

IQP-43-DSA-1461  
IQP-43-DSA-7296

# TRANSGENIC ANIMALS

An Interactive Qualifying Project Report

Submitted to the Faculty of

WORCESTER POLYTECHNIC INSTITUTE

In partial fulfillment of the requirements for the

Degree of Bachelor of Science

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August 24, 2005

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## **ABSTRACT**

The purpose of this IQP was to discuss the topic of transgenic animals, explore the reason for creating them, and assess their impact on society. This paper examines how transgenic animals are made and the main types that have been created. It also looks at their ethical and legal issues, and the impact of this new technology on society. This paper concludes that, with oversight caution, transgenic technology should continue. Transgenic animals can have enormous medical benefits and could help curing disease.

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## EXECUTIVE SUMMARY

A transgenic animal is an animal that has been genetically altered so that it will produce a specific protein. Foreign DNA has been inserted into the animal's DNA so it will produce a protein it does not normally have. They can be used for studying human diseases that the animals are not normally susceptible to, and can have strong medical benefits. The foreign DNA can be inserted in a number of different ways. It can be inserted by microinjection into a fertilized egg, where the DNA sequence is injected directly into the male pronucleus, or it can be created by delivering DNA *in vitro* to ES cells, then the ES cells are grown to the blastocyst stage and inserted in the uterus of a surrogate mother. Adding and deleting genes in these animals provides them with new properties that make them useful for better understanding disease or manufacturing a cure.

Different types of transgenic animals have been invented to cater to specific societal needs. Transgenic disease models are animals genetically altered to exhibit human pathologies that they do not normally have. This can be helpful in studying a disease so that we may understand them and develop treatments. Some human disease models that have been created so far in mice include HIV, Alzheimer's, and Oncogenes. These models help us gain insight to what causes the disease and its progression. Transpharmers, Xenotransplanters, and transgenic food sources are other types of transgenic animals that have also been created. Transpharmers are used for medicinal purpose by producing specific pharmaceutical proteins in their mammary gland to be consumed in the milk by people. Xenotransplanters are animals, usually pigs because of their similar blood physiology to humans, which have been genetically altered to better prepare their organs for transplantation. These are helpful because normally animal's

organs are quickly rejected by the human body, so this could be a way to prevent that from happening. The nearly limitless possibilities of the different transgenic animals that can be made will help serve society in the future when combating illness.

But should we make such animals? Since it is unethical and immoral to use humans for testing new therapeutic drugs, disease model animals serve a strong benefit to society. Models like Alzheimer's mouse do not suffer noticeably, so with strong medical benefit and little animal suffering, such experiments should be allowed to proceed. For models like oncomouse that involve tumor formation, the potential for suffering is much greater, so we are in favor of legislation mandating a minimization of animal suffering using pain killers and early sacrifice when possible. Since transpharmer animals likely don't even realize they are manufacturing the drug in their milk, and since such drugs can save thousands of lives, we are also strongly in favor of transpharming. Transgenic animals are a very controversial topic which requires close watch so that the animal has very minimal or if possible no suffering while maximizing the medical benefit.

Superpigs are an example of animals that were given human growth hormone and they had to be put to sleep because they were immobile and their organs failed. Thus we agree with the current moratorium on creating any new transgenic animals for food sources, other than fish which seem to tolerate growth hormone with few bad effects.

The legislation behind transgenic animals helps to keep this new technology from becoming an unethical source of scientific study, and we support strong legislative oversight of transgenic experiments. Patents on transgenic animals are a controversial topic. Patents are a way to increase incentive for creating them, but some animal rights activists think that it is unethical to patent animal life. There is no law that says living

things can't be patented however. Genetically altered bacteria, created for breaking down crude oil, have been patented for over 20 years now. Initially turned down because they were thought to be a creation of nature, they were eventually allowed to be patented because it was recognized that they met all requirements under section 101 under Title 35 of the United States code. The oncomouse was also eventually patented in the US and Europe by Harvard. It was controversial because it gave ownership of a mammalian species to a corporation for the first time. This landmark case lead the way for allowing transgenic animals to be patented.

Transgenic animals have had enormous benefits to our society. The methods used in creating them have opened up new possibilities in genetic research. This new technology has opened up new doors in the study of human disease and treatment. In our opinions, the benefits of transgenic animals out weigh the negatives so long as strong oversight is mandated minimizing potential animal suffering, and the impact that they have in helping treat disease is makes it almost unwise not to allow their use. This new technology will become more defined as time progresses. They will not only help save lives but they will improve upon the quality of life.

## **PROJECT OBJECTIVE**

The objective of this IQP was to investigate various topics dealing with transgenic animal research providing a paper that should educate the reader about the kinds of transgenic animals that have been invented, familiarize the reader with the laws governing transgenic animals, and their moral and ethical issues. Transgenic technology has been shown to have both positive and negative implications on society, making this new technology very controversial. The research of this paper also helped the authors come to their own conclusion on whether to support transgenic technology.

## **CHAPTER 1: INTRODUCTION TO TRANSGENIC ANIMALS**

A transgenic animal is an animal where foreign DNA has been inserted into the animal's DNA. Transgenic animals are created so that the DNA in the animal expresses a specific protein that is not normally produced in that animal. The uses of this technology are numerous. The purpose of this chapter is to briefly introduce the topic of transgenesis, and to describe and contrast the main ways transgenic animals are created.

One of the most common uses of transgenic animals is to model human disease. Because the testing of new vaccines and drugs must first be performed on animals, animal disease models are required. Yet many human diseases do not occur in animals, especially those animals convenient to work with like mice, so transgenic animals are created to mimic some aspect of human disease. A gene deficiency is created so that the animal is more susceptible to a disease, or genes can be added to get the same result.

### **Methods for Making Transgenic Animals**

Transgenic animals are usually made by cloning the transgene of interest (for example human insulin) then inserting that gene into the genome of a newly fertilized egg. The egg is then cultured to the blastocyst stage and implanted into the uterus of a surrogate mother.

One method of inserting foreign DNA into an egg is microinjecting it into the male pronucleus. The male pronucleus is used because it is larger than the female pronucleus. Eggs are matured using hormones to increase ovulation in a group of

animals. Then the eggs are harvested and injected with hundreds of copies of the desired DNA using a micropipette (Transgenic Animals 2003) (Figure-1).



Figure-1: Microinjection of DNA into a Pronucleus.  
<http://www.medecine.unige.ch/transgenese/microinj.jpg>

If the procedure is a success, the animal produced will have altered DNA throughout every cell in its body. But because of the randomness of this procedure, in some cases the DNA doesn't integrate or only some of the cells have the new DNA sequence. When this occurs the resulting animal is a "mosaic animal", not all the cells contain the transgene DNA sequence. The offspring of these particular animals will sometimes carry the gene and sometimes not. A major benefit of making transgenic animals using microinjection is that it can be used on a wide variety of animals.

| Animal species | Number of ova injected | Number of offspring | Number of transgenic offspring |
|----------------|------------------------|---------------------|--------------------------------|
| rabbit         | 1907                   | 218 (11.4%)         | 28 (1.5%)                      |
| sheep          | 1032                   | 73 (7.1%)           | 1 (0.1%)                       |
| pig            | 2035                   | 192 (9.4%)          | 20 (1.0%)                      |

Table 1: The Efficiency of Microinjection period Figures in parentheses denote percent efficiency compared to original number of ova injected. (Transgenic Animals and Plants, 2005)

A second technique for making a transgenic animal uses embryonic stem cells or ES cells to introduce the new DNA sequence. ES cells are cells that regenerate and differentiate into other cells. They are undifferentiated cells that have the potential to differentiate into any type of cell, somatic cells or germ line cells, so if the transgene can be inserted into an ES cell, the cell can then be used to create the transgenic animal. These cells become very useful when it is important to target gene sequences to specific sites in the genome because ES cells can be incorporated into a blastocyst and differentiate normally or be grown in vitro (Figure-2, left side).

