are currently being used for research into diabetes[146].

Animal disease models are the most difficult application for which to find alternatives. However, reliance on animal models may not be the most effective way to discover human therapies and the concentration on experimental animals may lead to under-funding of clinical research. The situation is very different for safety testing, where alternatives already exist which may have greater predictive value than animal tests:

> ***"…many of these novel, advanced, in vitro approaches result in information which is often more relevant than animal studies for human hazard assessment, due to their use of human-derived proteins, cells and tissues."***
>
> Pfaller *et al*, 2001[130].

The current animal safety tests are beset with problems and the need for new tests has been recognised. Non-animal alternative tests are already in existence or could be developed. The requirements for testing can be met by *in vitro* systems with the added advantage that results can be obtained for a much wider range of doses and should be more easily reproducible[130].

The decision to use transgenic animals, once enshrined in regulations, would result in their continued use for a long time – regardless of whether it offers the best method. Test regimes

have persisted for many years despite poor applicability to humans. A good example is the Draize test, in which potential eye irritants are tested in rabbit's eyes. An interlaboratory study in 1971 demonstrated that test results were extremely variable both day by day and between different laboratories[147]. Another study showed that the correlation between rabbit and human eye responses was extremely poor - only 0.5[148] - so tossing a coin could produce results with as much value. It has been stated by personnel from the US Food and Drug Administration that there is no clear relationship between rabbit and human eye responses[149]. Despite all this, the test is still in routine use today.

5.6 Welfare

Laboratory animals used in medical experiments are bound to suffer to a greater or lesser extent. Is this suffering likely to be greater in transgenic animals? Potentially, yes, as animals can now be engineered to develop conditions they would otherwise not be subject to. There are certainly many examples of suffering in transgenic mice.

A transgenic mouse model of brittle bone disease has been produced at the University of London which causes mice to suffer extensive bone fracturing and even respiration can result in fractured ribs[150]. A model of Beckwith-Wiedemann syndrome has been developed at the Babraham Institute in Cambridge in which mice have, among other symptoms, tongue enlargement and skeletal abnormalities[151].

Unexpected effects often arise from transgenic insertions. For exmple, after insertion of the simian virus 40 gene (SV40), which is associated with carcinogenesis, unexpected mortality of mice was observed although the viral activity usually associated with gene expression was absent. It was noticed that several animals had hydrocephalus-like symptoms. When the cranium was removed, large amounts of fluid escaped and the brain collapsed. Despite the enormous pain that must be associated with brain tumours large enough to make the head bulge, researchers went on to breed a line of SV40 mice[101].

The transgenic mouse now proposed for use in rapid carcinogenicity testing was also an accidental development. Researchers at Harvard had intended to create a developmental model but noticed that the mouse had such raised susceptibility that it developed tumours from skin abrasions. In a validation study for carcinogenicity testing it was noted:

> ***"..when the tumour burden exceeds 30-40 per mouse, papillomas frequently coalesce and continue to grow as a single mass. Also advanced keratinisation of lesions results in sloughing or removal by biting or scratching."***
>
> R.W. Tennant, 1998[134].

Mutagenesis programmes also produce painful deformities which are selected for reproduction although transgenic and knockout techniques may increase the pace and range of introduced disabilities. All three have become almost routine in basic biological research with little thought of limiting the potential damage to the animals. There is a danger that the fundamental requirement to 'replace, reduce and refine' could get lost in the momentum of the programmes.

5.7 Conclusion

> ***"..the use of transgenic animal models could lead to refinement and reduction in the numbers of animals used in experiments. There is, however, a substantial risk that the current intense interest in developing novel transgenic strains will, in fact, result in an overall increase in experimental animal use."***
>
> Mepham *et al*, 1998[102].

Genetic modification of mice is an assault on species integrity as is the deliberate, large-scale genetic mutation in the mutagenesis programme. This programme may be seen as the watershed in our relationship to the mouse, with the insertion of genes from different species a significant further abuse. However, allowing incremental abuse based on past actions has no moral justification. Instead, the ethical issues raised by the creation of transgenic animals should precipitate a reassessment of the mutagenesis programme and of our entire relationship to the laboratory mouse. The convenience of mice as experimental animals should not lead to their neglect as a species.

It is difficult to find replacements for animal models for human diseases and certainly they will continue to be used in the short term. However - quite apart from ethical issues - there is a danger that transgenic animal models will be seen as a panacea for all the problems in extrapolating from one species to another. However good the model, a mouse is a mouse, even when it has some human genes. There is also a danger that genes will increasingly be seen as determinants of disease, neglecting environment and prevention. It is estimated that two-thirds of cancer deaths in the developed world could be prevented by lifestyle changes, essentially diet, exercise and reduction of tobacco and alcohol consumption[152]. Perhaps the concentration on 'cure' is misplaced.

In toxicity testing, there is an opportunity to develop non-animal alternatives which offer greater accuracy in predicting human response. However, there is a good possibility that this will be lost in the rush for transgenic development and the misapprehension that genetic modification will 'fix' the problems of animal toxicity testing.

Transgenic techniques may have potential to reduce the numbers of animals used in some instances. However, there is much more likelihood that the lure of genetic modification will lead to far greater use of animals and less investigation of alternatives. Rigorous assessments are needed to determine whether each experiment is the only, or even the best, means of achieving a particular end. Only those medical applications which reduce animal use or offer very convincing advances should be allowed.

Transgenic work is seductive, fashionable – and expensive. It is frequently linked to drug development, which is generally concentrated on those diseases for which there will be adequate financial returns. There is a danger that the glamour associated with transgenic technology and the potential profits in pharmaceuticals will drive development choices rather than medical or social need.

GeneWatch believes that each use of transgenic technology in laboratory animals should be scrutinised very carefully – particularly in mice because their interests are so easily overlooked.

6. PHARMACEUTICAL PRODUCTION – 'PHARMING'

"Will new animal reservoirs of fatal human disease be created? Will more virulent pathogens be artificially created? What is the environmental impact of the 'release' of genetically engineered animals? Perhaps most importantly...do the advantages of a bioengineered product outweigh potential consequences of its use?"

Pinkert and Murray, 1999[153]

The production of a foreign protein in the milk of transgenic animals was first demonstrated in 1987 when scientists at the National Institute of Health in the USA reported the production of human tissue plasminogen activator in mouse milk[154]. Since then, at least 29 human therapeutic proteins have been produced in transgenic animals (see Table 5), most of them in milk, but some in blood, urine, or sperm[155,88,156]. 'Pharming' is the application closest to commercialisation and it is likely that drugs will be the first product derived from transgenic animals to reach the market place.

There are several reasons put forward for using transgenic animals for drug production. It has been presented as the only means to supply large quantities of some proteins[157] by arguing that production would be cheap and could be scaled up easily if demand increased, and production in mammalian cells means that the correct form of the molecules is produced[158,159].

These arguments need to be scrutinised carefully if it is accepted that genetic modification of animals should only be undertaken when there is no reasonable alternative. Balancing the needs of people for drugs with the welfare and integrity of animal species is a complex ethical dilemma. However, if drug requirements could be equally well met by other means and if the driving force behind pharming is primarily to make profits for the companies concerned, arguments in favour of using animals for drug production cannot be justified.

6.1 Commercialisation

There are potentially large profits to be made from transgenic pharmaceutical production. The annual market for just six of the products currently produced in transgenic animal milk is $2.6 billion (see Table 4) and supplying five of the six drugs listed - with a total market of $1.4 billion - would theoretically only require 24 cows or 105 goats[159].

Table 4: Animals required and potential value for transgenic pharmaceuticals

	F-VIII	F-IX	Protein C	AT III	Fibrinogen	Albumin
Current annual market (million $)	$882	$160	$100	$150	$150	$1,120
Estimated number of transgenic animals to supply world demand						
Rabbit	217	2,857	7,143	15,000	107×10^3	225×10^6
Pig	2	15	38	81	577	1212×10^3
Goat	1	3	6	12	83	175,000
Sheep	1	1	3	6	45	93,000
Cattle	1	1	2	3	17	35,000

Table from Wall, 1999 [159]

Outlay is high and the companies developing transgenic animals have a large investment to recoup. Cost estimates to produce one transgenic founder cow are $300,000 to $500,000[84,22]. There is also the cost of failed experiments – for each transgenic founder which produces a 'useful' protein, there will be many which do not express the protein sufficiently, do not perform the correct processing of the protein, or cannot form the basis for a breeding line. There is also the series of experiments on other animals which precede gene insertion in the species of interest.

Three products from transgenic animals have reached clinical trials:

- alpha-1-antitrypsin from transgenic sheep is being produced by PPL, Edinburgh;
- antithrombin III from transgenic goats by Genzyme Transgenics, Massachusetts;
- alpha–glucosidase from transgenic rabbits was being produced in Holland in a joint venture between Pharming and Genzyme Transgenics until Pharming went into receivership in August 2000. Genzyme is funding the continuation of the trial during the transition to cell culture production[183].

In early 2001, PPL was unable to raise the £45 million needed to begin construction of a factory to scale up alpha-1-antitrypsin production[184]. They went on to secure non-binding commitments to £30 million in September 2001 but funding is still far from certain. The money is needed to finance PPL until the scheduled launch of its cystic fibrosis treatment in 2005[185].

The prospect of genetically modified poultry as 'bioreactors' for the production of pharmaceuticals is very attractive commercially because of the high proportion of protein in eggs as well as the convenience of eggs as a delivery system. A number of researchers around the world have reported success with experimental production of transgenic poultry[43,37,186] and some have reported the production of pharmaceutical proteins in eggs[163]. The health, or even survival rates, of transgenic birds have not generally been disclosed, perhaps because the focus is still on whether the technique has worked at all. Some researchers have reported producing transgenic flocks that are near to commercialisation, although details have not yet appeared in peer reviewed journals[163].

Table 5: Pharmaceutical production in transgenic animals (Page 1 of 4)

ANIMAL	TRANSGENE/ SOURCE (PROMOTER/ SOURCE)	PURPOSE	COMPANY	COUNTRY/ COMMENTS	REF
Cattle	Human serum albumin	Blood volume replacement	Genzyme / Fresenius AG	USA. Used during surgery and serious burns.	160
Cattle	Myelin basic protein: Human	Treatment of multiple sclerosis	AgResearch	New Zealand. Application accepted in March 1999 and given approval in July 2000. The High Court ordered a temporary moratorium and referred the decision for reconsideration after an appeal by a group of environmental activists and Maoris; ERMA upheld the original decision despite opposition from its Maori Advisory Committee.	161
Cattle	Lactoferrin: Human (α_{s1}-casein: Bovine)	Improved infant formula	Pharming	Holland. Lactoferrin increases anti bacterial properties of milk and could potentially offer some mastitis protection.	162
Chicken	alpha interferon in eggs	Hepatitis, some cancers	AviGenics	USA. Company claims they have established flock of transgenic chickens with germline transmission. However, no further details released since 1999 press release.	163
Chicken	Antibodies/ human growth factor	Immunity	GeneWorks	USA. Claim to have established flock of transgenic chickens; no details disclosed re which proteins to be expressed, or progress.	163
Chicken	Humanized antibody (GD3)	Cancer treatment	Viragen/ Roslin	Edinburgh. 'Proof of principle' rather than close to commercialisation - Viragen wish to become a 'contract manufacturer' for pharmaceuticals using transgenic chickens.	164
Chicken	Gene copies for additional and altered lysozyme (no information on source or construct)	Altered egg composition	Uni of Guelph	Canada. Lysozyme is routinely extracted from eggs and used as a food preservative. Intention to raise amount and alter properties. Still in development stage.	165
Goat	α-proteinase inhibitor: Human (β-casein: Goat)	Cystic fibrosis treatment	Genzyme	USA	93
Goat	a) 7 different Monoclonal antibodies b) Monoclonal antibodies (β-casein: Goat)	Therapies for range of diseases	Genzyme with various partners	Genzyme has patent on production of monoclonal antibodies in the milk of transgenic animals. Target diseases for antibody development include Crohns disease, rheumatoid arthritis, neurological disorders, psioriasis, breast and colon cancer, and HIV/ Aids. Target antigens not always disclosed.	a) 160 b) 93

Table 5: Pharmaceutical production in transgenic animals (Page 2 of 4)

ANIMAL	TRANSGENE/ SOURCE (PROMOTER/ SOURCE)	PURPOSE	COMPANY	COUNTRY/ COMMENTS	REF
Goat	Antithrombin III: Human (β-casein: Goat)	Thrombosis/ embolism	Genzyme	USA. Phase III clinical trials started May 1998.	93
Goat	Hepatitis B surface antigen: Human (α_{s1}-casein: Bovine)	Hepatitis	Chinese Academy of Sciences, Beijing	China. Suface antigen also deteced in saliva, blood, and at low levels in the tongue, kidneys, brain, and skin.	166
Goat	Tissue plasminogen activator, long acting variant: Human	Anti clotting agent	Genzyme	USA. Used after heart attacks.	167
Goat	Surface protein: malaria parasite, *Plasmodium falciparum*	Malaria vaccine	National Institute of Allergy and Infectious Disease	USA. Preliminary reports of use of technique developed in mice being applied to goats to produce malaria vaccine in goat milk.	168
Pig	Factor VIII (Whey Acidic Protein: Mouse)	Haemophilia treatment	Virginia Polytechnic Institute & State University (VPISU)	USA. One of the most complex transgenic proteins expressed. Recombinant Factor VIII produced in cell culture supplies approximately 50% of patients and has been in use since 1993. It is hard to meet the demand as cell culture production is low.	169
Pig	Fibrinogen (Whey Acidic Protein: Mouse)	Tissue sealant and drug delivery matrix	VPISU	USA. 3 fibrinogen chain constructs were microinjected into 320 embryos; 10 live births, 5 transgenic and only 1 germ line. None of the transgenic pigs expressed all three fibrinogen types.	62
Pig	Growth hormone: Human (Whey Acidic Protein: Mouse)	Faster growth	Munchen Uni	Growth hormone was intended to be expressed only in milk, to develop production of pharmaceuticals in milk. However, HGH was also expressed at high levels in the brains of transgenic mice.	213
Pig	Haemoglobin: Human (β-globin LCR: Human)	Oxygen delivery blood products for transfusion	DNX Inc, New Jersey, USA (now acquired by Xenogen)	USA. Primarily to develop transgenic techniques. Human haemoglobin is unstable once removed from red blood cells, so DNX planned further genetic alterations. However, a modified version can already be produced in both bacterial and yeast cells [170].	155

Table 5: Pharmaceutical production in transgenic animals (Page 3 of 4)