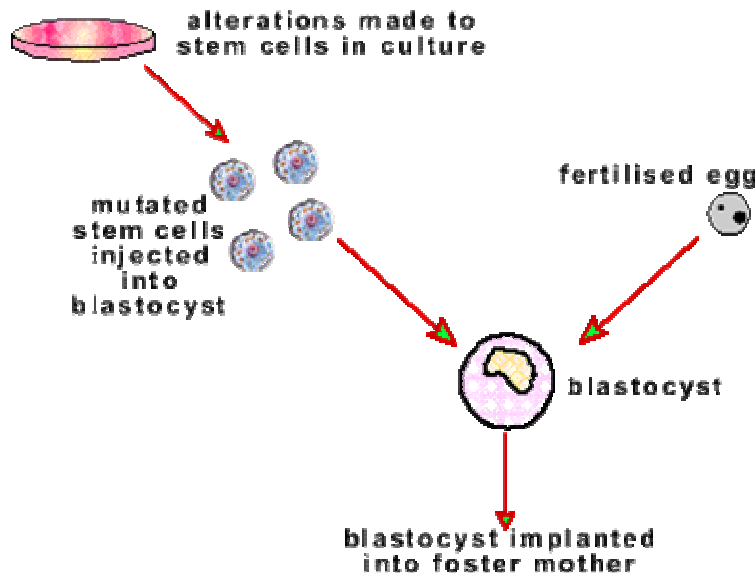


Figure-2: The Two main Ways to Create a Transgenic Animal. The left side depicts the injection of ES cells into a blastocyst. The right side depicts the natural maturation of a fertilized egg into a blastocyst. ([http://www.agresearch.co.nz/scied/search/biotech/gene\\_gmomaking\\_animal.htm#stem](http://www.agresearch.co.nz/scied/search/biotech/gene_gmomaking_animal.htm#stem))

The process of making a transgenic animal using ES cells starts by obtaining ES cells from the blastocyst of donor mice. The cells are taken from an embryo that has not yet been implanted in the uterine wall. The fertilized embryos are collected from female mice a few days after fertilization or the mice are given steroids that prevent implantation

while embryonic cell division occurs. The embryos are harvested and cultured using an embryonic fibroblast feeder layer that produces leukemia inhibitory factor. This factor will help reduce the differentiation of the cells. The inner cell mass of the harvested embryos dissociates from Trophectoderm cells. This allows the ES cells from the inner cell mass to be selected individually for undifferentiated morphology, indicating their likely pluripotency, then they are allowed to grow and multiply. The DNA is introduced into the ES cells by microinjection, viruses, chemicals, or electroporation. Electroporation is a process that uses a pulse of high voltage to make cell membranes permeable and allow the introduction of new DNA. The electric charge is passed through a plate with DNA layered on top of cells. The negative charge of DNA caused by its phosphate residues is attracted to positive electrodes. Once the DNA is absorbed in the cell, the DNA moves into the cytoplasm and integrates into the cellular DNA (Taconic, 2003).

The cells become ES cell lines that can be evaluated for their potential as gene targeting tools or used in studies of cellular differentiation. If the transgene successfully incorporated, the ES cells can be injected into a blastocyst, and the embryo implanted as before to make the transgenic animal.

**Microinjection of Eggs vs. ES Cells for Creating Transgenic Animals**

| <b>Microinjection of Eggs</b>                              |                                                                           | <b>ES Cell-Mediated Gene Transfer</b>              |                                   |
|------------------------------------------------------------|---------------------------------------------------------------------------|----------------------------------------------------|-----------------------------------|
| <b>Pros</b>                                                | <b>Cons</b>                                                               | <b>Pros</b>                                        | <b>Cons</b>                       |
| One of the first technique to prove successful in mammals. | Sometimes genes are over or under expressed resulting in a mosaic animal. | Works well in mice, which are often used.          | Doesn't work well in all mammals. |
| Works in a wide variety of mammals.                        | DNA integration is a random process and DNA                               | Helps in studying genetic control of developmental |                                   |

sometimes inserts in processes.  
 a place where it's  
 not expressed.

Allows for precise  
 targeting of defined  
 mutations in the  
 gene.

|                       | <b>TRANSGENIC</b>                             | <b>ES DERIVED</b>                     |
|-----------------------|-----------------------------------------------|---------------------------------------|
| <b>Genetic action</b> | <b>Dominant</b>                               | <b>Recessive or dominant</b>          |
| <b>Insertion site</b> | <b>Random</b>                                 | <b>Targeted to endogenous</b>         |
| <b>Copy number</b>    | <b>Vari dominant able,<br/>1 to &gt;100</b>   | <b>Heterozygotes 1, homozygotes 2</b> |
| <b>Expression</b>     | <b>Usually integration site<br/>dependent</b> | <b>Usually same as endogenous</b>     |
| <b>Time</b>           | <b>6-9 months</b>                             | <b>1.5 -2 years</b>                   |
| <b>Cost</b>           | <b>&gt;\$3000</b>                             | <b>&gt;\$12,000</b>                   |
| <b>Other</b>          | <b>Insertional mutation</b>                   | <b>Effects on neighboring genes</b>   |

Table 2: Comparison of Genetically Engineered Mice Created by Pronuclear Injection Vs ES Cells (Camper, 2005) period

A gene targeting process is used to introduce the desired DNA sequence into ES cells. In gene targeting, a piece of DNA that carries the transgene or DNA sequence of interest is introduced into the ES cells of a mouse by electroporation. Inside the ES cell the DNA sequence can insert directly in the intact genome at a specific region. ES cells that successfully incorporate the sequence are added to an embryo in its early stages. This is done by either injecting the ES cells in an embryo or culturing them with it. The ES cells and embryo cell develop together into a chimeric embryo. The chimeric embryo

is implanted in a surrogate mother. When the chimeric mouse is born the color is usually different from that of the host because the gene for the coat color is often chosen to be from the ES cells genetic makeup and not the host. This is an easy way to confirm the success of the procedure. The chimera then carries the new gene in its germ line and can be selectively bred to establish the gene permanently in a new mouse (Taconic, 2003).

Another method for inserting the foreign DNA into the ES cells is to use viruses. A retrovirus can be modified so that it carries a desired DNA sequence in its DNA. The disease causing genes are removed from the virus so disease is not introduced to the transgenic animal. The virus can infect the ES cells with a specific gene but does not infect all of the ES cells with the retroviral DNA. Germ line cells need to be infected for the procedure to be successful.

A third method used to deliver the new DNA into the ES cells is DNA microinjection. This is done by microinjection of the DNA sequence into the ES cells in the blastocyst of the host animal. This process produces a mosaic animal that does not always carry the transgene in its germ line. The process is often repeated to gain more transgenic animals.

A versatile approach to enhance the delivery of nonviral DNA involves complexation with cationic polymers, which can be designed to overcome the barriers to effective gene transfer. DNA is packaged into a polymeric or scaffolding. Liposomes are usually used to fuse to the ES cell membrane and insert the DNA. Polylysine and plasmid DNA can also be used to make an attraction between biotin and avidin. A mixture of the biotin-modified and unmodified cationic polymers are electrostatically complexed with non-viral DNA and tethered to a substrate containing nonglycosylated

avidin. The cells that were used, HEK293T and NIH/3T3, grew along the surface, then were directly exposed to the tethered complexes and are easily transformed. Transfection is a function of the surface DNA quantities and the number of tethers in the complex. This process has shown to be much more efficient than traditional bulk delivery (Segura and Shea, 2002).

The success rate for these methods of creating transgenic animals that can birth animals containing the transgene is very low. If the genetic manipulation does not cause an abortion, the result is a first generation of animals that need to be tested for the expression of the transgene. Depending on the technique used, the first generation may result in chimeras, and various tissues need to be tested for transgene insertion. When the transgene has integrated into the germ cells, the so-called germ line chimeras are then inbred for 10 to 20 generations until homozygous transgenic animals are obtained and the transgene is present in every cell. At this stage, embryos carrying the transgene can be frozen and stored for subsequent implantation (Buy, 1997).

### **Assays Used for Screening Transgenic Animals**

There are several ways of testing the effectiveness of the transgenic method used to determine whether the transgene incorporated into the various tissues of the animal. Foreign DNA can be detected in the genome of a transgenic animal using either a Southern assay, or PCR. One of the more reliable tests is the "Southern Blot" test. A Southern blot is a method of identifying a specific DNA band on an agarose gel electrophoresis by marking specific DNA sequences (Answers.com). This technique uses a restriction enzyme to split DNA at specific locations, and then the fragmented DNA is

put through an agarose gel (Figure 3). An electric current is run through the agarose gel. DNA moves towards the positive electrodes because it is negatively charged. The size of the DNA affects how far it migrates through the gel, the smaller DNA fragments have an easier time migrating through the gel, so those fragments move the farthest on the gel. The pattern of DNA in the gel is then blotted to a membrane that retains the pattern, but allows hybridization to a probe to illuminate a specific gene (like the transgene). The DNA from the animal is compared with the position of the DNA of the transgenic DNA. If the sample contains the transgene, the transgenic DNA was successfully integrated into the animal's cell.