ANIMAL	TRANSGENE/ SOURCE (PROMOTER/ SOURCE)	PURPOSE	COMPANY	COUNTRY/ COMMENTS	REF
Pig	Protein C: Human (Whey Acidic Protein: Mouse)	Blood coagulation	VPISU, American Red Cross	USA	89
Rabbit	α-glucosidase	Treatment for Pompe's disease	Pharming, Genzyme	Holland. Pharming / Genzyme joint venture running clinical trials until Pharming went into receivership in August 2001. Genzyme is funding continued production for nine patients in the trials until the transition to a form derived from CHO cell culture[193].	171
Rabbit	Calcitonin: Salmon (β-lactoglobulin: Sheep)	Treatment of osteoporosis & Pagets disease	PPL	Edinburgh. Calcitonin produced in 'fusion gene' with human a-lactalbumin to make biologically stable; this also made the calcitonin biologically inactive in the transgenic rabbit.	172
Rabbit	Erythropoieten (EPO): Human a) (Whey acidic protein: Rabbit) b) (β-lactoglobulin: Bovine)	Anaemia associated with kidney failure, chemotherapy or compromised immunity	a) Unite d'endocrinologie de l'embryon b) Universities of Kuopio and Helsinki, Finland	a) France and b) Finland. EPO stimulates red blood cell production. Excess as a result of 'leaking' of the transgene can cause death and has caused severe polycythemia in transgenic animals. Most died prematurely and could not reproduce[97]. To avoid this, in (b) they used a construct for a less biologically active form of EPO; animals still developed moderate polycythemia.	a) 97 b) 173
Rabbit	Extracellular superoxide dismutase (SOD): Human (Whey Acidic Protein: Mouse)	Blood purification	Astra Hessle AB, Mölndal	Sweden. Transgenic protein was detected in ovaries as well as in milk. Glycosolation patterns slightly different in each species: rabbits, hamster cells and human cells.	92
Rabbit	Growth hormone: Human (Whey Acidic Protein: Mouse)	Trial of rabbits as bioreactor	Centro de Ingeniería Genética y Biotecnología, Havana	Cuba. 51 pups born; 11 transgenic; 4 died immediately after birth, and another 2 subsequently.	174
Rabbit	Insulin-like growth factor: Human (α_{s1}-casein: Bovine)	Develop transgenic techniques	Munchen Uni/ Uni of Vet. Science, Budapest	Hungary. Purpose to develop transgenic production in ruminants.	175

Table 5: Pharmaceutical production in transgenic animals (Page 4 of 4)

ANIMAL	TRANSGENE/ SOURCE (PROMOTER/ SOURCE)	PURPOSE	COMPANY	COUNTRY/ COMMENTS	REF
Rabbit	Interleukin-2 (β-casein: Rabbit)	Cancer treatment	Federal Institute of Technology/ Sandoz	Switzerland.	176
Rabbit	Tissue plasminogen activator: Human (α_{s1}-casein: Bovine)	Anti clotting agent	Cientro de Investigaciones para el Mejoramiento Animal, Havana	Cuba. 388 embryos injected; 59 pups born; 3 transgenic but only 2 stillborn. Very low level of transgene expression.	177
Sheep	Alpha-1-antitrypsin: Human (β-lactoglobulin: Sheep)	Cystic fibrosis/ emphysema	PPL	Edinburgh. Clinical trials under way.	178
Sheep	Factor VIII: Human (β-lactoglobulin: Sheep)	Blood coagulation/ haemophilia	Institute für Tierzucht und Tierverhalten, Neustadt, Fraunhofer Institut, Hanover	Germany. The gene construct was expressed in most major organs as well as in the mammary gland. Although the gene construct was confirmed integrated in mammary gland cells, it was not yet determined whether Factor VIII was present in milk.	179
Sheep	Factor IX: Human (β-lactoglobulin: Sheep)	Blood coagulation/ haemophilia	AFRC, PPL	Edinburgh.	180
Sheep	Fibrinogen (β-lactoglobulin: Sheep)	Tissue sealant and drug delivery	Infigen Inc, VPISU	USA	Note 1

Abbreviations:
AFRC = Agricultural and Food Research Council (now renamed the Biotechnology and Biological Sciences Research Council)
CHO = Chinese Hamster Ovary
PPL = Pharmaceutical Proteins Limited
Uni = University
VPISU = Virginia Polytechnic Institute & State University

Note 1: Cottingham et al, reported in ref 62

Table adapted from: Rudolph (1999)[181], Wall (1999)[159], Pursel and Rexroad (1993)[182].

6.2 Alternatives

> ***"Almost any living organism, or part thereof, could serve as a bioreactor. Bacteria, yeast, insect cells, mammalian cells in culture, plants, and chicken eggs are all potential competing production systems."***
>
> R.J. Wall, US Department of Agriculture 1999[159].

Human therapeutic proteins can be produced in many systems – in mammalian cell culture, in bacteria, in plant cell culture, in transgenic plants, and in transgenic animals.

The most established systems are bacteria and mammalian cell cultures. Insulin and growth hormone were among the first therapeutic proteins to be produced in bacteria in the early 1980s – today, over thirty pharmaceuticals are produced commercially in cell culture[187]. Bacterial systems are efficient, high producers and many years experience has been gained in large scale production systems. However, the failure of bacterial systems to perform correct processing of human proteins led to the development of mammalian cell cultures. There are now several therapeutic proteins commercially produced by mammalian cells, including erythropoietin, Factors VIII and IX, human serum albumin and tissue plasminogen activator[160,169,187].

Antibodies are complex proteins, which are potentially required in large quantities. Richard Francis, the head of purification development for GlaxoSmithKline, described mammalian cell culture as "the present gold standard" for antibody production[188] and went on to compare the alternatives. The advantages and disadvantages are summarised in Table 6.

Table 6: Comparison between different pharmaceutical production methods

	ADVANTAGES	DISADVANTAGES
Bacterial cell culture	Cheap. Easy to produce.	Do not perform proper glycosolation.
Mammalian cell culture	Correct glycosolation of proteins possible. Highly controllable production conditions.	Expensive. Difficult to produce large quantities. Long time-lag for scale-up (plant construction). Risk of contamination.
Transgenic plant cell culture	Medium cost. Highly controlled production conditions. No risk of contamination with human or animal pathogens. Reduced environmental risk. Easy to modify transgenes.	Glycosolation very similar to mammalian cells but some further processing may be required.
Transgenic plants	Cheap. Easy to scale up. Storage and distribution cheap and easy. No risk of contamination with human or animal pathogens. Easy to modify transgenes.	Still in development. Glycosolation very similar to mammalian cells but some further processing may be required. May be environmental risks from cultivation.
Transgenic animals	Expected to be cheap and easy to scale up once development process complete.	Development expensive. Animal welfare and integrity may be compromised. Possibility of cross species transmission of pathogens.

Transgenic plants are being developed to express therapeutic proteins and clinical trials are underway for the first such bio-pharmaceuticals[187,189]. A range of proteins have been produced experimentally in transgenic plants, including 19 vaccines[190], 14 antibodies[190], and 15 human therapeutic proteins such as erythropoietin, Protein C, hirudin, interferon, haemoglobin, human serum albumin, and alpha-1-antitrypsin[191,190]. Transgenic plants are expected to be the cheapest method for production of pharmaceutical proteins, estimated at approximately half the cost of production in transgenic animal milk[189].

Pharmaceuticals have been produced in tobacco plants, potatoes, oilseed, thale cress, mustard, rice, turnip, cow-pea, black eyed bean, cereals, and maize[191]. Tobacco is the most common plant experimentally, but large scale commercial production would probably require production in grain or oilseed crops as extraction from tobacco requires the removal of various toxins, such as nicotine[191]. There are attempts to engineer vaccines into food plants which are edible raw, such as bananas, to facilitate the delivery process.

Plants can express, fold and process proteins in a similar way to mammals and differences in structure are few compared to bacterial processing. It may be possible to further engineer plants to improve processing in instances where, for example, glycosolation differs[191]. If proteins can be expressed in seeds, there may be significant advantages for storage or transport.

The use of transgenic plants has environmental risks, primarily of the transgene outcrossing and causing genetic pollution of wild species[192]. There is also the risk of consumption by animals, or indeed inadvertent consumption by humans – which could be serious in the case of proteins toxic at high dose. There would also be highly damaging consequences if the GM plants cross-pollinated non-GM crops. Growing plants in closed greenhouses goes some way to reducing these risks, although it is likely that this would also raise production costs.

The production of proteins in plant cell culture is also possible, although less developed than whole plant systems. Suspended plant cells have been used to produce recombinant antibodies, enzymes, and therapeutic proteins including human interleukin and human alpha-1-antitrypsin[189]. Plant cell culture avoids the environmental risks associated with agricultural cultivation of genetically modified plants. It would be cheaper than mammalian cell culture, although considerably more expensive than open cultivation of plants[189]. In situations where batch consistency is extremely important, any cell culture system is likely to be advantageous as conditions can be tightly controlled and reproduced.

6.3 Effect on animals' welfare

> ***"Examination of a great number of transgenic lines has shown that mammary gland specific expression of a target protein is associated with increased plasma levels of this protein, even in the absence of ectopic expression."***
>
> H.M.Meade, Genzyme Transgenics, 1999[93].

It has been argued that the mammary gland makes an ideal production system because it is isolated from the circulatory system and the milk is continually removed, so the expressed protein is unlikely to impact on the animal[9]. However, there is often some expression in non target tissue[92] and nearly always some 'leaking' into the circulatory system[93]. Endogenous milk proteins are found in the circulation in cattle, especially during late pregnancy, and both transgenes and milk protein genes are transiently expressed during oestrus[159].

This can have serious effects on the animal concerned. One research group reports production of erythropoietin (EPO) in transgenic rabbit milk with some associated expression affecting red blood cell production. Their health was severely compromised, resulting in death in one of the

experimental groups. The researcher suggested that EPO was not a suitable candidate for mammary gland expression[97].

In severe cases, the animal will not be used for breeding because such compromised animals could not produce a viable line. It is of concern that less extreme effects may go un-noticed either because of poor monitoring or because subtle effects are difficult to detect.

6.4 Safety

> ***"This method [transgenic expression in milk] permits flexible scale-up of protein manufacturing to meet increasing production needs throughout the product development process. Scale-up is as simple as breeding more transgenic animals."***
>
> Genzyme Transgenics website, 30th September 2001[193].

> ***"Phenotype and genotype cannot be reliably defined in transgenic animals until successive generations of offspring obtained from outbreeding with nontransgenic animals are analyzed."***
>
> Butler *et al*, 1997[62].

The potential risks to human health from pharmaceutical production in GM animals give cause for concern. There are two main areas of risk - cross species disease transmission and that altered or novel proteins from descendants of GM founder animals may surface after the initial regulatory period has passed.

There is a risk of cross species pathogen transmission or viral contamination with any animal product[93]. A small possibility of catching a new disease may be a justifiable risk for someone already suffering from a life threatening illness. However, new pathogens crossing the species barrier could enter the population at large because of the risk of onward transmission. This is especially worrying in the light of recent experiences with BSE.

Any new pharmaceutical product is required to pass extensive safety tests before being made available for public use; and release may still result in unexpected, and sometimes serious, effects as the drug is given to a wide variety of people in greatly different circumstances. Subtle changes in a protein's structure can significantly alter it's effects. The assumption that descendants of a transgenic founder will produce identical proteins in their milk may not be justified.

The transgenic process is random, frequently resulting in integration of multiple copies of the transgene and damage to the animal's own genes[59,69]. All of these effects can be masked by heterozygosity so that effects on both the transgenic product and the animal may not surface for generations. It has to be asked at what point the regulatory process regarding transgenic pharmaceuticals will be completed – is a product line regarded as stable after three generations? Seven generations? Will the products resulting from the 'simple scale-up' referred to in the Genzyme Transgenics website be subject to the same rigorous scrutiny as the initial product?

Much is also made of the ability of transgenic animals to correctly process human proteins. It is likely that proteins produced in transgenic animals – and the other production systems – are each different[93]. In one study, glycosolation and functional properties were compared between native (human) superoxide dismutase (SOD), SOD produced in Chinese hamster ovary cells, and transgenic SOD produced in rabbit milk. (Superoxide dismutase is a major enzyme in plasma, lymph and synovial fluid.) Functional properties were similar although glycosolation was slightly different in all three cases[92]. The closest match is likely to be human cells in culture.

6.5 Conclusion

Pharmaceutical production in transgenic animals is one method of supplying drugs, which may in some instances be the easiest means of meeting bulk requirements. However, there are alternative production systems that could meet requirements – bacterial and mammalian cell cultures, transgenic plants, and transgenic plant cell cultures. All of these systems have drawbacks as well as advantages. At present, by far the most important deciding factor on which system is developed is potential profit.

There is a need for a systematic appraisal of the different systems which takes into account the technical, social and ethical aspects of how society is to meet the need for drugs. It is likely that the first choice would be mammalian cell culture, which may also have the potential to offer the highest quality product[187].

7. AGRICULTURE

> ***"To date attempts [to engineer livestock for use in agriculture] have failed to result in the production of genetically superior livestock (sheep and pigs) due to a variety of undesirable side effects in these animals, although the transgenic animals have been more feed efficient and leaner."***
>
> Pinkert and Murray, 1999[153].

Most of the transgenic animals being developed are either for medical purposes (research or organ production) or for the production of high value pharmaceuticals. However, several research groups around the world, including major government and academic laboratories (e.g. CSIRO in Australia and USDA in USA), are attempting to develop transgenic cows, sheep and pigs with increased agricultural productivity. There are also attempts to engineer increased disease resistance. Table 7 lists the transgenic animals which have been produced and their intended agricultural use. Twenty-one of the thirty-four genetic modifications listed are aimed at increased productivity.

There are two fundamental questions regarding transgenic growth enhancement – firstly, and most importantly, is it necessary? - Does the world need a marginal decrease in the time taken for pigs to reach slaughter weight or a 10% increase in wool production or in the protein content of milk? Secondly, are transgenic attempts to enhance productivity likely to be successful without severely compromising the welfare of the animals involved?

Feeding an increasing world population is often put forward as a reason to develop transgenic agriculture [e.g.6,7]. Global food production will need to rise as population increases but global output is not the key determinant of whether people are adequately nourished. 790 million people in developing countries and 34 million people in developed countries are malnourished now, despite the fact that gross world food production is sufficient to feed every person on the planet adequately[194]. The reasons for malnutrition are chiefly poverty, in that access to food is determined by income, and the disparity in regional agricultural productivity[195] – neither of which will be affected by the gene constructs which are currently being inserted into animals.