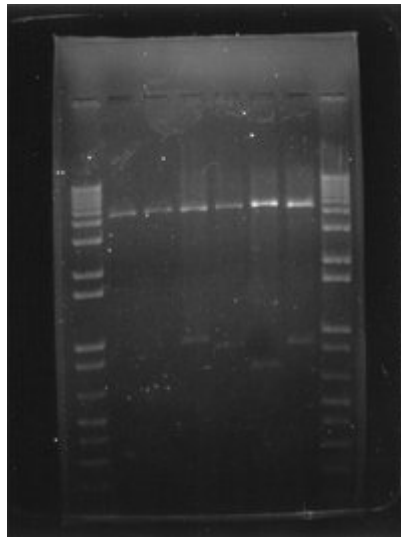


Figure-3: Digital Printout of an Agarose Gel Electrophoresis Performed on Plasmid DNA (Answers.com).

A Western Blot is another way to determine if an animal's tissue is expressing the transgene, i.e. is making the transgenic protein. This method takes into account that transgenic animals are engineered to produce specific protein. These proteins can be detected in a similar way as the DNA in the southern blot. In the western blot, whole cell

extracts containing protein are electrophoresed to separate the proteins by size, and then blotted. Lighter proteins will travel further. The protein is then blotted to membrane made of nitrocellulose and a specific protein is visualized among the bands present using an antibody solution. The antibody recognizes and attaches to the transgenic protein, allowing its detection. If the protein that the antibody binds to is present in the sample the antibody will bind to it and it will create a dark spot in the gel (Figure-4) (Answers.com).

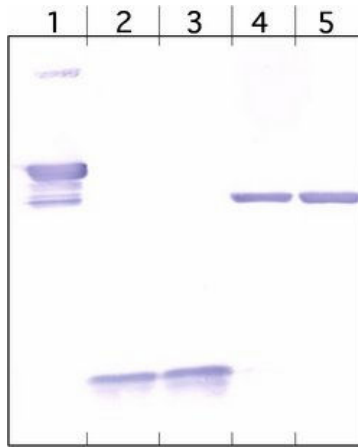


Figure-4: Picture of a Western Blot With 5 Vertical Lanes (Answers.com).

An Enzyme Linked Immunosorbent Assay or ELISA is another technique used for screening transgenic animals. It is used to measure the levels of antibodies found in the transgenic animal's serums, blood and urine. Animals produce antibodies when substances are introduced which are foreign to the body (Agresearch, 2001). An antigen or protein is added to a plastic well to which it attaches. The serum of the animal is then added and if the animal is positive for the antibodies to the protein, they will bind to the proteins. Antigens attached to an enzyme are then added to the mixture and they will bind the antibodies. The enzymes react with a chromogen substrate to produce a color

(Figure-5). If the color changes then the animal is positive for the antibodies against the transgenic protein.

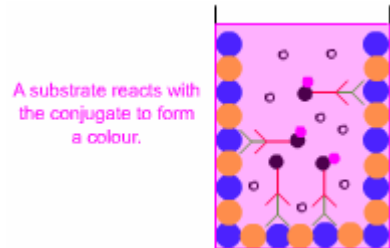


Figure-5: Diagram of an ELISA Test. An antigen (usually the transgenic protein) (blue sphere) is used to coat the well of a microtiter dish. Blood or urine from the test animal is added to the well. If it contains antibodies against the transgenic protein they bind the walls of the dish (green). Antibodies containing enzymes are then used to bind the former antibodies (pink). Substrate is added. This part is a chemical reaction. The substrate reacts with the conjugate and forms a colour that you can see. By using a colourimeter and measuring the optical density of the solution, the technician is able to determine the number of antibody sites available (Agresearch, 2001).

The type of transgenic animal to be made is a very important question. There are several factors that need to be taken into account when deciding what animals need to be used. Disease models are usually mice since they have a short gestation time which allows more data to be obtained. Transpharmers are usually cows, sheep, or goats since they produce large quantities of milk containing the product. It is important to look at the gestation time, number of progeny, and amount of milk per year of the animal depending on what it is to be used for. Transgenic animals are very useful and help scientists gain a better understanding of animal and human biological function.

## CHAPTER 2: TRANSGENIC ANIMAL EXAMPLES

This chapter will describe some of the categories of transgenic animals, and which transgenic animals have been made so far. Some of the uses include disease models, food sources, and bioreactors for producing medication for humans. Transgenic animals help us to better understand human disease and improve health care.

### **Transgenic Disease Models**

Transgenic animal disease models are animals that have been genetically altered to have traits that mimic the symptoms of specific human pathologies. The disease models are needed so that we can better understand the disease for treatment. Many animals do not normally exhibit the equivalent of certain human diseases. So a human transgene specific to the disease needs to be expressed in the animal. This allows for pathological characteristics in the animal so that it can be studied. Animal disease models are very useful in that they allow us to screen drugs that may be harmful or have bad side effects. Once the therapeutic agents have been discovered and tested, human cells may then be tested, followed by human test subjects in clinical trials. But because it is not ethical or safe to perform the initial tests in humans, we use transgenic animals.

### *AIDS MOUSE*

This animal is a mouse that was used for studying human immunodeficiency virus or HIV. The mouse has a transgene that encodes for the genome of type 1 HIV. Mice normally lack the receptor and co-receptor that allows them to be infected with HIV. The mice were genetically altered to contain the gene for human CD4 promoter upstream of the human CD4 gene and human CXCR-5 co-receptor gene. These genes were inserted

into a newly fertilized mouse zygote and then transferred into the uterus of a female mouse. Pups from the mouse litter were selected for their phenotypic correlation with human HIV (see below). The production of the co-receptors in the mice allows for the HIV virus to attach to the T cells. The mouse will then have the ability to synthesize all the viral proteins that aid the HIV in successfully infecting it (Hanna, 1998).

The AIDS mouse was designed to exhibit symptoms similar to human AIDS such as wasting, atrophic lymphoid organs, atrophic kidneys, and early death. Studying these mice has led to the identification of human host factors that play critical roles in the type 1 HIV replication cycle. Thus, these studies have not only contributed towards our basic understanding of type 1 HIV life-cycle, they have also provided us with novel targets for future therapeutic intervention. Table 1 below shows various types of HIV animal models.

| <b>Animal Model</b>                                     | <b>Virus Challenge</b> | <b>Model Characteristics</b>                                                                                                  | <b>Comments</b>                                                                       |
|---------------------------------------------------------|------------------------|-------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| Mouse<br>Transgenic<br>HIV-1<br>transgenic<br>Xenograft | HIV-1<br>None<br>HIV-1 | Expression of human CD4, human co-receptor<br>Expression of HIV-1 gene products<br>Varies, dependent on tissue type engrafted | No spreading infection<br>Developed AIDS-like pathologies<br>T cell depletion         |
| Rat<br>Transgenic<br>HIV-1<br>Transgenic                | HIV-1<br>None          | Expression huCD4 and huCCR5<br>LTR-driven expressions of HIV-1 gene product                                                   | Poor virus spread, no replication of T lymphocytes<br>Developed AIDS-like pathologies |
| Rabbit<br>Transgenic                                    | HIV-1                  | Expression of huCD4 and T lymphocytes                                                                                         | Inefficient virus spread                                                              |
| Drosophila                                              | None                   | Inducible, ubiquitous expressions of HIV-1 Tat                                                                                | Similarities to AIDS-like neuropathologies                                            |
| Cat                                                     | FIV                    | Replication of FIV, a related lentivirus                                                                                      | Development of immunodeficiency, somewhat similar to AIDS                             |

Table 1: Small animal models for lentiviral disease have been developed that permit a certain level of in vivo analysis. They all suffer from one major limitation: it is unclear how relevant they are in terms of HIV-1 infection in man (Van Maanen, 2005).

## *ALZHEIMER'S MOUSE*

Alzheimer's is a disease marked by the loss of cognitive ability, and associated with the development of abnormal tissues and protein deposits in the cerebral cortex. It is a neurological disease that affects the memory. The impairment to the brain is due to the accumulation of neurotoxic precursors to and build up of amyloid proteins, which form a plaque in the brain (Figure-1).

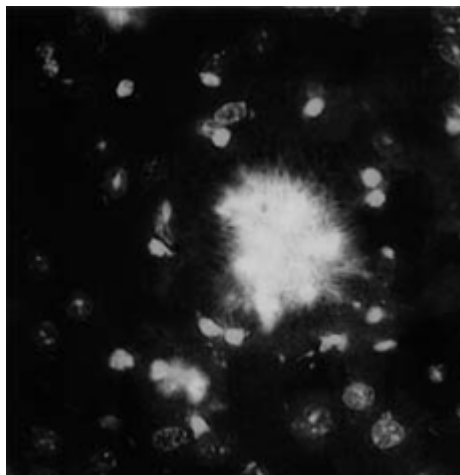


Figure-1: A large human amyloid deposit (lighter color) in the frontal cortex of the novel Alzheimer's mouse (Photo courtesy of Bruce T. Lamb, Ph.D.) (Bowers, 1999).

The amyloid-beta protein is initially soluble (and highly toxic to neuronal cells), and its buildup eventually causes the degeneration of neurons and their neurotransmitters in the brain (Games et al, 1995).

Expression of a mutant version of human amyloid precursor protein (APP) mRNA, holo-APP, and A-beta in the brains of the mice (which are associated with an aggressive early onset type of Alzheimer's disease) causes them to exhibit human-like Alzheimer's disease symptoms (Games et al, 1995). The mice also develop

neurofibrillary tangles. The mouse model develops many of the neuropathological symptoms of AD in a temporal and regional dependent manner.

This model has been very useful for AD research. The Alzheimer's mouse in one experiment developed most of the pathologic changes seen in human brains with the exception of neurofibrillary tangles. This observation may cause a reconsideration of the pathogenesis of the disease, suggesting that these tangles are a result of a destructive neurological process rather than a direct cause (Alzheimer's Breakthrough, 2005). The AD mouse model was also used in a vaccine trial to clear the plaques once they had formed (Schenk et al, 1999). The vaccine restored neurological performance in the mice, and is currently in phase II human clinical trials at Elan Pharmaceuticals.

### *ONCOMOUSE*

A third mouse that was used as a disease model is the Oncomouse. This mouse was originally created by Harvard and was the first animal to ever be patented (Leder and Stewart, 1984). Oncomouse has been genetically engineered to develop specific forms of cancer. This mouse's germ cells and somatic cells contain an activated human oncogene sequence (discussed below) that has been introduced into the animal at an early embryonic stage to ensure that the oncogene is present in all the animals' cells. This will increase the chances of the mouse developing malignant tumors, so it can be used to test various potential anti-cancer treatments.

Oncomice have been created that carry either the v-Ha-ras or the c-myc gene driven by the mouse mammary tumor virus (MMTV) promoter/enhancer (Sinn, 1987). These two genes are important to cellular growth, and ras has been found to cause cancer

when mutated. When these two mouse strains are crossed, the mouse develops accelerated tumor formation in its cells. Using this animal model cancer can be studied, and better insight to what causes cancer can be learned. Potential anti-tumor compounds can be tested on the animals to see if the animal has any sign of reduced carcinogenesis.

### *SMART MOUSE*

Although not a disease model, this animal was used as a model for what could be done to help memory loss in humans. Joe Z. Tsien, a researcher at Princeton University, genetically engineered a smart mouse. He named the mouse "Doogie" after the boy genius in the TV series Doogie Howser, MD (BBC News, 1999). Doogie was able to navigate through mazes better than regular mice, and has shown signs of better intelligence and memory through other tests. This strain of mice also retained into adulthood certain brain features of juvenile mice, which, like young humans, are widely believed to be better than adults at grasping large amounts of new information.

During embryogenesis, a gene in the brain called the NR2B codes for a protein that covers the surface of neurons. It is an efficient receptor for the chemical signal N-methyl-D-aspartate (NMDA), a neuronal channel protein, which opens when it is bound by the amino acid glutamate after its neuron multiply fired. Active NMDA receptors allow calcium ions to enter the neuron, making it more sensitive to stimulation. This effect is theorized to be responsible for associative memory and thought in the brain. If two signals arrive at the same time, maybe one results from seeing a lit match and the other results from a sensation of pain, then the receptor is triggered and a memory is

formed (Harmon, 1999). Research has shown that in young animals memory is triggered even when the input signals are relatively far apart.



Figure-2: Doogie the mouse who exhibited enhanced memory and intelligence (BBC News).

This use of transgenic animals is ethically debatable. Even if this experiment is not performed in humans, it is very useful in the study of memory. It shows that some day we may be able boost human intelligence and it could be used in gene therapy for such areas as dementia.

### **Transpharmers**

Transpharmers are transgenic animals that are genetically altered to produce pharmaceutical compounds in either the milk, eggs, or blood. Gene pharming is a technology that scientists use to alter an animal's DNA. These genetically modified animals are mostly used to make human proteins that have medicinal value. The protein encoded by the transgene is engineered to be secreted in the animal's milk, eggs, or blood, and then collected and purified. However, the mammary gland is the most common place they are made to produce the protein. Livestock such as cattle, sheep,

goats, chickens, rabbits, and pigs have already been modified in this way to produce several useful proteins and drugs (Gillespie, 2005).

The most effective approach for creating a transpharmer is to express a protein in the mammary gland by using a promoter from a milk protein gene to direct expression. An example of this would be Herman the bull, which produced the human protein lactoferrin under a beta casein promoter, and his female progeny produced it in their milk (Biotech Notes, 1994). This protein binds to iron, which is an essential part of infant growth. Cow milk does not contain lactoferrin, so infants are given other iron rich sources, mother's milk or formula. Baby Herman's offspring however produced lactoferrin at such a low rate that it was not commercialized. The company which created baby Herman is Leiden-based Gene Pharming Europe BV who plan on artificial inseminating 60 cows with Herman's sperm (Reuters, 1992).

The human gene for lactoferrin was inserted into baby Herman's genes at the embryonic stage. Herman was to have children that would produce the pharmaceutical compound in their milk. Even though the children did not produce enough for it to be worth commercializing, the scientific benefits from this are numerous. The same technology could be used in making other medications produced in milk and then taken and purified in large quantities.

### **Xenotransplanters**

Xenotransplantation describes when an animal organ is transplanted into a human. Xenotransplanters are animals genetically altered to better prepare their organs for transplantation into human recipients. This is a very useful technology because there is

an enormous backlog of patients needing organ transplants, and the body normally rejects the foreign animal organs. When this occurs, the body could go into hyperacute rejection and the immune system will kill the organ by wiping out all cells in it. The organ will turn black and die.

With past xenotransplants, surgeons could avoid hyperacute rejection by using organs from other primates with similar genomes as humans. But for numerous reasons like size of organs and availability due to extinction, biotechnologists have turned to pigs. A pig's physiology is similar to that of humans (Pearson, 2003). However, there are drawbacks that come with using pig organs because the surfaces of porcine endothelial cells, which line the blood vessels of the donor organs, have molecules that humans don't. This can lead to hyperacute rejection or delayed xenografted rejection, which causes antibodies and other killer cells to attack the organ.

A major cause for these rejections of pig organs is due to a gene in the pigs that codes for the enzyme alpha-1,3-galactosyltransferase (GGTA1). This enzyme codes for the sugar alpha-1,3-galactose that is on the surface of the cells in pigs. This sugar is recognized as foreign in humans and causes hyperacute rejection of the organs. David Ayares of Revivicor in Blacksburg, Virginia has found preliminary evidence that pig organs engineered to lack GGTA1 can bypass rejection (Pearson, 2003). Xenotransplantation has many risks involved, deadly rejection or viruses, but if this technology were to be successful the benefits are great.

## Transgenic Food Sources

This classification of transgenic animals involves genetically modifying animals to better accommodate the needs of human consumption. An example of this is when growth hormone is incorporated into an animal's genome. Gene constructs encoding growth hormone have been incorporated into the genome of several species of salmon to create “superfish”. These animals show increased growth rates, improved flesh color and increased disease resistance (Devlin et al, 1997).

One way of getting salmon to grow is to add an antifreeze protein promoter, which produces a growth-stimulating hormone in the fish. This was discovered when Choy Hew, Ph.D. accidentally froze a tank of flounder. When the fish tank was thawed, the fish were still alive. This particular species of fish had a gene that produced an antifreeze protein that prevented its tissues from dieing under freezing conditions. The gene that activated this protein, which is normally turned off, was then isolated. This gene was used like a switch to activate the growth-stimulating hormone in the salmon. In the resulting salmon the switch remained on. These fish grow much faster than conventional salmon, but they do not reach the same physical maturity (Lewis, 2001).

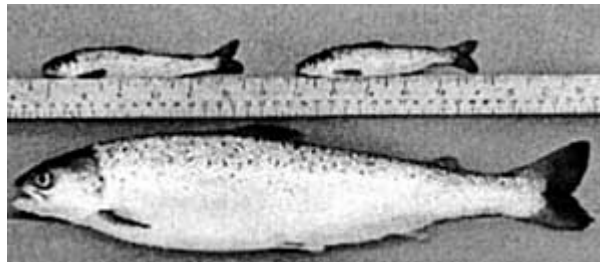


Figure-3: A transgenic Atlantic salmon, containing an antifreeze protein promoter, measured against control siblings (Rome, 2001).