Growth enhancement work is aimed at breeds that are already very high producers, dependent on intensive farming methods including high protein diets. Any meaningful rise in agricultural productivity would need to be in areas of the world which are currently experiencing low food availability[195]. However, these are generally not areas in which intensive livestock systems are used. Nor would a switch to intensive methods increase food availability because of the relationship between meat production and protein in such systems. The production of one kilogram of beef in the developed world is estimated to require five kilograms of plant protein, most of which could be eaten directly by humans[196]. Increasing intensive meat production is not a recipe for improving food availability. The same argument applies to milk production, with the further dimension that milk is currently produced on a quota system in most OECD countries to restrict supplies[197]. It is hard to see the logic in increasing either milk production *per se* or the protein content of milk in areas of the world where there is already an oversupply. Increases in transgenic breeds' productivity are likely to be profitable for the intensive livestock industry but there is no reason to think they would improve world food security.

Is it likely that productivity can be directly enhanced by transgenic methods without detrimental effects on the animals? With the exception of alterations to milk composition, the traits to be altered are complex – growth or metabolism of nutrients. Modern agricultural breeds in intensive livestock systems have already been pushed to their limits to increase production - a dairy cow in an intensive system can now produce 6,400 litres of milk per season compared with 2,000 only 70 years ago[198], and a broiler chicken reaches slaughter weight in just 6 weeks, compared to 12 weeks 30 years ago[12]. It is unlikely that an introduced gene would be able to increase growth

without compromising the animal. In the words of a keen proponent of the technology:

> ***"Transgenic research for livestock is directed towards improved animal productivity….To achieve this it is usually necessary to modify some component of the animal's physiology, thus potentially altering the existing delicate balance of nutrition, endocrinology and metabolism. Since this balance has been established through many generations of selection for superior performance and environmental compatibility, it represents a wide range of optimised gene combinations that are difficult to perturb without causing unexpected deleterious effects on animal phenotype."***
>
> Kevin Ward, CSIRO, 1999[199].

7.1 Meat production

Much of the earlier work on transgenic livestock revolved around growth enhancement, both for the potential commercial advantage and because growth hormones had been used in the early experiments on transgenic mice[2]. Genes coding for growth hormones from humans, sheep, pigs and cows have been inserted into sheep, pigs, cows and rabbits. Animals have displayed enhanced growth, an increased lean meat/fat ratio and increased efficiency of feed conversion. However, there have been high costs for the animals involved. The Beltsville pigs, the first transgenic pigs genetically modified to express growth hormones, are notorious for the range of problems they developed. These included gastric ulcers, liver and kidney damage, degenerative joint disease, lameness, lethargy, loss of co-ordination, sensitivity to pneumonia, damaged vision, diabetic conditions, dermatitis, and loss of libido[96]. Another research group reported enhanced growth and no detrimental effects, but also reported that the one transgenic pig expressing the transgene was killed at 18 weeks after contracting pneumonia, precluding further study[200]. Transgenic sheep expressing either a human or a bovine growth hormone developed diabetes and/ or a range of other pathologies and all died young[201].

Some researchers, including CSIRO in Australia and USDA in the USA, are continuing their work to develop transgenic animals with enhanced growth. In the latest reported experiments, the USDA facility (which produced the Beltsville pigs) reported insertion of an insulin-like growth factor gene. 17 transgenic piglets resulted from injection of 1,207 zygotes and 6 of the 17 were either born dead or died before reaching adulthood. The remaining 11 animals were reported as healthy but did not show enhanced growth, although the females did have leaner meat[202]. CSIRO have reported more success with sheep. They have bred a transgenic line to three generations and reported both enhanced growth and sheep which appear healthy[203].

The production of transgenic animals expressing additional growth hormones has not been taken up commercially and seems unlikely to prove successful. At present, most instances in which transgenic animals without severe pathologies are reported are those in which the transgene does not have the desired effect. Wherever the transgene does affect growth, the fitness of the animals seems compromised.

Various researchers are also working on enhanced growth via gene therapy, in which there is direct injection of plasmid DNA into the muscles, and on administration of growth hormones via injections. The same questions remain with regard to the necessity for growth enhancement within our farming systems, the potential effects on the animals concerned, and public acceptability of growth enhancement - particularly in light of the conflicts over the use of BST (bovine somatatrophin) in cows' milk[204,205].

Japanese researchers are reported to have genetically modified pigs with a gene from a spinach plant, FAD2, which produces an enzyme involved in fat metabolism to produce pigs which have less fat and so are 'healthier' to eat[206].

Table 7: Agricultural applications - transgenic animals (Page 1 of 4)

ANIMAL	TRANSGENE/ SOURCE (PROMOTER/ SOURCE)	PURPOSE	COMPANY	COUNTRY/ COMMENTS	REF
GROWTH HORMONES					
Cattle	Growth hormone: Human (Transferrin: Mouse)	Faster growth	Unknown	USA	226
Cattle	Insulin-like growth factor: Human (Actin: Chicken)	Development of techniques	Granada Biosciences Inc	USA. Abortion rate varied from 13% to 75% for the different gene constructs attempted.	227
Chicken	Growth hormone: Bovine (Rous sarcoma virus/ mouse promoter)	Faster growth/ disease resistance	Merck Laboratories	USA. Increased resistance to infection with one virus strain. Increased leg growth in 2 of 13 chickens.	228
Pig	*cSKI* transgene: Chicken (Mouse Sarcoma virus)	Leaner meat	USDA, Beltsville	USA. 29 transgenic piglets born - 10 exhibited varying degrees of abnormalities including muscle enlargement, muscle weakness and weakness in front and back legs.	28
Pig	Growth hormone: Bovine (Methallionein: Mouse)	Faster growth	USDA, Beltsville	Some transgenic lines gained weight faster, and made leaner meat. However, additional GH also caused lameness, lethargy, gastric ulcers and infertility.	229
Pig	Growth hormone: Human (Methallionein: Mouse) (Albumin: Mouse)	Faster growth	USDA, Beltsville	As above.	229
Pig	Growth hormone: Rat (Moloney leukaemia virus: Mouse)	Faster growth	Tufts Univeristy School of Veterinary Medicine, Massachusetts	USA. Only one transgenic pig was produced, which was killed at nine months. Postmortem showed joint damage characteristic of osteochondritis dissecans and impaired fertility.	230
Pig	Growth hormone: Pig (Methallionein: Human)	Faster growth/ leaner meat	Uni of Adelaide/ Metrofarms (a) BresaGen (b)	Australia. Target to have a growth hormone regulated by dietary zinc. Vize et al (a) reports weight gain in 1 of the 4 transgenic pigs, which died of pneumonia at 18 weeks.	a) 200 b) 231

Table 7: Agricultural applications - transgenic animals (Page 2 of 4)

ANIMAL	TRANSGENE/ SOURCE (PROMOTER/ SOURCE)	PURPOSE	COMPANY	COUNTRY/ COMMENTS	REF
Pig	Insulin-like growth factor: Human a) (Methallionein: Mouse) b) (Skeletal α-Actin: Avian)	Leaner meat	USDA Beltsville	USA. b) 14 transgenic pigs live born; 3 died before parturition. The rest seemed healthy. The desired effect of the transgene was absent in male pigs; female pigs had 6% more lean meat than controls[202].	202
Pig	Growth hormone: Human (Methallionein: Mouse)	Development of techniques	Uni of Pennsylvania/ USDA Beltsville	USA. Transgene expressed but did not cause dramatic increases in growth.	232
Rabbit	Growth hormone: Human (Methallionein: Mouse)	Development of techniques	Uni of Pennsylvania/ USDA Beltsville	USA. Although 28 transgenic rabbits were reported, this included 2 stillbirths and 5 killed as fetuses. Only one live rabbit had detectable expression of HGH.	232
Sheep	Growth hormone releasing factor: Human (Albumin: Mouse)	Faster growth	USDA, Beltsville	USA. All the sheep which expressed the transgene developed diabetes and/or a range of pathologies, and died early.	201
Sheep	Growth hormone releasing factor: Human (Methallionein: Mouse)	Faster growth	USDA Beltsville	USA. Only one lamb expressed the transgene and did not show enhanced growth.	233
Sheep	Growth hormone: Bovine (Transferrin: Mouse)	Faster growth	USDA, Beltsville	USA. All the sheep which expressed the transgene developed diabetes and/or a range of pathologies, and died early.	201
Sheep	Growth hormone: Bovine (Methallionein: Mouse)	Faster growth	USDA, Beltsville	USA. The two transgenic lambs died before two months. There was no effect on growth and the transgene was expressed in most tissues tested.	233
Sheep	Growth hormone: Human (Methallionein: Mouse)	Development of techniques	Uni of Pennsylvania	USA. Only 1 transgenic sheep resulted from 10,332 injected embryos but the transgenic product was not expressed.	232
Sheep	Insulin-like growth factor: Sheep (Keratin: Mouse)	Increased wool growth	Lincoln Uni, NZ	New Zealand. Initially reported 6% increase in wool growth; however, this was not maintained in later years or in offspring[209].	90

Table 7: Agricultural applications - transgenic animals (Page 3 of 4)

ANIMAL	TRANSGENE/ SOURCE (PROMOTER/ SOURCE)	PURPOSE	COMPANY	COUNTRY/ COMMENTS	REF
MILK COMPOSITION/ WOOL PRODUCTION /'BIOSTEEL'					
Sheep	Marker gene: Bacteria (Keratin: Mouse)	Increased wool growth (R&D)	Lincoln Uni, NZ	Intention to target expression specifically in wool follicle to avoid more systemic effects. However, marker gene was also expressed in other organs (spleen, liver, kidneys).	234
Sheep	Cysteine synthesis gene: Bacteria - *E. Coli* and *Salmonella typhimurium* (Rous sarcoma virus/ mouse promoter)	Increased wool growth	Uni of Adelaide	Australia. Transgenic expression was universally extremely low; suggested this may be because higher level expression damaged embryos *in utero*.	235
Cattle	Additional copies of bovine a- and κ-casein genes (in development)	Altered milk composition	AgResearch	New Zealand. Application accepted in March 1999 and finally approved May 25th 2001.	161
Cattle	Disruption of β-lactoglobulin gene (in development)	Altered milk composition	AgResearch	New Zealand. Application accepted in March 1999 and finally approved May 25th 2001.	161
Goat	Spider gene	'BioSteel' production	Nexia Biotechnologies Inc	Canada. Nexia are working to develop wound closure applications and also with the Canadian military to produce lightweight body armour. Spider silk can also be produced in tobacco and potatoes[225].	223
Pig	Phytase gene: *Escherichia Coli*	Reduce phosphorus in pig excrement	Guelph Uni	Canada. Pigs cannot digest phytate - about 80% of the phosphorus in common grains. Transgenically adding phytate resulted in approximately 75% reduction in faecal phosphate and removed the need for dietary supplement.	219
DISEASE RESISTANCE					
Chicken	Avian Sarcoma Virus envelope: Avian leukosis virus	Resistance to ALV/ develop techniques.	USDA	USA. Increased resistance to one strain of ALV. However, 25% went on to develop cancer and 18% to die from it.	39

Table 7: Agricultural applications - transgenic animals (Page 4 of 4)

ANIMAL	TRANSGENE/ SOURCE (PROMOTER/ SOURCE)	PURPOSE	COMPANY	COUNTRY/ COMMENTS	REF
Pig	Mx : Mouse (Methallionein: Human) (SV 40: Human) (Mx promoter: Mouse)	Influenza resistance/ transgenic techniques	Munchen Uni	Germany. Attempts to induce resistance to influenza failed as the mouse protein Mx1 was not expressed in the transgenic pigs.	213
Sheep	Visna virus envelope (Ovine retrovirus)	Develop transgenic strategies for immunity to ovine lentivirus	John Hopkins Uni, Baltimore/ Kansas Uni/ USDA Beltsville	USA. Ovine lentivirus causes encephalitis, pneumonia and arthritis in sheep, and control of viruses has not proved possible with vaccination. Transgene primarily to study lentivirus in sheep and whether a transgenic product would confer immunity. No results re immunity, although it is possible that transgenic sheep have some enhanced resistance.	29
Pig, Rabbit, Sheep	a) Immunoglobulin A (α and κ): Mouse b) Monoclonal anitibody: Mouse	Develop expression of transgenic antibodies in non mouse species	a) Uni of Veterinary Science, Budapest b) Munchen Uni	Both Weidle *et al* (pigs, rabbits)[212] and Lo *et al* (mice, pigs, sheep)[211] reported transgene expression but no enhanced immunity, and both reported unexpected results: aberrant sizes of the transgenic antibody or little antigen binding capacity. A 1998 review of transgenic disease resistance in farm animals cited no further work[31].	a) 211 b) 212
DEVELOPMENT OF TRANSGENIC POULTRY					
Chicken	Range of marker genes, most commonly LacZ (bacteria) and neomycin resistance gene (bacteria). Replication competent and defective avian viruses: Leukosis Virus, Reticuloendotheliosis Virus, Spleen Necrosis Virus	Develop transgenic techniques	a) Roslin Institute b) Amgen Inc, California Institute of Technology, Uni of Georgia c) Institute of Cancer Research, France d) Uni's of Tsukauba and Kumamoto, Japan e) Seoul National Uni	UK, USA, France, Japan, Korea. So far the modifications carried out have primarily been to develop transgenic techniques, which are proving unsuccessful in poultry because the early embryos are very inaccessible.	a) 26 b) 41 c) 30 d) 236 e) 42

Uni = University

Table adapted from: Rudolph (1999)[191], Wall (1999)[159], and Pursel and Rexroad (1993)[192].

7.2 Wool production

Two types of transgenic sheep are being developed for increased wool production. One method is to introduce bacterial genes into sheep so that they can directly produce the protein cysteine, often the limiting factor in the rate of wool production[207]. This method is primarily being developed by CSIRO in Australia and Lincoln University in New Zealand. The first GM sheep for cysteine biosynthesis were reported in 1995 but of the 28 sheep produced so far, none have shown the desired effects. The percentage of transgenic animals produced is even lower than usual[207]. One of the proponents of this approach – CSIRO's Kevin Ward - suggests the following explanation:

> ***"An obvious explanation for this is that high levels of expression of a cysteine biosynthesis pathway in sheep embryos are lethal and therefore the only transgenic animals obtained are those in which the genes have been inserted into a region of the genome that prevents their expression or, at best, allows only low levels of expression"*** [207].

This approach - to re-introduce metabolic pathways from bacteria to mammals - has been suggested as potentially profitable for the livestock industry, and described as 'metabolic repair'[207]. However, it is not surprising that a complex function like cysteine production, lost during millions of years of evolution, cannot be casually re-inserted without disrupting the whole metabolism of the animal.

The other approach for increasing wool production has been to introduce an insulin-like growth factor into the sheep. Forty-eight 1st and 2nd generation transgenic sheep have been produced in New Zealand and wool production measured over 3 years. An initial increase of 6% in fleece weights reported in 1996[90] was not present in the 2nd or 3rd shearing and there was no significant difference in fleece weight between transgenic and non-transgenic 2nd generation sheep[208].

7.3 Milk production

Most of the development work on transgenic animals for protein production in milk has been directed at the production of high value pharmaceutical products. However, the potential to alter the composition of milk for dairy uses is also being investigated.

There are three main proposals for the transgenic modification of milk for the dairy industry - 'humanising' cows' milk (primarily to enhance the properties of infant formula); increasing the proportion of the more valuable protein component; and reducing lactose to increase potential markets for milk. The primary contender for serious industry attention is increasing protein content[209].

- **'Humanisation'** of milk by the addition of genes coding for human lactoferrin and lysozyme is aimed at increasing the anti-bacterial properties of milk and therefore the resistance to infection which is imparted to the baby by infant formula. Increased lactoferrin is also expected to improve iron absorption in infants fed on formula. Lysozyme is present in human milk at 3,000 times the concentration found in cows' milk, while lactoferrin is present at 8-80 times. Increased levels of lactoferrin and lysozyme may also have the effect of increasing resistance to mastitis[210]. However, it is hard to imagine public acceptance of milk from genetically modified cows being used in infant formula.

- **Increasing the protein content** of milk (specifically the casein content) is considered the most desirable modification to milk content[209]. Protein is the most valuable component of milk to the dairy industry, particularly for cheese production[9]. It is estimated that a 10% increase in protein content would raise dairy industry profits by £60 million in the US[9] and by

£24 million in Australia[209].

- **Decreasing the lactose content** to make milk more palatable to the estimated 70% of the world's population (mainly in Asia) who have some degree of lactose intolerance would have a large impact on potential markets[9].

As yet, genetically modified milk is not close to reaching the table and the ideas for altering milk properties remain research projects. Firstly, the animals are too expensive to be used in normal agricultural systems. Secondly, an approval process would be needed before milk with altered properties could be used as food. Perhaps most importantly, there is also serious doubt over whether consumers would accept genetically modified milk – especially to feed to babies.

7.4 Disease resistance

> ***"So far, no successful resistance enhancing gene transfer experiment in farm animals has been reported."***
> Muller and Brem, 1998[31].

Using transgenic techniques to create disease resistant animals is frequently cited as a potential application of genetic modification that would be beneficial for animal welfare as well as being profitable in terms of agricultural productivity.

There are relatively few reports of attempts to introduce disease resistance traits into animals – only 9 out of the 77 modifications to farm animals listed in Table 1 and no improved immunity was reported in 8 of the 9 instances[211,212,213]. None of these applications are the subject of commercial development as far as can be ascertained from information in the public domain.

It is undoubtedly true that breeding animals with increased disease resistance would be of benefit to both humans and animals – perhaps with the exception of the battery hen and other similar systems, where disease resistance may simply allow the overcrowded conditions which breed disease to continue or even deteriorate. However, it is questionable whether transgenic strategies are the best way to achieve enhanced disease resistance.

The International Livestock Research Institute in Kenya is dedicated to improving sustainable livestock production in developing countries. As well as developing vaccines and diagnostic tests, they are using genetic identification of disease resistant traits to facilitate breeding programmes. Indigenous breeds are the most likely to possess genetic traits both for resistance and dealing with environmental stress as animals have been subjected to intense selection pressure whenever there have been epidemics or droughts[214]. Conservation is urgently needed as it is estimated that one third of indigenous African livestock breeds are threatened with extinction, but such conservation is expensive[214].

The results of breed evaluation research in Africa indicated that smallholder farmers should utilise local indigenous breeds more widely to control gastro-intestinal worms, arguably one of the most damaging diseases to livestock. An economic assessment conservatively estimates that this strategy would bring net benefits of $52 million from sheep production in Sub Saharan Africa[215].

Expansion of programmes to identify indigenous breeds with natural immunity, ensuring that existing genetic diversity is maintained, and pursuing conventional breeding programmes - combined with programmes to promote breeds and practices that require low inputs - would almost certainly be a more effective use of resources than developing transgenic animals.

> ***"Gene transfer programmes for the generation of animals carrying disease resistance constructs must consider carefully the possibility of creating or***

accumulating pathogenic agents able to escape the introduced host defence gene. For example, the strategy of introducing mutated pathogen genes into animals might result in recombination events with wild type pathogens, thus creating resistant strains or even altered species specificity."

Muller and Brem, 1998[31].

Increasing poultry resistance to strains of the Avian Leukosis Virus (ALV) or Reticuloendotheliosis Virus (REV) using GM versions of the viruses has been investigated[39]. However, the use of even defective versions of the viruses could increase the risk of developing cancer as it is thought that the integration of either ALV or REV next to endogenous cancer genes triggers the disease[40]. In one experiment where increased resistance was reported, 25% of the resistant birds went on to develop cancer[39]. Perhaps even more seriously, there is a risk of creating new pathogens by recombination of the GM virus with the wild types which are widespread in commercial flocks[37,39].

There are other cautionary notes about transgenic programmes to develop disease resistance[61,31]. These centre on the potential to accelerate the development of new pathogens or to increase the likelihood of transfer between species. This could happen in a number of ways - via pathogen mutation as a response to immunity in the host animal; by the creation of a disease reservoir in the resistant host (i.e. the animal does not display symptoms but continues to carry the disease)[61,216,217]; or as a result of recombination with the wild pathogen. While it is hard to evaluate the likelihood of this happening, recent experiences with BSE would suggest a precautionary approach.

7.5 Phosphate reduction - the 'enviro-pig'

Pollution of waterways with phosphate and nitrate - eutrophication - is recognised as a national and international environmental problem and livestock account for 34% of the phosphate pollution in the European Union[218]. Pigs contribute significantly to this problem as they cannot digest phosphorous as phytate - the form it takes in plants - so the plant phosphorous in their diets is excreted as phosphate. The level of pollution has been further exacerbated by the increasing practice of feeding pigs with mineral phosphate supplements in order to maximise growth.

In an attempt to address this problem, researchers at the University of Guelph in Ontario have introduced a gene from the bacteria *Escherichia coli* (coding for the phytase enzyme) into the salivary glands of pigs so that they can digest plant phytate. A reduction of approximately 65% in the phosphate content of pig manure was reported in transgenic pigs expressing phytase and virtual removal of the need for phosphate supplements[219]. No health effects were reported in the 12 G_1 pigs (the generation bred from the transgenic founder), although slightly elevated levels of phytase were found in tissues other than the salivary gland. Further investigation on several more generations is required to determine whether there are adverse effects on the pig or on humans consuming the pork.

There are several other strategies for resolving the problem of phosphate pollution from pigs. One is to supplement feed with phytase, the enzyme which allows digestion of plant phytate. This is reported to bring about a reduction of phosphate in manure of 60%[220]. Another strategy is to genetically modify corn to reduce phytate content[221] - but this has the environmental risks associated with genetic modification of any crop plant[192].

All of these strategies potentially resolve one aspect of the environmental pollution from pigs but all represent a symptomatic approach to a more fundamental problem - the increasing intensification of farming and the attendant environmental and welfare impacts.

"The challenge in relation to the maintenance of a sustainable resource base, is to

devise effective and practical means to return the wastes from lowland animal production systems to crop production."

Atkinson and Watson, 1996[222].

A different approach is to modify the farming system rather than the pigs by reducing the intensity at which they are reared and linking pig farming to crop systems which can utilise the wastes produced. The great majority of the 8 million pigs in Europe are kept indoors for most of their lives[68] on farms which can neither produce the feed they need nor absorb the waste that they produce[222]. This clearly impacts on the pigs and, in some systems, it is almost impossible for them to achieve any semblance of normal behaviour[68]. The high protein diets they receive mean there is a nutrient imbalance, with nitrogen and phosphorous inputs commonly exceeding - by 4.2 and 2.7 times respectively - the amount of these nutrients in the pork produced[222]. Most of these nutrient inputs are therefore excreted on small areas where the waste cannot be absorbed. Manure from the UK pig population would need to be spread over an estimated 190,000 hectares of crop land[222]. In addition, the high protein requirement for intensive production means that the UK imports 358,000 tonnes of soya beans for pig feed annually, presumably contributing to nutrient depletion in the countries of origin.

Another alternative to genetic modification is the proposal from European detergent manufacturers to recycle the phosphorous from intensive livestock wastes and human sewage (the other major source of phosphate pollution) for use as a feedstock. This would be a shorter term approach to reabsorbing the outputs from excess nutrients[218].

Genetic modification of farm animals to digest phytate offers a partial solution to one aspect of the environmental problems which intensive farming creates. A much more effective solution would be to develop farming systems which match nutrient outputs from livestock to nutrient requirements for crops - thereby avoiding the problem of nutrient outflows and improving animal welfare at the same time.

7.6 BioSteel® production

Nexia Biotechnologies Inc has engineered goats to produce spiders' silk in their milk, which the company has called BioSteel®. The protein is the 'dragline' silk used in spiders' webs and is one of the strongest materials in the world. Nexia is working with the Canadian military to produce lightweight body armour from BioSteel® and is also investigating medical applications, including wound closure systems and ligament prosthetic devices[223]. Nexia has licensed Geron Coporation's nuclear transfer patents to attempt to clone the transgenic goats[224] since, in order to go into production, Nexia would need a sizeable herd, which would be difficult to obtain without cloning technology. As yet, there are no published reports on the health of the genetically modified goats.

Spiders' silk has remarkable properties and will undoubtedly be a very useful material because of its extreme strength, flexibility and light weight. It is not possible to 'farm' spiders because they are aggressively territorial and it is very difficult to produce the silk successfully in bacteria because of its structure. However, large scale production is possible in plants and Nexia is collaborating with the Institute of Plant Genetics and Crop Plant Research in Germany, who have announced successful production in both tobacco leaves and potato tubers[225].

7.7 Conclusion

There are currently no transgenic mammals or birds in use in agriculture, although research groups around the world are working on their development. Quite apart from ethical

considerations, transgenic animals would have to be healthy, confer sufficient advantage, not require special care and be of an equivalent cost to their non-transgenic counterparts in order to be of any benefit to agriculture. In addition, there must be no possibility that the transgene could compromise the welfare of the animal and the transgenic product would have to be demonstrated to be safe for human consumption.

None of these conditions have been met at present. Animals with inserted growth hormones have such compromised health that they would be non-viable. Even transgenic lines which appear healthy would require careful monitoring through generations as the random integration of transgenes means that damaging effects can surface in later generations[61].

Are there compelling arguments for the agricultural applications described? None are necessary to feed the world's growing population. With the possible exception of disease resistance, none even impart a significant advantage to people in general, as opposed to increased profits in subsections of industry.

The development of transgenic animals is likely to contribute further to the intensification of agriculture and may contribute to a loss of agricultural genetic diversity. The priority for developing disease resistance is to preserve the genetic diversity of farm animal breeds. However, it appears that much scope remains for enhancing disease resistance in agricultural breeds without transgenic technology, although genetic techniques can certainly offer significant advantages - in identification of distinct gene types, for example.

GeneWatch does not consider that any of the agricultural applications are sufficiently imperative to justify the use of GM technology or the cost to the animals involved. There are not only adequate, but better, alternatives.

8. XENOTRANSPLANTATION

Xenotransplantation is the term used to describe the transfer of organs, tissues or cells between species and from animals to humans. Since there is a shortfall in the number of human organs available for transplantation, it has been proposed that organs from animals could be used instead. Because of problems with the rejection of animal organs by the human immune system, attempts are being made to genetically engineer animals (mainly pigs) to make them more suitable as organ donors.

8.1 The organ 'gap'

Organ transplantation has progressed since the 1960s through increased understanding of the immunology of organ rejection, the development of immunosuppressive drugs and improved methods of tissue matching, organ storage and transport[237,238]. Another important factor in the success of heart and other transplants was the acceptance of criteria to demonstrate brain stem death, which allowed the use of organs from so-called 'heart-beating' patients[239].

In 2000, there were 1,487 kidney transplants and 217 heart transplants in the UK. However, there were 6,284 people on the kidney transplant waiting list and 178 waiting for hearts[240]. This disparity in numbers between those in need of organ transplants and organs available is known as the 'organ gap'. Improvements in road safety leading to fewer deaths and thus fewer organs for transplantation has been blamed, in part, for this shortfall. It is against this background of an organ gap that new technologies are being researched and promoted, one of which is xenotransplantation.

8.2 History and the application of genetic technologies

Attempts to use animals as kidney and heart donors for humans date back to the early 1900s when primates such as chimpanzees and baboons were used[241]. Survival times were very low - often less than a day – and even with high doses of immunosuppressive drugs, maximum survival times were about two months. The most famous experiment was the transfer of a baboon heart into a newborn baby (Baby Fae) in 1984, who died 20 days later. Pig heart valves are now routinely used as replacements in cases of human heart disease, but the valves are not living since the tissue has been fixed and preserved and infectious organisms killed with the use of a chemical, glutaraldheyde. However, animal-to-human organ transplantation is still highly experimental and organs will have to be living and functional to be successful.

Many practical obstacles therefore have to be overcome if xenotransplantation is ever to work. The main barrier is thought to be organ rejection because the transplanted organ is detected as 'foreign' by the immune system and attacked. This immunological reaction to a xenotransplant has three stages[242]:

- hyperacute rejection – occurring very soon after transplantation and involving an antibody response which then triggers the activity of a molecule called 'complement' and a series of damaging reactions;
- delayed rejection – involves antibodies and cells in a rejection response;
- cell-mediated rejection – where immune system cells attack the transplanted organ.

These reactions are thought to be triggered because certain molecules on the surface of cells differ from species to species. The immune system detects these differences in the transplanted organ and a whole cascade of reactions begins as the body tries to kill what it sees as a foreign invader.

To try to overcome this, scientists are genetically modifying animals in one of two ways:

- to remove molecules that mark other species as foreign to the human immune system – in the case of pigs this is known as α-gal [243];
- to include a gene for a human protein - either CD55 (DAF – decay-activating factor) or CD59 - which inhibits the complement system[244,245,246].

Genetic modification is also being used to inhibit other parts of the rejection response and boost protective mechanisms. Research typically involves experiments to produce GM mice, followed by mouse-to-rat transplants and then - to test xenotransplantation techniques further for their suitability for humans - pig-to-primate transplants. Pigs have been chosen as the species of choice as organ donors for humans because their organs are about the right size (miniature breeds of pig are often used as other breeds may become too large), they are relatively cheap and are thought not to pose the same ethical concerns as primates. Importantly, using pigs rather than primates should also reduce the chance of disease-causing viruses being transferred along with the organ (but see Section 8.4 below).

As well as whole organs, xenotransplantation of pig nervous tissue to treat Parkinson's and Huntington's diseases, and pig pancreatic islet cells (the cells which produce insulin) to treat diabetes are also under investigation[247].