## CHAPTER 3: TRANSGENIC ETHICS

It is true that transgenic animals hold the potential for enormous contributions to science and medicine. What remains uncertain is whether engineering new life and modifying existing forms is ethical in modern society. Technicalities in genetic engineering no longer limit the ability of scientists to manipulate life forms. So now that we know how to make such animals, we now use ethics to determine what bounds we should use with this new technology. This chapter will first explore the advantages and benefits of creating transgenic animals, along with some concerns from the public and other sources. It will also be helpful to observe how people from different religions, cultures, and backgrounds perceive this issue because basic principles may be derived from past values (Curran and Koszarycz, 2004). To effectively reason which new animals are ethically acceptable and which should be avoided is a balance of their medical benefits versus any detriments such as animal suffering. This chapter will list specific transgenic examples and individual applications which cover the varying levels of animal suffering ranging from none at all to life threatening. The final segment of the chapter will consist of an evaluation of current practices along with suggested plans of action regarding how to proceed with animal transgenesis.

### **Transgenic Positives**

The advantages of using transgenic animals can be divided into three broad categories: medical, scientific, and food benefits. As discussed in Chapter-2, medical advantages are seen with disease models like Alzheimer's mouse and Oncomouse that

teach us how diseases initiate and progress (Lemonick et al, 2001). Such models are required for performing experiments not ethical in human beings. Medical advantages are also seen in transpharming models that produce life-saving pharmaceuticals in their milk (D’Silva, 1998), and Xenotransplanters that grow organs for human transplants (Butler, 2002). Scientific benefits are seen in some animals engineered to over-express a specific protein (or knockouts engineered not to produce a specific protein) to help elicit a newly discovered protein’s function. Food benefits are seen in superfish that grow faster and larger than regular fish. Basic science and medical research would benefit from fewer required lab animals and generation of more accurate data because of a greater genetic similarity in the test subjects, for example mice or monkeys (Taconic.com). Agriculturally, farm animals that can produce better products, more efficiently while consuming less food themselves would be a valuable commodity. Additionally, disease resistance and faster production times offer even more incentive to progress (Mephram, 1994). The potential to successfully utilize transgenic animals is huge, as is the possibility of exploiting them.

### **Transgenic Negatives**

There are many concerns involved with making transgenic animals, some real some products of fear. Commonly applied to the practice of genetic modification is the phrase “playing God,” which can mean that man has become arrogant and disregarded his respect for nature, or has otherwise violated nature in some way (Macer, 1990). Destroying the “integrity” of the animal genome, in this case applied to the intactness of a genome, is a worry of environmentalists (Vorstenbosch, 1993). The question of interest

arises, who is really benefiting: the human species or biotech investors? The worry that animals may be reduced to instruments or tools is important to consider. Then there is the slippery slope argument which says that what can be done with animals may someday be done with humans (Schroten, 1997). The most widely accepted arguments against the technology object to animal suffering, and point to preservation of the welfare of transgenic animals which is rooted in modern environmental philosophy (Rollin, 1996). Also loss of genetic diversity, environmental hazards, and human health risks pose questions that must be addressed (Mepham, 1994). In any case, the primary worry remains; will a given experiment result in mutant animals with increased mortality or other negative effects on the health and well-being of that animal? Unfortunately, we can't know until we try.

Another fact to keep in mind when discussing the tampering with genes and genomes is that we have been doing it for centuries via selective breeding. Selective breeding has given us the broiler chicken which grows to approximately 2 kg in about 40 days, half the time it took 30 years ago. The chickens grow muscle faster than the skeletal and cardiovascular systems which support it and end up with leg problems and heart failure (Christiansen and Sadoe, 2000). With effects like these on the welfare of non-genetically altered animals, few would permit the use of a controversial new method to again push the animals to their production limits.

### **The Beltsville Pigs Ethics**

The dramatic case of the Beltsville pigs is an example of how a promising experiment can result in a reduction of animal welfare. Engineered to produce human

growth hormone, they were expected to grow faster and produce leaner meat (Figure 1). Although these goals were accomplished, the pigs suffered from arthritis, stomach lesions and gastric ulcers, lack of coordination and muscle weakness (D’Silva, 1998). It is generally agreed that the costs in the suffering of the animals outweighed any benefits offered by the transgene recipients, and scientists thus imposed a voluntary moratorium

on growth hormone experiments.

Rollin (1996) notes that it is the *effects* of the transgene transfer and not the transfer itself that is objectionable in this case. A counterargument is that manipulation of embryos *in vitro* results in stress to the birth mother and presumably also

### TRANSGENIC PIGS more lean - less fat



Control Transgenic  
Figure 1. Leaner pork chops from a transgenic pig.  
USDA Agricultural Research Service, 1980.

to the unborn animal.

### Alzheimer’s Mouse Ethics

Not all animals are required to suffer to serve the greater good. In the author’s view, an example of an acceptable transgenic animal model for human disease is Alzheimer’s mouse. Created by a team of experts from across the country, including Prof. Adams of WPI, the mouse models “express high levels of human mutant  $\beta$ -

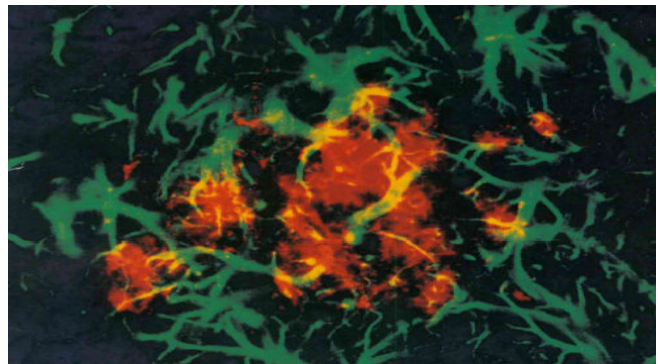
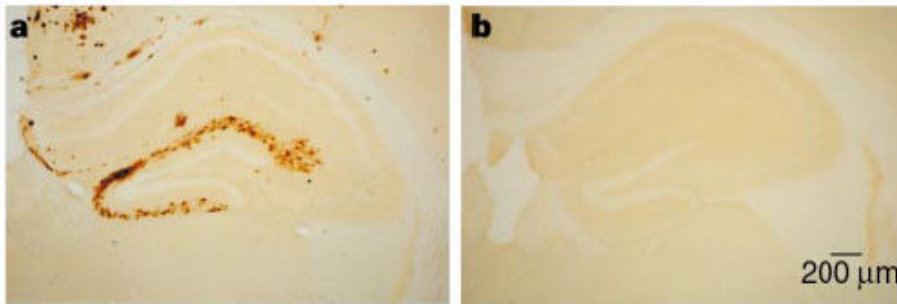


Figure 2. A laser scanning confocal image of an Alzheimer’s mouse brain showing normal mouse neurons (green), human amyloid beta protein (red), and distorted neurites (yellow) telltale features of Alzheimer’s disease (Games, Adams, et al, 1995).

amyloid precursor protein, and the animals progressively develop many of the pathological hallmarks of Alzheimer's disease" (Figure 2) (Games, Adams, *et al*, 1995). What makes this model an ideal candidate for ethical acceptance is that the mice do not suffer to any measurable degree, yet this model is essential for continuing research into Alzheimer's therapeutic compounds. For example, using this mouse model, Elan Pharmaceuticals has already developed a vaccine that "can markedly reduce pathology in an animal model of the disease" and "may prove beneficial for both the treatment and prevention of Alzheimer's disease" (Schenk *et al*, 1999). Elan is currently in phase II human clinical trials with their second version of this vaccine. Figure 3 clearly shows that the vaccine works to reduce, even eliminate A $\beta$  plaque formation (panel B, loss of brown color) when performed early enough.



**Figure 3. Panel A shows the hippocampal region of an untreated Alzheimer's mouse brain with many plaques (dark brown), while panel B shows the same area of an Alzheimer's mouse**

**brain that has been vaccinated (Schenk *et al*, 1999).**

Since this model provides a very strong medical benefit, with no observable animal suffering, the authors of this report support the continuance of these Alzheimer's experiments.

### **Transpharming Ethics**

Transgenic cows also offer hope to applied transgenesis as they do not suffer in producing valuable exogenous proteins in their milk. These examples make clear that it is not unacceptable to create animals with new traits; it becomes objectionable when the animals are put in circumstances that are detrimental to their health or cause suffering.