However, if xenotransplantation technology is to be economically viable, it has to be able to supply genetically modified pigs on demand. Because genetic modification of embryos is technically difficult, the nuclear transfer technique (cloning) is being used to produce GM pigs from GM cells. The cloned GM animals will then be bred naturally to produce a herd of GM pigs.

8.3 Success rates

There has been much hype about the promise of xenotransplantation. In 1995, a leading xenotransplantation company, Imutran, was reported to have claimed that the technology was *"ready for testing in humans"* because monkeys receiving GM pig hearts survived for 60 days rather than the usual one hour[248]. However, this was when the monkey's own heart was still in place to pump blood and survival was only for 5-9 days when the transplanted heart had to pump blood. Progress has therefore not been as rapid or smooth as the proponents of xenotransplantation promised. The UK's regulatory authority, UKXIRA (UK Xenotransplantation Interim Regulatory Authority), was established in 1997 to oversee xenotransplantation in the UK and its 1999/2000 Annual Report concluded that:

> ***"In summary, the evidence of efficacy has not advanced at the rate predicted when the UKXIRA was established some three years ago. Clinical trials involving whole organs are clearly still some way off."*** [249]

Originally, single gene changes, altering one key surface marker molecule (α-gal which is present in pigs but not humans), or expressing the human protein suppressing the complement reaction were expected to overcome the problems of hyperacute rejection and allow progress. But despite some success, it is evident that the later stages of rejection pose more serious problems than anticipated and are triggered by many diverse factors, not α–gal and complement alone[242]. These problems have not yet been overcome either through further genetic modification or immunosuppressive regimes. Therefore, it is evident that much more complex genetic modifications will be needed than originally predicted or other strategies adopted, although rejection problems are not as serious with cell transplants as they are for whole organs[249].

Approaches which are being investigated include attempts to 'educate' the body to accept pig cells. For example, by infusing the patient's bone marrow cells into a pig foetus it is hoped that

both the pig and human cells would come to consider each other as compatible. The pig/human hybrid bone marrow would then be infused into the patient before organ transplantation. Infusing pig bone marrow cells into the patient sometime before organ transplant and using anti-rejection drugs whilst the body adapts to the pig cells has also been proposed[250].

All these approaches are highly speculative and the prospects for animal to human whole organ transplants remain extremely remote. However, much hype continues. In March 2000, when PPL Therapeutics announced that it had successfully cloned pigs at its laboratories in the USA, claims were made that human experiments could start in six years[251] even though cloning itself cannot overcome the obstacles facing continued rejection of foreign transplants. In January 2002, PPL announced the birth of cloned piglets with the α-gal gene 'knocked out'. The press release went on to claim that: *"the promise of xenotransplantation is now a reality"* [252]. This report was quickly followed by a publication by their rivals, Immerge Biotherapeutics, describing the birth of 4 cloned piglets also with the α-gal gene knocked out[253]. However, in both cases, only one of the pair of α-gal genes is knocked out and all of the piglets still produce α-gal. They will have to be cross bred with other cloned GM pigs and then piglets with 2 copies of the genes selected for future use. However, there are many other molecules which trigger rejection so a single genetic modification is unlikely to be successful[254]. PPL's announcement was widely interpreted as having commercial reasons in order to boost its share price which rose by 46% on the day of the announcement but fell back later the same week when news of Dolly's arthritis was announced (see Section 9).

Despite the poor performance of xenotransplantation trials, there is still considerable investment in research. For example, from January 2001 Novartis has committed $10 million per year for three years to the xenotransplantation company, Immerge BioTherapeutics, a joint venture with BioTransplant.

8.4 Risks of xenotransplantation

In addition to the practical question of whether a human body will ever accept a different species' organ, there are other serious risks and ethical concerns including the possibility of transferring disease-causing organisms and incompatible physiology.

8.4.1 Transfer of disease-causing organisms

One of the most serious risks of xenotransplantation is that a disease-causing organism could be transferred with the organ and the dangers of cross infection are greater the more closely species are related. Primates have been rejected as donors because they are so closely related to humans. Although pigs were considered safer in this respect, it was shown in 1997 that pigs can carry certain viruses (porcine endogenous retroviruses – PERV) that can infect human cells in laboratory tests[255]. These have been found in a variety of pig tissues including pig pancreatic islet cells which have been proposed to treat diabetes[256].

Retroviruses become part of the host's genetic material and so are still found in animals even in conditions which usually exclude most disease-causing organisms. These viruses do not usually cause disease in the natural host but may cause disease if they spread to another species. Whilst many retroviruses remain harmless, some can:

- cause tumours;
- combine with other retroviruses to produce novel viruses with unexpected properties;
- alter gene expression[257].

Because transplant patients have their immune system suppressed with drugs, they may be especially vulnerable to the effects of retroviruses and any infection could then spread in the

population. Such cross-species transfers have caused widespread disease outbreaks in the past. For example, Ebola and Marburg monkey viruses have caused outbreaks of disease in humans; HIV may have originated from monkey retroviruses; and in the 1950s, millions of people were infected with simian virus 40, a monkey virus which contaminated vaccines made in monkey cell lines[247]. A review of 159 patients who had been in contact with pig cells in experimental treatments for liver, spleen and kidney failure (their blood was passed through pig organs outside the patients' bodies); burns (pig skin grafts); or islet cell transplants for diabetes showed no sign of their having acquired pig retroviruses[258]. However, the majority of exposure times were low (hours rather than days) with only one case of islet cell transplant extending to 460 days. Pig cells were also found in the blood of over 20 patients.

The risk of PERV transfer is likely to remain unquantifiable and may only be determined via direct observation of the outcomes of animal-to-human transplants. Whether it is ethically justifiable to allow such risks to the whole population to save one life has been questioned[259]. In 2000, the Roslin Institute pulled out of xenotransplantation research because of the risks from retroviruses, focusing instead on tissue regeneration from stem cells through its alliance with the US biotech company, Geron[260].

8.4.2 Incompatible physiology

Even if an animal's organ is not rejected and it carries no infectious agents, it may simply not work properly in a different species because their physiology is not identical to a human's. This is particularly important for the kidney and liver, which carry out complex biochemical functions in the body. For example, there are small but important differences between the structure of the porcine and human hormone, vasopressin, which controls urine production, and whether a pig's kidney will respond to human vasopressin is unclear. How well the hormones produced by the pig kidney (renin to control blood pressure and erythropoietin to stimulate red blood cell formation) will work in humans is also not known. Therefore, animal organs may not be able to support life in humans. Similar problems may arise with pancreatic islet cell transplants if the pig insulin produced acts differently to human insulin. Insulin for the treatment of diabetes used to be isolated from pig or cattle pancreases but has largely been replaced by artificial insulin made by genetically modified organisms in contained facilities. Artificial insulin was considered to be an advance as it avoided side effects caused by bovine or porcine insulin.

8.5 Threats to animal welfare

Thousands of animals have been used in xenotransplantation research ranging from mice to chimpanzees. For example, kidneys have been transferred between sheep, tiger, pig, cat, lion, wolf, fox and dingo to dog; dog to wolf; cat, hare and pig to rabbit; rabbit to cat; pig to dog, baboon, monkey, goat and rabbit; sheep and pig to goat; and guinea pig and mouse to rat[261]. Many of the recipients will not only have endured surgery but will also have suffered the effects of organ failure and the side effects of immunosuppressive drug regimes. Because genetic modification techniques are variable in their effectiveness, many animal 'failures' will also have been destroyed. For example, when the α-gal gene was removed from mice, all the mice developed cataracts and became blind[262].

The cloning process is also inefficient, with many offspring dying around the time of birth (see Section 9). Whether the prospects for xenotransplantation justify the scale of animal suffering seems questionable to say the least. Using pigs as organ donors would also change our relationship with them, further treating them as commodities for human use. Whether pigs deserve less moral attention than primates is also questionable[263].

Table 8: Companies involved in xenotransplantation research

COMPANY	LOCATION	ORGANS	COMMENTS
Advanced Cell Technology	Worcester, MA	Kidney, heart	Using cloning and GM techniques.
Alexion Pharmaceuticals	New Haven, CT	Nerve cell based therapies	Focusing on Parkinson's Disease and spinal cord damage using GM pigs.
Algenix	Shoreview, MN	Liver	Developing bio-artificial livers using pig cells for external use.
Circe Biomedical	Waltham, MA	Liver, pancreas	Developing bio-artificial livers using pig cells.
Diacrin	Charlestown, MA	Nerve, liver and retina cell based therapies	In partnership with Genzyme Corp using tissue from GM pigs for treatment of neurological disorders.
Immerge BioTherapeutics	Charlestown, MA	Kidney, heart	Joint venture between Novartis and BioTransplant Inc. Agreement with Infigen (an animal cloning company) to collaborate on the production of GM miniature pigs for xenotransplantation.
Nextran/Baxter	Princeton, NJ/Deerfield, IL	Liver	Uses GM pigs and has tested pig liver as an external support for liver failure.
PPL Therapeutics	Edinburgh, Scotland	Kidney, heart	Combining cloning and genetic modification technologies on pigs.
ReNeuron	England	Nerve cell therapies for stroke victims	Developing mouse stem cell lines.
Ximerex	Omaha, NE	Liver	Formed by a scientist from the University of Nebraska Medical Center. Uses GM pigs to produce human/pig hybrid liver by introducing human cells into foetal pigs.

8.6 Who's involved?

Supplying organs or replacement tissues is seen as a potentially lucrative market and has led to considerable commercial investment in the technology. In 1998, the xenotransplantation market was predicted to be worth up to $6 billion in 2010[247]. Several companies - all except two of which are located in the USA - are developing xenotransplantation techniques to use for a variety of organs and tissues (see Table 8).

PPL Therapeutics is the only company in the UK involved in whole organ xenotransplantation research. Another UK company, ReNeuron, is developing mouse stem cell lines to develop nerve tissue to treat stroke patients. In 2000, Novartis closed its UK division of Imutran, which has now been incorporated into Immerge BioTherapeutics. In 1992, at its UK research base in Cambridgeshire, Imutran had been the first to produce a genetically modified pig (called 'Astrid') designed to reduce rejection by expressing a human complement inhibiting protein, CD55.

The companies involved are trying to develop whole organ transplantation; tissues for use in nervous system disease or damage; or bio-artificial machines outside the body which use animal

cells to support liver or kidney function as the patient's blood is passed through them. GM pigs are the most commonly used donor animal. All approaches for organ transplantation envisage using immunosuppressive drugs in partnership with xenotransplanation because the problems of rejection are not considered to be completely resolvable – even patients with human organ transplants require lifelong immunosuppresion drugs. Many companies have research collaborations with universities and hospitals in the US.

8.7 Alternatives

An important question when considering whether xenotransplantation should be pursued is whether there are other options for improving the availability of organs for transplantation. Some of these may themselves raise animal welfare issues resulting from animal experimentation, which will need to be addressed. Alternatives that could be used to fill the organ gap include:

- ***Prevention*** – to address the root causes that lead to the need for organ transplantation. These include life-style improvements to reduce heart disease and early diagnosis of diabetes (which is an important cause of kidney failure).
- ***Better transplantation services*** – The BMA and others have called for a range of measures to improve services, including better coordination and increased provision of intensive care beds[264]. In Spain, such measures - together with new ways of increasing organ donation - led to 33.6 organs per million of the population being transplanted in 1999 compared to 13 per million in the UK[265].
- ***Increasing organ donation rates*** – An opt-out scheme has been proposed where it would be assumed that a person would be willing to donate their organs after death unless they specifically registered that they did not wish this to happen[266]. Whilst this approach raises important questions about the moral acceptability of such presumed consent[267], other options include mandated choice[268] (where a person's willingness to donate cannot be overridden by their relatives' wishes), and increased use of altruistic donation by living donors in the case of kidney transplants (people have two kidneys but can survive with one).
- ***Biomechanical devices*** – Improvements in artificial heart technology[269,270], in dialysis machines and artificial livers may also lead to more effective ways of treating organ failure. Miniaturisation of artificial livers and kidneys could lead to people being able to move around while they are using them and living a more normal life.
- ***Stem cell technologies*** – Attempts are being made to regenerate tissues from stem cells, a type of cell that retains the ability to develop into different cell types[271]. Stem cells would be 'reprogrammed' to develop into the tissues required. To avoid the problems of rejection, the stem cells could either be genetically engineered or the nucleus from a cell of the patient could be used with an empty egg to produce a compatible tissue. (This latter approach is called 'therapeutic cloning' to distinguish it from 'reproductive cloning', where an individual would be created.) Stem cells can be isolated from embryos or adults but embryo research raises particular ethical concerns about the creation of embryos for use by another person. All such research is a long way from producing whole organs but the production of heart or liver tissue to support failing organs, nerve cells to treat neurological disease and islet cells to treat diabetes is more realistic in the medium term.
- ***Improving transplant tolerance*** – Ways of promoting tolerance so that cross-matching and anti-rejection drugs are no longer required are being investigated in experimental animals. This includes injection of donor cells into the recipient and modifying the transplanted organ using targeted gene therapy so that it produces proteins which interfere with the rejection response[272].

8.8 Conclusion

As the population ages and technological advances allow us to keep people alive longer, the demand for new organs is likely to keep on increasing. Filling the organ gap through the production and sale of genetically modified animal organs, rather than through unpaid donations, is an attractive prospect for the biotechnology industry. However, the prospects for xenotransplantation are poor and research involves a vast number of animals in painful experimentation each year. It may be impossible to remove the risks of transfer of diseases which could threaten not only the patient but the wider population and incompatible physiological differences may also obstruct development. There are alternatives - some of which could address need immediately - such as improvements to the provision of NHS services and encouraging donation. Other areas of science, such as the regeneration of tissues from stem cells, also offer solutions for the future. Therefore, GeneWatch believes that the risks to human health and the suffering of animals involved in xenotransplantation research cannot be justified.

9. CLONING

"Cloning by nuclear transfer now offers a new, cell-based route for transgenesis and will undoubtedly accelerate the progress of this technology in farm animal species"

John Clark, Roslin Institute[273].

Cloning is the synthetic production of genetically identical organisms. This is being achieved by 'nuclear transfer', the transfer of cell nuclei from cultured cell lines and adult cells into recipient de-nucleated cells. Although cloning in itself need not be 'transgenic', it is seen as a major step forward in the development of transgenic technology because it offers the ability to multiply desirable GM animals cheaply. It therefore makes it more likely that the transgenics industry will be commercially successful.

Cloning research on embryos was originally driven by the lure of producing large numbers of elite, identical animals - which were not transgenic - at low costs[274]. This has been overtaken by the potential impact of nuclear transfer techniques on transgenic animals. Cloning from cultured cell lines and adult cells holds the promise that transgenic technology could become much more efficient and that targeted manipulation could become possible[275]. PPL Therapeutics first reported producing cloned lambs from cultured embryo cells in 1996[276] and then a cloned sheep – the famous Dolly - from adult cells in 1996[46]. Until that time, cloning had only been possible from embryonic cells. Cloning from non-embryonic cells is necessary if it is to be useful for GM techniques. Genetic modification and gene targeting in cultured cells, followed by nuclear transfer, were reported in 2000[277,278]. However, serious doubts remain over the effects of cloning on the health of the animal. A high proportion of clones from non-embryonic cells result in late abortions or stillbirth, or have post-natal abnormalities[279,280]. It has recently been reported that Dolly has developed arthritis of the hip and knee, which could be a result of genetic abnormalities from the cloning process[281]. A sheep of Dolly's age would not be expected to develop arthritis, raising the question of whether the cloning process or the use of an adult cell has increased the ageing process.

In addition to work on cloning as part of the process of producing transgenic animals, some research groups are using cloning techniques with the intention of developing a cost-effective means of reproducing expensive agricultural animals. Genetics Australia Ltd is working with Monash University to this end and considers the cost at which cloned embryos could be sold is approaching that of artificial insemination costs - although the success rate (pregnancies and live births) are still unacceptably low[274]. Another Australian company, RAB Australia, in collaboration with Clone International, is reported to have sold 2 clones of a top Holstein bull to China[282].

While these animals would not be transgenic, the prospect of cloned transgenic lines of livestock raises worrying questions about the loss of genetic diversity within farm animals. However, there is still a long way to go before the practical difficulties of the technique are overcome. At present levels of efficiency, 65 slaughtered cows are needed to extract sufficient eggs to produce 10 cloned calves[274].

9.1 Abnormalities

"Cloning by nuclear transfer is an inefficient process in which most clones die before birth and survivors often display growth abnormalities."

Humpherys *et al*, 2001[283].

The reprogramming required for an adult cell to revert to an undifferentiated state and then develop into a range of new cell types may offer an explanation for the large number of

abnormalities associated with cloned animals[279]. Cells from very early embryos are 'undifferentiated' - that is, they have the potential to develop into any of the cells in the body and most of the 40,000 or so genes they contain still have the potential to be expressed. As the cell develops into a particular organ or tissue, genes are progressively 'switched on' or 'switched off' until only those genes required for the correct functioning of the differentiated cell will operate. Until the report of the production of Dolly from an adult (and therefore differentiated) sheep cell in 1997, it was thought that this progression was irreversible. While this is obviously not the case, the processes involved are still not understood.

The efficiencies of cloning are extremely low, presumably as a result of the abnormalities in the developing embryos. Table 9 is taken from a recent review of mammalian cloning[52] and shows that the percentage of animals reaching adulthood per manipulated egg ranges from 0.5% in cows to 1% in sheep.

Table 9: Cloned animals reaching adulthood

	EGGS MANIPULATED	LIVE BIRTHS	ANIMALS REACHING ADULTHOOD
Sheep	988	26	10
Cow	3524	24	17
Pig	511	6	6
Mouse	5354	65	32
Goat	285	3	Not known

Data from Solter (2000)[52], except data for goats from Baguisi et al (1999)[284] .

A range of defects has been reported for cloned animals. In one report of 13 cloned calves, all 8 calves born live required oxygen. 2 subsequently died and the dead calves and aborted foetuses all showed cardiovascular and placental abnormalities. The maternal cows also underwent considerable hardship. 48 cows were impregnated, of which 18 became pregnant. 6 aborted, leaving 12 included in the study. Of these, 3 aborted and died and 1 died after giving birth by Caesarean[54].

In another study of 40 cloned calves, 34 showed one or more of the following peri-natal abnormalities: hypoxia, hypoglycaemia, metabolic acidosis and/or hypothermia. 8 calves died before 14 weeks, 1 calf could not stand without external support, and 4 calves had minor limb deformities. Most calves did not suckle vigorously, did not display normal behaviour patterns and would be described as slow or weak. Some required tube feeding. Birth weight and other characteristics varied considerably even in clones from the same embryo[53].

Of 80 genetically modified and cloned lamb embryos transferred to surrogate mothers, only 14 lambs were born alive. All but 3 died before 12 weeks of age with abnormal kidneys, brain or liver[278].

A recent study[283] compared the expression of various genes in mice cloned from embryonic stem (ES) cells with those in the donor ES cell line. It found gene expression in the cloned mice was extremely disturbed and varied wildly even in clones from the same cell line, which should theoretically be genetically identical. Many clones survived to adulthood despite widespread disruption of gene regulation, showing that even apparently normal animals may have subtle abnormalities. This uncontrolled defective gene regulation can (at least in mice) be transmitted to offspring[285]. The use of nuclear transfer techniques for genetic modification may introduce another element of unpredictability into the genetic outcome even while offering a route to more targeted manipulations.

Many reports have recorded abnormally long gestation and high birth weights followed by difficult births as well as peri-natal deaths[286,287]. These effects are also found in bovine pregnancies resulting from *in vitro* fertilisation and it has been suggested that the *in vitro* process could be the cause[287]. While this may be a contributory factor, abnormalities have been found to be markedly greater in clones produced from adult cells rather than embryos with the same *in vitro* processes[279]. The act of cloning from adult differentiated cells therefore seems to be causal[279].

Whilst some have claimed that the evidence suggests that surviving clones develop and progress normally[288], recent research with cloned mice suggests that their life span can be reduced suggesting that some deleterious impacts of cloning may not become evident for some time[289] underlying the anxiety created by Dolly's development of arthritis at an early age.

9.2 Companion animals

There are proposals to clone pets and to produce genetically modified pet animals. Genetic Savings and Clone (GSC), for instance, is planning to make a considerable profit from people's attachment to their pets. The company has a dog and a cat cloning development programme underway as an offshoot from the $2.3 million 'Missyplicity' project funded by the wealthy owner of a dog called Missy[290]. They already offer a service for cryo-storage of tissue from pets in anticipation of the time when cloning will be possible – for a fee. They anticipate that the cost for cloning a pet will be approximately $25,000 and that they may be cloning dogs as early as 2003, a service which they plan to offer commercially as soon as they can[291]. The first live cloned kitten was reported in February 2002 by a group working at Texas A&M University, with the intellectual property owned by GSC[292,293]. The kitten was the only live birth from 87 transferred embryos.

Another company, Transgenic Pets of Syracuse in New York, hope to produce GM cats within three years. The company plans to disrupt the production of *Feld1,* the allergen thought to be responsible for a high proportion of people's allergic reactions to cats. It is also thought to play *"a minor role in protecting the cat from bacteria"*[294]. The company's approach is essentially to 'knock the gene out and see what happens'[294]. They are planning to sell these GM cats for about $1,000.

GeneWatch considers that the use of either genetic modification or cloning to 'tailor' or reproduce pets is completely without justification and that it exploits both the animals and people's love of them. It is unlikely that animal lovers would wish their pet to be cloned – or allergy free – if they were aware of the unpredictability of the technology or the likelihood of ill effects on the animals produced. It is also unlikely that they would wish their cat or dog to be cloned – or non-allergenic – at the cost of suffering caused to the many hundreds of cats, dogs and other species during the development of the technology.

9.3 Cloning extinct and endangered species

There have been a number of attempts to clone extinct and endangered animals, including the Asian gaur[295] (an endangered wild ox), the mouflon lamb[296] (a rare breed of sheep), the woolly mammoth[297] and the panda[298]. Only the gaur and the mouflon were born live and only the mouflon has survived for more than a few days. There are also plans to clone the Indian cheetah, which became extinct 50 years ago[299]. Given the considerable problems with producing healthy offspring even in well known species, cloning extinct animals is extremely unlikely to be successful. Not only will it be difficult to assess whether live offspring properly represent their species, but the technology ignores the fact that a species is a product of an interaction between genes and environment. A cloned extinct animal will not have the opportunity to develop normally as it will have no other members of its species from which to learn and, in most cases, will not be able to live in the environment in which it evolved. These factors are likely to have a considerable negative impact on its welfare.

Using cloning to ‘rescue’ endangered species is a bizarre strategy. The major factor which renders a species endangered is habitat loss. In the same habitat as every endangered large animal - the ones that are usually noticed - there are almost always many other species of animals, insects and plants that will be lost. Cloning the ‘headline’ species does nothing to preserve the habitat or the associated species. The considerable resources used for cloning would be better spent contributing to more effective habitat management and preservation. Even if clones of extinct animals were successful, there would usually be no habitat left in which they could survive. In addition, endangered species have drastically reduced gene pools and it is certainly possible that cloning will introduce further genetic weakness.

Lastly, there is the danger that proposing cloning to ‘save’ endangered species will actually undermine efforts to protect habitats by giving people the false impression that extinctions are reversible.

9.4 Conclusion

At present, cloning technology is fraught with difficulties. The reasons for embryo abnormalities and peri-natal death are poorly understood, but the problems have appeared in all species which have been cloned and each cloned animal is produced at great expense to the welfare of many others. GeneWatch does not believe that cloning of animals is justifiable because of the suffering involved for the individual animals in the short and long term and the wider dangers that it brings. Further narrowing of gene pools in agricultural animals will not improve animal welfare nor increase world food security. The production of designer pets will involve yet more animal suffering. Cloning extinct animals encourages the pretence that endangered species can be saved. Cloning also normalises a technology which could at some point be extended to humans. None of this can be justified by the narrow commercial interests at stake.

10. REGULATION

Genetic modification of animals in the UK is regulated by several pieces of legislation. The most important are the Animals (Scientific Procedures) Act 1986 and the regulations for the contained use and deliberate release of GM organisms, issued under the Health and Safety at Work Act 1974 and the Environmental Protection Act 1990 respectively. The GM regulations are also established in accordance with the relevant European Directives.

Xenotransplantation is covered by the Medicines Act 1968, which would also cover the products of pharming. Any parts or products of GM animals intended to enter the food chain (e.g. food additives) would also be covered by the Novel Foods and Novel Food Ingredients Regulations 1997.

GM animals fall within the remit of a plethora of UK committees with regulatory or advisory roles because they are covered by such an array of different regulations. The committees and the regulations themselves are summarised in Appendix B.

10.1 UK legislation

10.1.1 Animals (Scientific Procedures) Act 1986

The production of genetically modified animals is regulated in the first instance by the Animals (Scientific Procedures) Act 1986 (ASPA). Under this Act, all experiments carried out on living animals must be licensed by the Home Office if they may cause pain, suffering, or lasting harm to the animal. Such experiments must be part of a programme of work with a project licence, the person applying must hold a personal licence, and the premises must be designated as a scientific procedure establishment. The Animal Procedures Committee (APC) is charged with advising the Home Office on matters relating to the Act.

The scientific justification of all project proposals should be subject to rigorous assessment, including an analysis of the potential benefit and the likely effect on the animal as well as consideration of alternatives. Regardless of justification, the Home Secretary should be satisfied that the '3Rs' (reduce, replace, refine – see Section 4) have been properly applied before project licenses are granted.

Proposals are required to undergo a Local Ethical Review process, which has been approved by the Home Office Animals Inspectorate. This is to support project staff and particularly to encourage continued review of proposals with regard to the 3Rs. The Local Ethical Review Committee is encouraged to have as many non-project staff as possible and representation from outside the establishment if possible.

Section 2(3) of the Act states that anything done for the purpose of, or liable to result in, the birth or hatching of a protected animal is also regulated if it may have the effect of causing pain, suffering, distress or lasting harm. Thus, all activities involving the creation or subsequent breeding of genetically modified animals and all cloning or breeding of animals for xenotransplantation are covered because such activities may result in harm to the modified animal. Offspring from genetically modified animals also come under the control of ASPA unless they are specifically discharged.

10.1.2 Protection of Animals Act 1911

No genetically modified animal has yet been released from the ASPA, which requires health and

welfare records for the preceding two generations. If an animal (or more realistically, a line of animals) was released, it would then come under the Protection of Animals Act 1911, which makes it an offence to cause unnecessary suffering to any animal. Responsibility for enforcement was transferred to the Department for the Environment, Food and Rural Affairs (DEFRA) in June 2001.

If defined as livestock, genetically modified animals will also be covered by the Agriculture (Miscellaneous Provisions) Act 1968, under which it is an offence to cause unnecessary pain or distress to any livestock kept on agricultural land. The Welfare of Farmed Animals (England) Regulations 2000 state that: *"no animals shall be kept for farming purposes unless it can be reasonably expected, on the basis of their genotype or phenotype, that they can be kept without detrimental effect on their health or welfare"*. (Similar legislation is being drawn up in Scotland, Wales and Northern Ireland.) It is unclear how this requirement would be either assessed or enforced in relation to genetically modified animals because it may take generations for genetic defects to surface and harmful effects may be subtle enough to go undetected. Animals kept for pharming (see Section 6) would also appear to be excluded from the regulations as the current definition of livestock is 'animals kept for the production of food, wool, skin or fur or for use in the farming of land'.

10.1.3 GMOs (Contained Use) Regulations 2000

Genetically modified animals are also covered by the GMOs (Contained Use) Regulations 2000, issued under the Health and Safety Act 1974. These regulations focus on the protection of human health and safety and, for micro-organisms only, environmental safety. The Health and Safety Executive (HSE) must be notified the first time a premises is used for genetic modification. A risk assessment must be carried out for every new modification and the HSE notified if the modified organism would pose a greater risk to human health than its unmodified parent. In practice, animal genetic modification rarely requires notification. The regulations cover the original modification process, any subsequent breeding and any GM animals supplied by others (unless the animals have a marketing consent granted under the Deliberate Release Directive).

Part VI of the Environmental Protection Act (EPA) 1990 and the GMOs (Risk Assessment) (Records and Exemptions) Regulations 1996 also require risk assessment for each activity involving GM animals, although no notification is required. Inspectors from the HSE enforce both the Contained Use Regulations and Part VI of the EPA 1990 on behalf of DEFRA.

The Advisory Committee on Genetic Modification (ACGM) advises the Health and Safety Commission about all aspects of human and environmental safety relating to the contained use of GMOs. At local level, Genetic Modification Safety Committees advise on the risk assessments prepared under the regulations.

10.1.4 GM Organisms (Deliberate Release) Regulations 1992

Deliberate releases of GMOs are regulated by the Genetically Modified Organisms (Deliberate Release) Regulations 1992 (as amended in 1995 and 1997) and are primarily intended for environmental protection. It is an offence under Part VI of the Environmental Protection Act (EPA) to release a GMO into the environment without the prior consent of the Secretary of State, the National Assembly for Wales or the Scottish Executive.

Deliberate releases for research and development purposes require a Part B consent, which means that applicants must undertake and submit to DEFRA a full assessment of the risk both to human health and to the environment. This will then be considered by the Advisory Committee on Releases into the Environment (ACRE). If consent is granted, conditions may be attached to the

release. There have been no experimental releases of GM animals in the UK or the rest of Europe – all the GM animal work to date has been considered to be 'contained' and thus falls under the Contained Use Regulations and the Environmental Protection Act.