The art of making transgenic animals has by no means reached perfection.

Herman, a bull given the human gene for lactoferrin with hopes of producing milk more similar to the human variety, was the only one out of 46 calves to receive the gene. That's about a 2% success rate, not



**Figure 4. Herman the transgenic bull with five daughters. Natuurinformatie, 2004.**

very reliable. Herman's semen was used to artificially inseminate other cows to produce more altered cattle so the females could transpharm lactoferrin in their milk. A study on the welfare of the transgenic cows was conducted and found a higher frequency of anomalies, greater mortality rate in comparison with controls, and higher birth weights which often necessitated caesarean section, and increased stress to the mother cow (van Reenan and Blokhuis, 1997). The authors offer three suggestions to prevent harmful consequences of transgenesis. First, improve techniques for handling and manipulating embryos to minimize embryo loss; second, systematically evaluate animal health and welfare to minimize potential animal suffering; and lastly, use the results of health and welfare screenings to positively improve conditions and promote transgenic animal welfare. The authors of this report support the construction of transpharmers, so long as

there is no evidence of suffering, and the transpharmed product is used for the common good.

### **Oncomouse Ethics**

The oncomouse is one of the most controversial transgenic animal models in use today. By incorporating an activated oncogene sequence into the germ cells of a mouse, researchers hope to ascertain more about carcinogenesis and cancer formation (Leder and



**Figure 5. A mouse with advanced tumor formation. PETA photo gallery, 2002.**

Stewart, 1984). Obtaining a more complete understanding of what causes cancer, and being able to test anti-cancer drugs in mice represent strong medical benefits. But the ethical concern with the oncomouse is that it usually suffers in order to collect relevant information, which is in opposition to the principles of animal rights (Figure 5). It

becomes necessary to consider the moral implications of producing such a species as well as measures of reducing animal suffering (Salvi, 2001).

By strongly regulating the use of transgenic mice like the oncomouse, it is possible to find a middle-ground where creating and employing the mice is acceptable. An approach offered by David Porter (1992) is assigning ethical scores to animal experiments. Several categories are scored from 1 to 5 with 1 being most tolerable and 5 being the least animal-friendly. For example, pain likely to be involved may be rated

from none at all (1), to severe (5). Other categories are duration of distress, duration of the experiment, and number of animals per experiment. The idea is to deliberately create tension between the two opposing positions (Porter, 1992). Once a system has been set up to regulate experimentation, methods can be sought that minimize animal suffering. These may include painkillers and sacrificing the animal before severe damage can be done. Human and animal ethics require that the oncomouse be used cautiously and sensibly in research.

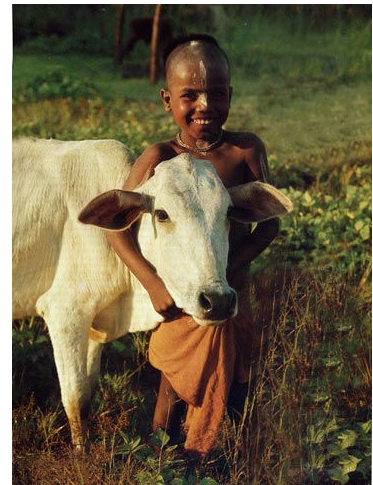
### **Religion and Transgenic Ethics**

Religions may form a base upon which morals and ethics can be founded, therefore in a discussion of transgenic animal ethics it is important to take a look at how some of the major religions of the world have regarded animals in the past, in addition to their current views on transgenesis.

Jews and Christians have traditionally opposed animal cruelty and taken the stance that animals are not considered sacred as the human soul is. Also, because they lack reason, animals may be reasonably used for human benefit. The role of the domesticated animal has special significance for Christians, for example as the shepherd tends to his flock (Curran and Koszarycz, 2004). This image aims toward love and care for all creatures. Stewardship of the animals of the earth requires responsibility in our actions towards them, but helpful interventions are welcomed as God's will to alleviate the pain and suffering caused by sin (Loma Linda University, 1997). Support for transgenic animals relies on their value for the common good.

Muslim ethics are guided by the Qur'an and hadith. The controlling concept is that of tawhid or the absolute unity of God. This principle states that whatever has not been forbidden by God is allowed within the boundaries of the Qur'an, meaning that animals may be used for the benefit of mankind (Curran and Koszarycz, 2004). Islam welcomes genetic engineering as it does all new discoveries that help ease the suffering of humanity. This justification comes from the hadith, "There should be neither harm nor reciprocating injury;" taken to mean "prevention is better than cure" (Islam Online, 2004).

Hinduism and Buddhism are two great religions of India. Both believe animals to be vital energetic beings and treat them as such. Reincarnation aids in this concern for creatures. In the Hindu faith, Kaamdhenu is the sacred cow of Gods which can fulfill all wishes and desires and is considered the mother of all cows (Curran and Koszarycz, 2004). Village life upholds the tradition of cow protection (Figure 6), not slaughtering the animal but instead taking dairy products as protein sources (Sager, 2003). This simpler way of life would reject the idea of bovine transpharmers in favor of naturally raising cattle.



**Figure 6. A Hindu child embraces a cow (Sacred Cow, 2004).**

### **Xenotransplantation Ethics**

13 people in the United States die everyday while waiting for a vital organ transplant (Carnell, 2000). With this in mind, researchers have begun



**Figure 7. One of the five cloned single knock-out piglets produced by PPL Therapeutics. AAAS, 2005.**

work on overcoming the vast rejection mechanisms inherent in xenografts (animal organs used in humans). On January 2, 2002 PPL Therapeutics announced the births of 5 cloned transgenic piglets. Each had one inactive copy of the gene  $\alpha$  1-3 galactosyl transferase which adds the sugar alpha-gal to the surface of pig cells (Butler, 2002). Later in July of 2002 PPL announced that four more piglets had been born, this time with both copies of the alpha-gal enzyme gene knocked-out, another critical step towards harvesting animal parts since this enzyme normally adds a type of sugar to the surface of pig cells viewed as foreign in humans (Pig donor 'breakthrough' claimed, 2002). Although there is promise, the success of xenotransplantation is a long way off. The main difficulty of hyper-acute rejection remains the immune system's T-cells which can affect the organ months or years after the transplant.

As xenotransplantation matures and it becomes practicable, the principal worry is that diseases present in animal organs could cross over into humans. It is already known that the influenza virus thrives in several species such as humans, pigs and birds. It has also been shown that pig retroviruses can infect human cell lines in vitro. Instead of rejecting the idea because of the possible risks, perhaps we should try to minimize the risks with respect to the benefits. One simple answer would be to use disease free-animals and keep them in sterile conditions although this would arguably violate the welfare of the animal. A compromise would be to raise the pigs normally, but screen for known viruses. After the Public Health Service issued guidelines for xenotransplantation in 2000, activists were quick to opt for the "precautionary principle" meaning to always minimize the risk regardless of the possible benefit. This is not very widely accepted as if you normally drive a car you are implicitly rejecting the principle (Carnell, 2000). In

light of the dangers of pathogens potentially crossing the species barrier, other options could be morally more acceptable than xenografts, such as using artificial organs to help reduce the strain on the organ donation market (Society, Religion and Technology Project, 2001). The welfare of the animal donors themselves cannot be forgotten in the midst of these potential medical benefits to humans either.

As discussed in Chapter-4, the Animal Welfare Act of 1966 is the main piece of legislation that defines how research animals are to be treated. The act protects “all warm-blooded species” but leaves out rats, mice, and birds, all of which are warm-blooded. This controversial omission is seen by PETA as a loophole for researchers to subject certain species to procedures and conditions that would be illegal for all other animals protected by the act. Organizations like the National Association of Biomedical Research oppose changes in legislation arguing that guidelines for use of rats, mice, and birds already exist in the National Institutes of Health plan. The ‘arbitrary’ exclusion of these species from the AWA is actually not favored by most researchers. The majority want equal protection for all lab animals including rats, mice, and birds which together account for approximately 95 percent of all laboratory animals (“Why Include Rats...”, 2002). It is a fair question to ask then why all but 5 percent of research animals are excluded from research protection?

Overall, the public perception of transgenic animals has not been very favorable, and with associations like ‘frankenfoods’ to describe edible transgenic plants, more fears over the legitimacy of modifying animal genomes are likely. Granted, animal rights and welfare activists have got a point that animals deserve respect and good treatment, but these issues can often be controlled using sensible policies. Given the strong medical

potentials of the various kinds of transgenic animals, and the fact that many do not suffer at all, and that the suffering when it does occur can be controlled, an outright ban on transgenesis is not a good idea in this author's opinion. The potential for transgenics is enormous and should not be squelched by fears and lack of information. Scientists and the public can resolve their differences with discussion, and implement rational guidelines and regulations for ensuring the safe, effective and ethically acceptable use of genetically modified organisms.

## **CHAPTER 4: TRANSGENIC LEGALITIES**

As Chapter 3 has shown, the ethics of producing transgenic animals is a very controversial topic which requires the implementation of legal policies to enforce oversight of transgenic experiments to minimize animal suffering while maximizing medical benefit. This chapter will take a closer look at the legislation behind transgenic animals. One prominent concern resides in the intellectual property rights of genetically modified animals. On the positive side, patenting transgenics offers various incentives, including stimulating additional biomedical research. On the other hand, some activists have protested the authority of the Patent and Trademark Office to grant any patents on animal life. Both sides of the issue will be presented along with several landmark court cases. The United States, Europe and Canada have reached different conclusions with regard to issuing exclusive rights for newly created life forms. Each decision reflects upon differing interpretations of local laws and past rulings on similar cases.

### **General Patentability Issues**

In granting patents to inventors, the U.S. Patent and Trademark Office requires that a submission satisfy the three requirements of novelty, utility and non-obviousness (35 U.S.C. § 101, § 102, § 103). None of the requirements demands that the invention be inanimate or non-living. Title 35 United States Code § 101 states that “Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title” (Bitlaw, 2000).

## Diamond vs. Chakrabarty

Biotechnology patent issues did not begin with animals. In 1972, microbiologist Ananda M. Chakrabarty applied for a patent application for his invention of a genetically engineered bacteria created by adding two different plasmids to the wild-type organism, each of which provided a separate pathway for breaking down components of crude oil .



**Figure 8: An opportunity for Chakrabarty's microorganisms to fulfill the requirement for utility, an oil spill at Mouillie Point, South Africa (Richardson, 2000).**

The patent usefulness requirement was met by the bacteria's potential to help in the treatment of oil spills, novelty was also met as there are no naturally occurring bacteria with the same capabilities, and finally the non-obvious requirement was clearcut (see Figure 1).

When a patent examiner initially rejected Chakrabarty's claim for the bacteria on the grounds that transgenic microorganisms were products of

nature and not patentable, the scientist appealed and

took his case to the Supreme Court where Diamond

vs. Chakrabarty became a landmark case in patent

law. The court found that the claim met all three requirements set forth under section 101

disregarding the examiner's grounds for refusal, and granted patents for the bacteria

themselves in addition to exclusive rights for the method of producing it and a carrier

material floating on water with the bacteria (Diamond v. Chakrabarty, 1980). The court

also recognized that potential hazards that could be generated by genetic research should

be met by Congress and the Executive branches and not the Judiciary; thus the only obligation of the court was to use current legislation to determine patentability. The judge’s interpretation in this case that the microorganism constituted a “manufacture” or “composition of matter” paved the way for subsequent animal patents.

The Patent and Trademark Office confirmed the decision several years later when it issued a statement in the Official Gazette: “The Patent and Trademark Office now considers nonnaturally occurring non-human multicellular organisms, including animals, to be patentable subject matter within the scope of 35 U.S.C. s. 101.” Also, the animals must be “given a new form, quality, properties or combination not present in the original article existing in nature in accordance with existing law” (Patent and Trademark Office Notice, 1987). With the floodgates opened to patenting animal life, the applications surged in along with substantial criticism directed towards the Patent Office.

### Animal Patents

The first animal patent, on the Harvard oncomouse, was awarded in 1988 just one year after the Patent Office affirmed that creatures may be protected under patent law. The oncomouse is a mouse given the human *ras* gene (see Figure 2) which predisposes it to cancer with much greater frequency than unmodified mice

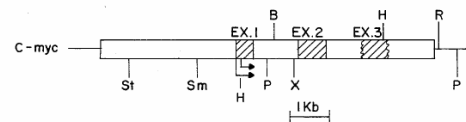


FIG 1

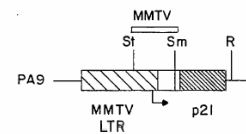


FIG 2

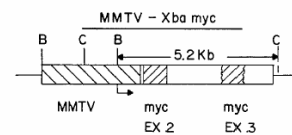


FIG 3

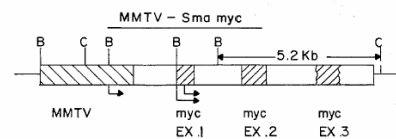


FIG 4

Figure 9: Four of the Oncomouse patent diagrams showing sequence placement within the mouse genome (Leder and Stewart, 1984).

(Anderson, 1988). More details will be discussed on the oncomouse case later. As of September 21, 2003 there have been 454 animal patents issued in the United States, of which over half (54%) are designated as disease models (American Anti-Vivisection Society, 2003). In addition to the oncomouse, some other mouse models that have been patented include an Alzheimer's mouse (Stern and Yan, 2000), a model for Kaposi's sarcoma (Lira and Yang, 2000) and an HIV mouse (incapable of viral transmission) (Jolicoeur, 1994) just to name a few. Other animals to receive patent protection now include cows, sheep, pigs, birds and fish, as well as macaques and chimpanzees. In addition to patents for animals themselves, new techniques and technologies that enable scientists to gain more power over animal genomes are being protected by patents. For example, Avigenics Incorporated has been awarded patents on a "Windowing Technology" for creating an aperture through egg shells which enables the creation of transgenic chickens, certain to be valuable in both food and drug production markets (Avigenics, 2000). As more and more transgenic applications are discovered, the number of biotechnology patents on animals is sure to rise as well.

### **Harvard and DuPont's Oncomouse**

The oncomouse was the first patented animal in the world. The famous "Harvard mouse" received patent number 4,736,866 on April 12 of 1988, and continues to be the center of the animal patent universe. This little mouse's big patent is controversial because it gives ownership of a species to a corporation for the first time. It encompasses wide claims which can lead to questions about accessibility, and it is a step forward into the uncertain future of biotechnology patent law. Claim 1 is as follows:

1. A transgenic non-human mammal all of whose germ cells and somatic cells contain a recombinant activated oncogene sequence introduced into said mammal, or an ancestor of said mammal, at an embryonic stage (Leder and Stewart, 1984).

This statement is enormously broad in that it covers any non-human mammal which has the sequence of interest and that it lays claim to all the offspring of the animal which also contain the activated oncogene. Given this fact DuPont, which now holds the patent, may assert its legal right to challenge anyone who uses the oncomouse without permission.

Control of who receives the mouse is another issue up for discussion. Distribution



**Figure 10: Taconic promotes the use of the oncomouse as a safer option than current methods of making cancer models (Taconic.com, 2005).**

of DuPont's oncomouse has been set up through Taconic, an international supplier of

Murine Pathogen-Free lab animals (see Figure 3) (Taconic,

1998). Taconic acquired the

license to distribute the mice with hopes for easier access to researchers. The company also promotes the use of the oncomouse as a promising alternative to exposing lab animals to high doses of carcinogenic compounds.

With such a revolutionary tool on hand, many researchers were concerned that DuPont's licensing of the oncomouse could slow the testing of new therapies. To overcome this problem, DuPont and the US National Institutes of Health negotiated a deal in 2000 giving non-for-profit researchers free access to the mouse with the stipulation that any commercial use must pay for the mice (Smaglik, 2000). Even so, some scientists who use the mice feel that DuPont's requirements for free licensing,

which force institutions to comply with a contract and submit an annual report, are too stringent. They argue that if required to pay the commercial licensing fee, it could well create an economic burden that will restrict research (Marshall, 2002). Unfortunately for these researchers the patent has been issued and the policies that DuPont requests must be followed. This does affect the future of



**Figure 11: Exclusively licensed by oncomouse patent holder Harvard University to distribute the oncomouse to researchers in both not-for-profit and commercial ventures, though with differing conditions and fees for each.**

animal patents though, because broad claims like the oncomouse patent will not be granted so casually anymore; being the first allowed for wider terms than would be acceptable today. Warren Woessner cites two more recent cases where the claims are limited to mice and to single genes: a mouse that develops an enlarged prostate gland (Pat. No. 5,175,383) and a mouse with depleted mature T-cells (Pat. No. 5,175,384) (Woessner, 1999). In another argument against the extensive scope of the patent, Richard Stallman calls the coverage of any mammalian species “arbitrary boundaries that extend far beyond what was invented by producing this strain” (Stallman, 2002). In that author’s opinion, patents should be issued on more narrow terms than was done in the oncomouse case so that patent holders are not given unreasonable power, to promote competition between techniques and technologies, and to reward creativity as was intended by the Constitution.