A Part C consent would be required before a GM animal could be marketed and, if granted, would allow marketing anywhere in the EU. To date, no applications to release a GM animal have been received in Europe. Enforcement of any conditions relating to import will be the responsibility of DEFRA. Import or export of GM animals for contained use does not require consent but must have Home Office approval. Imported animals are then covered by the ASPA.

The European Directive covering the deliberate release of GMOs has recently been revised (2001/18/EC) and new regulations have to be introduced by Member States by October 2002. Although there are considerable improvements in the environmental protection afforded by the new regulations, including a requirement for monitoring post-release, there are no changes which will be significant with regard to the animal welfare implications of the use of GM mammals and birds.

10.1.5 Xenotransplantation

In the UK, the United Kingdom Xenotransplantation Interim Regulatory Authority (UKXIRA) was established in 1997 to advise the Government on xenotransplantation issues. It considers the evidence on safety, efficacy and animal welfare, identifies research needs and considers whether clinical trials of xenotransplantation should take place in humans[300]. Their definition of xenotransplantation includes:

- solid organs (such as hearts and kidneys);
- cells (such as pig islet cells to treat diabetes);
- as a part of a biomedical device (such as an artificial liver outside the body which includes pig cells);
- animal cells used in gene therapies (such as mouse cells).

Up to November 2000, there had been three applications for clinical trials on humans, none of which have been approved[301]. The details of the application and identity of the company(s) seeking permission are not disclosed unless the trial is approved.

10.2 International legislation

The Convention on Biological Diversity[302] was adopted by 183 countries in May 1992 and came into force in December 1993. It requires contracting parties to regulate, manage and control the risks from living modified organisms. The Cartagena Protocol on Biosafety was adopted under this Convention in January 2000 and requires any country exporting 'live modified organisms' to gain the prior informed consent of the importing country. This Protocol – the emphasis of which is on environmental protection rather than animal welfare - has not yet been ratified but is expected to be so soon.

The relationship between environmental protection and free trade legislation is extremely uncomfortable and the regulations regarding GMOs are no exception. The Cartagena Protocol and the various EU Directives on releases of GMOs are framed to follow the precautionary principle. This requires that where there is a threat of serious or irreversible damage, the lack of scientific evidence of harm having occurred should not be used as a reason to postpone action. Decisions under trade law, on the other hand, are required to be based on scientific evidence of harm[303,304]. In direct reference to the World Trade Organisation, the Cartagena Protocol guidance states both that the agreement does not affect the rights and obligations of countries under

existing international agreements *and* that the protocol is not subordinate to other international agreements – arguably a very contradictory stance. Animal welfare and ethical issues are likely to have even less status than environmental protection.

10.3 Shortcomings in the regulatory system

> ***"…the demands of science and industry are met but.. the needs of animals and the concerns of the public are not. Far greater consideration should be given to questioning the necessity, intended benefits and long term social, ethical, and animal welfare implications of genetically engineering animals…"***
>
> RSPCA Submission to the APC, 2001[305].

Whilst there appears to be an array of regulations relating to GM animals, in terms of animal welfare it is only the ASPA provisions which apply to the current situation in the UK where GM animals are being produced in laboratories. The legislation specifically aimed at GMOs was framed for genetically modified plants and micro-organisms and focused exclusively on human and environmental safety.

The ASPA applies to animal experimentation generally, so the specific welfare and ethical concerns of GM animals are not addressed in law. The moral acceptability of genetic modification of animals and xenotransplantation – the question 'is it right or wrong?' - has been found to outweigh considerations of usefulness or safety for the majority of people[65]. However, legislation has been framed around cost benefit analysis and reducing animal suffering, so does not encompass the ethical appraisal that is needed.

There is a frustrating lack of information from the Home Office and the Animal Scientific Procedures Committee on the actual nature of the animal experimentation taking place and the way in which it has been justified. This makes independent evaluation extremely problematic. However, having reviewed the GM animal modification taking place, it is difficult to have confidence that the general air of scientific excitement about the technology (which may in any case be misplaced) is not influencing the decisions that are made. The ASPA theoretically requires both justification of a programme of work and application of the 3Rs (Reduce, Replace, Refine – see Section 4). However, within existing structures, the ability for the evaluation to do more than reflect the status quo in the scientific research community is questionable[306]. The crucial issue is the framework for evaluation and at present it seems that the necessity for research is frequently taken for granted, so the evaluation concerns whether the experimental design is the best that can deliver the research objective. It may not address whether the research itself is necessary or whether alternative approaches could achieve the same desired goal. For example, pharmaceutical companies may be developing drugs which will have only marginal advantages over those already available and which may also be too expensive to be widely available. In addition, research presented as vital for clinical outcomes may in fact only offer further basic biological information which is mainly of interest to the research team[306].

Furthermore, whilst the cost benefit assessment is required to take some account of the benefits accruing from the programme of work and the pain and suffering to the individual animal, the wider ethical questions are not considered. Indeed, it is hard to see how either the Home Office inspectors or insitutional Ethical Review Processes would be able to undertake the fundamental reviews that are required to address the ethical and moral concerns raised by the new technologies.

Looking to the future, the question of how animals are to be discharged from the ASPA and how their welfare will be monitored following approval has not been adequately addressed. The effects of genetic modification may not emerge for many generations as many genes other than

those targeted may have been affected. In addition, the effects of genetic mutations on welfare may not be apparent until animals have been exposed to a range of different conditions. The current requirement for two generations of records before discharge of GM animals from the Act therefore seems entirely inadequate. GeneWatch considers that all genetically modified animals should remain under the auspices of ASPA unless it were proven beyond reasonable doubt that animals would not suffer adverse affects under commercial conditions. A mechanism to monitor their fate under commercial conditions or as companion animals would also be needed to ensure that false assumptions had not been made.

10.4 Conclusions

For GM animals, there is a worrying mismatch between public expectations of regulatory controls and the reality that exists. The framework for assessment takes a very narrow perspective with no opportunity for reflection on the wider context of research. For example, under the existing system, xenotransplantation research continues to be licensed despite its very poor prospects and the existence of much more promising approaches to addressing the organ gap.

There have been calls in the Banner Report[307] for an overarching ethical body and by the Farm Animal Welfare Council for a body to oversee the implications of cloning technologies. However, unless such a committee was actually empowered to act and close down certain areas of research, the existing framework and interpretation of the ASPA would continue to facilitate the production of GM animals based on little critical evaluation of the promised benefits.

GeneWatch considers that the special ethical and welfare concerns raised by the genetic modification of animals mean that applications to carry out such work should undergo special scrutiny and that the appraisal should take account of the ethical boundaries transgressed. In addition, the scientific justification should be examined within a broad and rigorous framework with no *a priori* assumption that the research is justified.

11. CONCLUSIONS AND RECOMMENDATIONS

The genetic modification of animals is an assault on the integrity of another species. Not only can genetic modification cause considerable suffering to the animals involved, but it changes our relationship with the natural world and contributes to the commodification of animals by using them as we wish and for maximum commercial gain. The presumption in every case should be against such interventions in the absence of extremely compelling reasons for them.

There has been considerable hype and little substance to the claims surrounding the potential for the use of GM animals in agriculture or for drug production. Pharmaceutical production in transgenic animals is the closest to commercialisation and may, in some instances, be the easiest means of meeting bulk requirements. However, there are alternative production systems which may offer more reliable products with less associated risks. Bacterial and mammalian cell cultures, transgenic plants and transgenic plant cell cultures can all be used to produce human therapeutic proteins. At present, by far the most important deciding factor on which system will be developed is the potential profits for the companies concerned. There is a need for a systematic appraisal of alternatives which takes into account the technical, social and ethical aspects of how society wishes to meet the need for drugs.

If continued, the development of transgenic animals for agricultural purposes is likely to further intensify animal production and may lead to loss of genetic diversity. The requirement to increase food production to feed a growing world population is frequently put forward as a justification for GM animals. However, although the GM approaches under investigation could increase productivity in the breeds which are used in high input intensive agriculture and increase profits in subsections of the food production industry in the developed world, they are highly unlikely to impact on areas of the world that are currently experiencing food shortages. Modifications aimed at changing complex physiological processes such as growth are in any case likely to severely compromise an animal's health and welfare. Many animals are already at the limits of their productivity as the use of BST in dairy cows has demonstrated – whilst the drug itself may have no obviously harmful effects, the increased demands of milk production lead to a higher incidence of production diseases such as mastitis.

The priority for developing disease resistant breeds in the developing world is to preserve genetic diversity in farm animals. Expansion of programmes to identify indigenous breeds with natural immunity, ensuring that existing genetic diversity is maintained, and pursuing conventional breeding programmes - combined with promoting breeds and practices that require low inputs - would almost certainly be a more effective use of resources than developing transgenic animals.

The demand for new organs is likely to keep on increasing, but the prospects for xenotransplantation to fill the organ gap are poor. It may be impossible to remove the risks of transfer of diseases between species which could threaten not only the patient but the wider population, or to overcome the incompatible physiological differences between pigs and humans. There are alternatives - some of which could address need immediately - such as improvements to the provision of NHS services and encouraging donation. Other areas of science, such as the regeneration of tissues from stem cells, also offer possible solutions for the future. GeneWatch believes that the risks to human health and the suffering of animals involved in xenotransplantation research cannot be justified and that resources should be diverted to alternative methods.

Cloning technology is also fraught with problems. The reasons for embryo abnormalities and perinatal death are poorly understood but these have occurred in all species which have been cloned. GeneWatch does not believe that cloning of animals is justifiable because of the suffering involved to the individual animals in the short and long term and the wider dangers that it brings. Further narrowing of gene pools, encouraging the pretence that endangered species can be

saved and normalising the creation of copies - which itself has implications for the advancement of human cloning - cannot be justified by the narrow commercial interests at stake.

The use of transgenic animals in medicine is perhaps the most difficult and emotive area to assess. There are some applications which will advance medical knowledge. However, there is a danger that transgenic animal disease models will be seen as a panacea for all the problems involved in extrapolating from one species to another. There is also a danger that genes will increasingly be seen as determinants of disease, neglecting environmental factors and prevention. In safety testing, there is an opportunity to develop non-animal alternatives which offer greater accuracy in predicting human responses. However, it is likely that this potential benefit will be lost in the rush for transgenic development and the misapprehension that genetic modification will 'fix' the problems inherent in using different species to assess human safety.

Transgenic work is seductive, fashionable – and expensive. It is frequently linked to drug development, which is generally concentrated on those diseases for which there will be adequate financial returns. Research funding may be diverted from areas of medicine that are less patentable but which have more potential to improve health - for example, prevention, clinical studies, epidemiology and autopsy. There is a danger that the glamour associated with transgenic technology and the potential profits in pharmaceuticals will drive development choices rather than medical or social need.

99% of the animals which are genetically modified in the UK are mice. Both genetic modification and mutagenesis are an assault on species integrity and mutagenesis programmes may be seen as the watershed in our relationship to the mouse. Allowing incremental abuse based on past actions has no moral justification. Instead, the ethical issues raised by the creation of transgenic animals should precipitate a reassessment of mutagenesis programmes and of our entire relationship to the laboratory mouse. The convenience of mice as experimental animals should not lead to their neglect as a species.

Public opinion research indicates that the public are uneasy about the production of GM animals and do not believe that genetic modification should be allowed except under exceptional circumstances. Whilst the proponents of GM animals often claim that the benefits justify the means, for the majority of the public it is the *process* of genetic modification itself which raises ethical problems. Despite these concerns, and as this report has documented, genetic modification and allied technologies such as cloning have been seized upon by scientists and the agricultural and pharmaceutical industries as if they raised no special issues.

The ability to fundamentally change the genome of other species does raise new ethical issues and it appears these are not being properly addressed in the current regulatory system. The moral acceptability of genetically modifying animals and xenotransplantation – the question 'Is it right or wrong?' - has been found to outweigh considerations of usefulness or safety for many people. The current legislation is not encompassing the ethical appraisal that is needed and neither is the system asking sufficient questions about the justifications for experimentation. There is therefore a serious mismatch between public opinion and the operation of the regulatory system in this respect.

It appears that even in the UK, which prides itself on its animal welfare laws, the regulatory systems do not match public expectations and certainly do not appear to provide protection for animal welfare in the area of genetic modification. Whilst the principles of *Replace*, *Reduce* and *Refine* (the 3Rs) enshrined in the UK's approach to animal experimentation are intended to ensure proper protection of animals' interests, the new genetic technologies have reversed the trend of declining animal use. The poor provision of information about the nature and justification of experimentation precludes a detailed independent assessment, but the Animal Procedures Committee and the Home Office appear to be failing to ensure that the 3Rs are implemented in the application of the Animals (Scientific Procedures) Act (ASPA).

To meet the public's expectations that animals should be treated with respect, that animal welfare is prioritised, and that the grave ethical concerns about genetic modification are addressed, the Government should take the following steps:

1. **Introduce a requirement that broad ethical issues (including the use of genetic modification, its justification and the existence of alternatives) form an explicit part of the assessment of experimentation involving GM animals.**

2. **Establish boundaries for the genetic modification of animals and a framework for their evaluation including, as a minimum, that:**
   - **the genetic modification or cloning of companion animals (including dogs, cats and horses) is not allowed;**
   - **the genetic modification or cloning of farm animals is not allowed;**
   - **experiments intended to reduce the sentience of any species is not allowed;**
   - **explicit consideration of alternatives is included in each application, with the onus on the applicant to demonstrate that other approaches could not achieve broadly similar goals.**

3. **The Animal Procedures Committee or the Home Secretary should commission a detailed independent evaluation of the way the use of genetically modified animals has been justified under ASPA and the need to 'Reduce, Replace and Refine' the use of animals in experimentation. Xenotransplantation and GM animal disease models should be included in the scope of this study.**

4. **Provide public information about the nature of, and justification for, animal experimentation using GM and allied technologies.**

5. **Increase public debate about the use of genetic technologies on animals and involve the public in forming public policy and practice in this area.**

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APPENDIX A - ABBREVIATIONS AND GLOSSARY

ABBREVIATIONS

3 Rs	Replace, Reduce and Refine – the three principles of humane animal experimentation. (See Section 4 for explanation of the principles.)
AEBC	Agriculture and Environment Biotechnology Commission
APC	Animal Procedures Committee
ASPA	Animals (Scientific Procedures) Act 1986
BSE	Bovine Spongiform Encephalitis
CJD	Creudzfeldt Jacobs Disease
DEFRA	Department for Environment, Food and Rural Affairs
ERMA	Envionmental Risk Management Authority (the body which considers applications for release of GMO's in New Zealand)
ES cell	Embryo stem cell
FAO	Food and Agriculture Organisation of the United Nations
GM	Genetically modified
HIV	Human Immunodeficiency Virus
PGCs	Primordial germ cells

GLOSSARY

Acromegaly	A condition resulting from excess production of growth hormone, characterised by enlarged facial features, jaw, frontal bone of skull, widely spaced teeth and enlargement of the bones of the extremities.
Allergen	A substance (usually a protein) that triggers an allergic reaction in a susceptible person or animal.
Atherosclerosis	The progressive narrowing and hardening of the arteries over time.
Blastocyst	Early, pre-implanation embryo.
Carcinogen	A substance which causes cancer
Chimera	Organism composed of two genetically distinct types of cells. Chimeras result from situations where only a proportion of cells in an embryo are genetically modified. As the cells divide and the organism matures, some cells are unmodified and some contain the transgene.
Clone	A genetically identical gene, cell or organism.
Cloning	The production of genetically identical genes, cells or entire organisms which are derived from a single common gene or cell. Cloning of genes and cells to create many copies in the laboratory is a common procedure. It is used in this report to refer to the production of genetically identical organisms from single cells. *Nuclear transfer* is the mechanism which has been used to produce clones of adult organisms from a single somatic cell.