Europe and Canada have had tribulations of their own over patent protection of the Harvard oncomouse. In 1990, European Patent Office (EPO) examiners initially

refused Harvard's application for the oncomouse because: patents on plants and animals are forbidden by the European Patent Convention, the discovery had not been shown to be reproducible and that ethical questions regarding transgenic animals should not be overseen by patent law (Dickman, 1990). The appeals board later rejected these three grounds and suggested that the examiners scrutinize the issues of ethics and morality. DuPont argued for the patent because of the benefits that the European biotechnology market could reap from the incentives. Until the passage of the European oncomouse patent in 1992, DuPont protected itself with licensing agreements. A marked difference in the policies of US and European patent law is that the EPO has twice restricted the oncomouse patent, first in 2001 from covering all mammals to only rodents, and later in 2004 adding that mice specifically were the rodent being covered (Cyranoski, 2004). Both modifications to the patent came after objections against the existing patent were filed and examined.

In contrast to both the US and Europe, Canada completely rejected the patent for the oncomouse in 2002. The Canadian Supreme court said in its ruling that "A higher life form is not patentable because it is not a new 'manufacture' or 'composition of matter'" (Check, 2002). Important consequences to consider about this decision are the future of Canadian life-sciences research and biotechnology. One biotechnology association, BIOTECCanada, says the ruling will discourage researchers from creating better research models, especially transgenic animals with their benefits to science.

## **Negatives for Patenting Animals**

One grievance against the Patent and Trademark Office is for allowing that animals *are* patentable before ever examining whether they *should* be patentable (Edwards, 2001). In response, many argue that morality is not one of the three conditions that warrant patent protection; the issues of ethics and morality are not suited to the Patent and Trademark Office and should instead be taken up instead by Congress (Walter, 1998). As this hasn't yet been debated, animals are freely patentable as long as they fulfill the requirements of utility, novelty and non-obviousness.

Religions the world over object to the patenting of life, many taking it to be a devaluation of the individual by placing a price tag on it. Although many religious leaders see problems in making wealthier the companies who dump millions into research with hopes of wonder drugs and cancer cures, they less often perceive the benefits to human health. Then there is the general problem with how well humans have typically behaved when a new technology comes along that offers its users the advantage over those without it.

Another worry is that all transgenic farm animal patents will be held by a small number of corporations which will drive the family farm out of business. This dispute, although outwardly agreeable, does not stand up to examination. In reality, without patents, the owners of transgenic animals would only license their animals to those capable of paying: large companies (Walter, 1998). This latter case would result in real problems for the family farms as commercialized farmers would be at a significant advantage. It must also be noted here that currently, the main interest in transgenic animals is for medical and scientific research, not farming which further strengthens the

case for patentability. For the arguments against patenting animals, less are actually opposed to patents than are opposed to the technology itself, something that may not change anytime soon.

Then there is the slippery-slope argument again. Could patenting animals lead to human patents? It is already permissible to patent “purified” genes and animals with integrated human genes. The Patent Office considers non-human multicellular living organisms to be patentable. However, the PTO has not issued a statement defining the number of human genes required to make up a human-animal chimera (Edwards, 2001). Congress has not decided one way or the other that animals are patentable, only courts have ruled that existing laws do not forbid it.

### **Positives for Patenting Animals**

The process of creating a good disease model in a transgenic animal is no easy feat. It requires years of development in the lab, talented scientists, and money. The labs and scientists often find the funds they need from large corporations with the means to supply financial support both upfront and downstream for a project. Investments are inherently risky and the success of an idea is not always a simple path from point A to point B. Recall the complications of xenotransplantation, how only a few of the required steps to overcome hyperacute rejection have been achieved, and many challenges still stand in the way. With money flowing into Universities from industrial giants like DuPont, which provided \$6 million to Harvard University’s Philip Leder and Timothy Stewart (Blaug, 2004), the best case scenario would be a new invention with a patent capable of generating revenue. A further stimulus for allowing the patenting of animals

is that this return has the potential to be reinvested and thus help biotechnology grow even more. Without patent protection, these economic incentives would be nonexistent and biotechnology as a whole would suffer. With so much potential to help human health and treat disease, it is no longer a question of whether to pursue this type of animal research but how we will go about it.

Another aspect of patents is that of visibility of new technologies. More attention means less secrecy, and with any luck may lead to increased collaboration which would stimulate the field to an even greater extent (Walter, 1998). The public would also benefit from this situation as they would be better equipped to enter into the discussion of ethics and laws over transgenic animals, instead of being kept in the dark in the ever increasing pace of science. Against the possible pitfalls of patents granted to transgenic animals, the benefits are inescapably more promising. Current law may need a little reworking to be more fitting for biological patents, but what it comes down to is allowing the sciences to flourish through incentive programs and industry. Regarding the authors of this IQP, similar to our conclusions on transgenic ethics, we find that the benefits of patents covering transgenic animals outweigh the possible risks posed. Also, the risks should not be dismissed but rather incorporated into patent law just as the European Patent Office has taken objections towards the broadness of the oncomouse patent and reformed it to be more specific and reasonable.

## CONCLUSION

This report has explored the many facets of transgenic animals, from how they are made in the lab, and what uses we have found for them, to some of the ethical and legal issues surrounding their use. In Chapter 1, we looked at the different techniques that researchers have successfully used to integrate foreign genetic material into a new host. These core technologies have formed a base from which advances in the field of biotechnology have sprung. It must also be noted that the trial and error period involved in the early technology developmental stages has been the topic of intense debate which should lessen as the science becomes more efficient.

The various uses of genetically engineered animals were discussed in Chapter 2. Disease models such as the AIDS mouse and the Alzheimer's mouse have been hugely influential in the body of support for transgenic animals as they exhibit the most important of potential human benefits: aiding our understanding and treating life threatening illness. The use of animals for testing treatments is necessary because of the ethical gulf which prevents us from performing initial tests on humans. Transpharmers represent another means of improving the human condition by producing human proteins in their milk, blood or eggs. The resultant protein can then be purified and used to treat medical needs. Then there is the possibility of spawning animals with organs capable of transplantation into humans, xenotransplanters. With the great need for hearts, kidneys, livers and pancreases, a market for xenografts is already in place. All that remains now is the daunting task of overcoming the myriad of hyper-acute rejection mechanisms. The final category is that of transgenic food sources. Animals that can grow faster on the

same amount or less feed than wild type animals are a promising way to help feed the world's growing population.

Chapter 3 focused on the ethics of making and using transgenic animals, with pros and cons for each category. The most obvious benefit to humans is from continued research using disease models, but dilemmas ensue when people claim that animal suffering, "playing God," and destroying the animal's integrity are unethical in the use of transgenics. Not all disease models suffer, especially those transgenic animals engineered to mimic only a small aspect of a human disease. Alzheimer's mouse is such an example, only displaying plaque formation in the brain, strong medical benefit to serve as a model for blocking plaque formation, with little or no animal suffering. Animals modified for food sources are less beneficial towards humans and thus animal suffering in these cases does not represent an acceptable balance between the cost (in terms of animal suffering) and the benefits for humans. Not all animals suffer as shown by the Alzheimer's mouse case, but for those that do, like the Oncomouse, pain can be partially controlled to a certain extent with painkillers and early sacrifice before tumors get out of hand. Worries over transmission of animal viruses during xenografts are numerous but again, by testing and obtaining healthy animals and keeping them in clean conditions, these risks can be kept to a minimum. Ethics can become a major hurdle in biotechnology but with proper regulation, the worries posed by activists and others can be addressed.

Finally, in Chapter 4, patents and laws concerning genetically modified animals were introduced. Protection of laboratory animals has been regulated by the Animal Welfare Act, but activists have complained that by not covering mice, rats and birds, the

Act is basically useless because 95 percent of lab animals consist of these warm-blooded species. Patent law entered the biotech market in 1987 when it issued a statement claiming its authority to grant patents on transgenic animals. The famous oncomouse case ensued which was widely debated, and even rejected in Europe and Canada before receiving patent protection in the former market. Arguments that patents for creatures are not moral, hurt family farmers and could lead to patenting humans do not stand up to reason. The fact is, patents provide a means of compensation for the companies that invest millions of dollars in research which in turn stimulates further research and eventually better treatments. The benefits of patenting transgenic animals thus outweigh the risks.

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