Cytoplasm	The cell excluding the nucleus. The cytoplasm consists of a continuous aqueous solution (cytosol) and the organelles and inclusions suspended in it. It is the site of most of the chemical activities of the cell
DNA, Deoxyribonucleic acid	Deoxyribonucleic acid (DNA) is the chemical that makes up the chromosomes of almost all organisms. It contains the genetic information for cell structure, function and organisation. The DNA is made up of four bases - adenosine (A), cytosine (C), guanine (G) and thymine (T), which form two complementary chains in a spiral ladder (double helix) formation. A always pairs with C and G with T. The DNA forms a code using triplets. Each triplet (e.g. AAC, CCG, ATT) codes for one amino acid, which are molecules that, joined together, form proteins.
Ectopic	Occurring in an organ or other structure which is positioned abnormally within the body.
Embryo stem cells (ES Cells)	Cultured embryonic cells which are still able to develop into any cell in the organism - i.e. they are *undifferentiated*. They are taken from the inner cell mass of blastocyst stage embryos.
Endoscope	A surgical viewing instrument, generally flexible and thin.
Founder	The genetically modified animal which is used to breed a GM line.
Gene	The basic unit of heredity that transmits information from one generation to another. Genes consist of specific sequences of DNA nucleotides which code for the construction of a particular protein
Genetic modification	Alteration of the genome of an organism by inserting genes from another organism, or altered genes which are native to that organism. Genetic modification may also involve disruption of native genes.
Genome	The complete genetic material of an organism or a cell.
Genotype	The genetic make up of an organism or cell, as distinct from its expressed features or phenotype.
Germline	Inherited material that comes from the eggs or sperm and is passed on to offspring.
Glycosolation	The process of adding sugar units to protein molecules. The sugars will add to the protein molecule's shape and structural properties (e.g. what other molecules the protein will readily bind to). The correct glycosolation of transgenic human proteins is very important if the transgenic version is to match the activity and characteristics of the natural protein.
Heterozygous	See 'homozygous'.
Homozygous	Genes usually occur in pairs, one inherited from each parent, with one on each paired chromosome. The different forms of a particular gene are called *alleles*. If both alleles for a particular gene are the same, the organism is *homozygous* for that gene, while if they are different the organism is *heterozygous*. Only one allele can be effective at any given time, so if the organism is heterozygous, only the dominant allele will be active. Organisms *heterozygous* for a particular gene can pass either allele on to their offspring, so offspring may display the effects of a gene which the parent carries but which is not active because it is recessive. Organisms *homozygous* for a particular gene will definitely pass that gene to their offspring.
In vitro	Literally 'in glass', as opposed to *'in vivo'* which means in the living animal. Many experiments or procedures, including many reproductive functions, may be successfully carried out outside of the body in culture medium.
Insertional mutation	When a transgene integrates within one of the animal's own genes, disrupting its function. This happens in about 7% of cases of genetic modification.

Intracytoplasmic sperm injection (ICSI)	Direct injection of sperm into the egg.
Knock-out	Integration of genetic material into a gene so that the gene is rendered non-functional.
Laparoscopy	A surgical procedure in which a tiny scope is inserted into the abdomen through a small incision.
Laparotomy	General term for abdominal surgery.
Liposomes	Synthetic, fat membrane-bound vesicles developed for drug delivery.
Microinjection	In microinjection - also called pronuclear injection - DNA is injected into the nucleus of a single cell embryo using a very fine needle. Typically 200 - 500 copies of the gene construct are injected into each embryo. Injected DNA may be integrated randomly into the genome of the embryos, resulting in a genetically modified embryo. Microinjection was until recently the only successful method for producing GM livestock.
Mitochondria	A small intracellular organelle which is responsible for energy production and cellular respiration.
Murine	Pertaining to mice or rats.
Mutagen	A substance that can cause an increase in the rate of mutation.
Neonate	A newborn baby/baby animal.
Nuclear transfer	Nuclear transfer is the method which has been used to produce clones of animals. The nucleus of the cell to be cloned is inserted into an egg from which the nucleus has been removed (enucleated). An electric current is used to fuse the donor nucleus with the recipient cell and to start embryonic development. The resultant organism is a clone of the animal from which the donor cell was taken although it contains a small amount of DNA from the mitochondria in the original egg cytoplasm.
Oncogene	A gene that, if activated, can make a cell cancerous.
Oocyte	The developing egg in the ovary.
Outcrossing	The transfer of transgenes from genetically modified organisms to related species (e.g. by pollen transfer from genetically modified crop plants).
Papillomas	Tumours of the skin or mucous membranes.
Pathogen	Any disease producing microorganism.
Phenotype	The characteristics displayed by an organism under a particular set of environmental factors. The phenotype results from the interaction of the genotype and the environment.
Plasmid	A small, independently-replicating piece of DNA that can be transferred from one organism to another.
Polycythemia	Excess red blood cells.
Primordial germ cells (PGCs)	The embryonic cells which will develop into sperm and eggs.
Promoter	The part of a gene that contains the information to turn the gene on or off. The process of transcription is initiated at the promoter.
Retrovirus	A type of virus that contains RNA as its genetic material. The RNA of the virus is translated into DNA, which inserts itself into an infected cell's own DNA. Retroviruses can cause many diseases, including some cancers and AIDS.
Sentient	Able to feel and possessing consciousness.

Somatic cell	All body cells except eggs and sperm.
Superovulation	The stimulation of multiple ovulation with fertility drugs.
Teratogenic	Tending to cause abnormal development.
Transgene	The gene(s) transferred into another organism using genetic modification.
Transgenic	An organism that contains foreign genes created by the use of genetic modification.
Vector	In the context of genetic modification, a vector is something that can transfer DNA sequences from one organism to another. Different vectors may have properties particularly appropriate to different situations. Both viruses and plasmids are used as vectors.
Virus	An organism that is too small to be seen with a light microscope but is capable of independent metabolism and reproduction within a living cell. Viruses are parasites of animals, plants and some bacteria (known as bacteriophages) and may cause disease.
Xenotransplantation	The transfer of organs between species including from animals to humans.
Zygote	A single cell resulting from the fusion of the egg and sperm.

APPENDIX B - LEGISLATION

TABLE B1: EUROPEAN LEGISLATION RELATING TO GENETICALLY MODIFIED ANIMALS

LEGISLATION	SCOPE/PURPOSE
Council Directive on the Protection of Animals Used for Experimental and Other Scientific Purposes (86/609/EEC)	Covers experimental or other scientific procedures with animals.
Council Directive on the Protection of Animals Kept for Farming Purposes (98/58/EC)	Gives general rules for the protection of animals of all species kept for the production of food, wool, skin or fur or for other farming purposes, including fish, reptiles and amphibians. These rules are based on the European Convention for the Protection of Animals kept for Farming Purposes. They reflect the 'Five Freedoms' as adopted by the Farm Animal Welfare Council.
Council Directive on the Contained Use of Genetically Modified Micro-Organisms (90/219/EEC), (98/81/EC)	Covers the contained use of GM micro-organisms.
Council Directive on Genetically Modified Organisms (Deliberate Release) (2001/18/EC)	Covers the release and marketing of GMOs. No applications for GM animals in the EU to date.
Council Directive on Proprietary Medicinal Products (65/65/EEC, 93/42/EEC). Council Directive on the Implementation of Good Clinical Practice in the Conduct of Clinical Trials on Medicinal Products for Human Use (2001/20/EC)	65/65/EEC and 2001/20/EC cover some aspects of xenotransplantation (cell therapies, gene therapies involving viable animal tissue). 93/42/EEC covers potential xenotransplantation material involving the use of a medical device.
Council Directive Concerning Novel Foods and Novel Food Ingredients (EC 97/258)	Defines a novel food as a food which has not been used for human consumption to a significant degree within the Community (and falls within a number of specified categories, which include GMOs). Would cover GM animal material intended to enter the human food chain. No applications in the EU to date.

TABLE B2: UK COMMITTEES OVERSEEING GENETIC MODIFICATION OF ANIMALS

COMMITTEE	ROLE
Agriculture and Environment Biotechnology Commission (AEBC)	Independent body intended to give strategic overview to ministers on biotechnology and environmental issues.
Animal Procedures Committee (APC)	Set up under the auspices of the Animals (Scientific Procedures) Act to advise the Home Secretary about the functioning of the Act. Individual cases may be referred to the APC.
Advisory Committee on Genetic Modification (ACGM)	Advises Health and Safety Executive (HSE) on all aspects of the human and environmental safety of the contained use of genetically modified organisms.
Advisory Committee on Releases to the Environment (ACRE)	Set up under auspices of the Environmental Protection Act. Advises on any release of GMOs and approval must be given before consent issued.
United Kingdom Xenotransplantation Interim Regulatory Authority (UKXIRA)	Advises the Government on xenotransplantation and provides a means of regulating applications to undertake xenotransplantation on humans.
Advisory Committee on Novel Foods and Processes (ACNFP)	Advises the Food Standards Agency on applications to market under the Novel Foods Regulation. Would consider the human safety aspect of eating meat or other products from GM animals.

TABLE B3: UK LEGISLATION RELATING TO GENETICALLY MODIFIED ANIMALS

TITLE OF LEGISLATION	NOTES	LEAD DEPARTMENT/ ACT OF PARLIAMENT
Animals (Scientific Procedures) Act 1986 (revised 1993 and 1999). Advisory Committee - the Animal Procedures Committee (APC).	Any experiment or scientific procedure carried out on living animals must be licensed under the Act. All genetically modified or cloned animals fall under the Act. Proposals must take account of the 3 Rs (see Section 4).	Home Office.
Environmental Protection Act 1990 (Part VI).	Act under which the GMO regulations are introduced.	Department for the Environment, Food and Rural Affairs (DEFRA) (England); Scottish Executive (Scotland); National Assembly (Wales).
Genetically Modified Organisms (Deliberate Release) Regulations 1992 (SI 1992/3280) (amended by SI 1993/152, SI 1995/304, and SI 1997/1900). Genetically Modified Organisms (Risk Assessment) (Records and Exemptions) Regulations 1996 (SI 1996/3280) (amended 1997). Advisory Committee - Advisory Committee on Releases to the Environment (ACRE).	Aim of legislation is to protect the environment and human health. Applications for release must be made to the Secretary of State in writing and must contain risk assessment as well as details of modification. The regulations are primarily aimed at plants and the required information for risk assessment is largely irrelevant for animals.	DEFRA (England); Scottish Executive (Scotland); National Assembly (Wales). Regulations made under the Environmental Protection Act 1990.
Protection of Animals Act 1911.	Made it an offence to cause unnecessary suffering, or failing to prevent such suffering, to any animal, including farm animals and domestic animals but excluding vivisection, wild animals, the hunt and killing animals for food.	Responsibility not assigned.
Agriculture (Miscellaneous Provisions) Act 1968.	Made it an offence to cause unnecessary pain or distress to any livestock kept on agricultural land (but note that GM animals may not come under the definition of livestock).	DEFRA
Welfare of Farmed Animals (England) Regulations 2000 (SI No. 1870). Similar legislation is being drawn up for Wales, Scotland, and Northern Ireland.	States: "no animals shall be kept for farming purposes unless it can be reasonably expected, on the basis of their genotype or phenotype, that they can be kept without detrimental effect on their health or welfare".	DEFRA (England); Scottish Executive (Scotland); National Assembly (Wales).
Welfare of Animals (Transport) Order 1997, the Welfare of Animals at Markets Order 1990, the Welfare of Animals (Slaughter and Killing) Regulations 1995/6.	Covers all animals and would include GM animals.	DEFRA
Medicines Act 1968. Medicines for Human Use (Marketing Authorisations, etc) Regulations 1994.	Covers some aspects of xenotransplantation (cell therapies, gene therapies involving viable animal tissue).	Department of Health
The Novel Foods and Novel Food Ingredients Regulations 1997. Advisory Committee - Advisory Committee on Novel Foods and Processes (ACNFP).	Would cover GM animal material intended to enter the human food chain. No applications in the UK to date.	DEFRA

"…the demands of science and industry are met but.. the needs of animals and the concerns of the public are not. Far greater consideration should be given to questioning the necessity, intended benefits and long term social, ethical, and animal welfare implications of genetically engineering animals…"

RSPCA Submission to the Animal Procedures Committee, 2001

"There is a striking mismatch between the traditional concern of regulators with issues of risk and safety, and that of the public, which centres on questions of moral acceptability."

Biotechnology and the European Public Concerted Action Group, 1997

"The history of cancer research has been a history of curing cancer in the mouse. We have cured mice of cancer for decades - and it simply didn't work in humans."

Dr Richard Klausner, Director of the National Cancer Institute, USA, 1998

"Uncritical reliance on the results of animal tests can be dangerously misleading, and has cost the health and lives of tens of thousands of humans."

J. C. W. Salen, 1994

"..the use of transgenic animal models could lead to refinement and reduction in the numbers of animals used in experiments. There is, however, a substantial risk that the current intense interest in developing novel transgenic strains will, in fact, result in an overall increase in experimental animal use."

T. B. Mepham *et al*, 1998

"Research in transgenic farm animals has a unique character. Thousands of person years of effort, much of it from the private sector, have been expended without yielding any product."

G. E. Seidel, 1999

"To date attempts [to engineer livestock for use in agriculture] have failed to result in the production of genetically superior livestock (sheep and pigs) due to a variety of undesirable side effects in these animals, although the transgenic animals have been more feed efficient and leaner."

C. A. Pinkert and J. D. Murray, 1999

"Cloning by nuclear transfer is an inefficient process in which most clones die before birth and survivors often display growth abnormalities."

D. Humpherys *et al*, 2001

GeneWatch UK

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