

IQP-43-DSA-5263
IQP-43-DSA-2430
IQP-43-DSA-0687

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

By:

Matthew Doherty

Ntohmchukwu Izuchi

Tyson Nason

CDR Deadline: August 22, 2007

APPROVED:

Prof. David S. Adams, Ph.D.
WPI Project Advisor

ABSTRACT

This IQP examines the methods for creating transgenic animals, their medical benefits, and the result of this technology on society. Different categories of transgenic animals are described, along with the bioethics that surround their creation. Next we discussed the legal guidelines and patent laws for transgenesis, including examples of groundbreaking Supreme Court cases. We conclude that with the exception of mammalian growth factor experiments, most other transgenic experiments should be continued, with strong oversight.

TABLE OF CONTENTS

Signature Page	1
Abstract	2
Table of Contents	3
Project Objective	4
Chapter-1: Transgenic Animal Technology	5
Chapter-2: Transgenic Examples	15
Chapter-3: Transgenic Ethics	25
Chapter-4: Transgenic Legalities	39
Conclusions	55
Bibliography	56

PROJECT OBJECTIVE

The purpose of this project was to examine and research the effects of transgenic technology on society, and to discuss the legal and ethical issues that surround this topic. This report should clearly describe what a transgenic animal is, how they are created, the different categories of animals created, the bioethics behind experiments, and finally the legal guidelines and patent laws that pertain to specific animals. Technology is constantly growing, and so is our knowledge of certain diseases and potential cures. Transgenic technology can help add to that knowledge; the experiments have both positives and negatives both ethically and morally, making it a fascinating yet controversial topic. The information herein should provide the reader with a background to make an educated opinion on the topic of transgenic animals, to determine whether or not to support this technology.

Chapter 1: TRANSGENIC ANIMAL TECHNOLOGY

Transgenic animals have been engineered to contain foreign DNA in their cells. The aim of producing a transgenic animal is to add genetic material into an organism's genome to generate new traits. These animals are created for the purpose of manufacturing life saving pharmaceuticals in their milk, to serve as disease models for mimicking certain human disorders, to provide organ donors for transplantations, or to serve as food. The purpose of this chapter is to describe how such animals are created and screened.

DNA

All known living organisms develop and function from DNA, which is an extremely long polymer that is the main component of chromosomes and is the material that transfers genetic characteristics in all life forms (except the RNA viruses). DNA embodies two monomer units called nucleotides which are found in four different structures:

adenine and guanine which are purine bases, and thymine and cytosine which are pyrimidine bases. Though there are two different base categories, adenine from the purines pairs with thymine from the pyrimidines, and guanine from the purines pairs with cytosine from the pyrimidines, giving DNA a paired structure called a double helix (Figure 1). The DNA is compacted with proteins, including histones, to make chromosomes. (DNA, 2005)

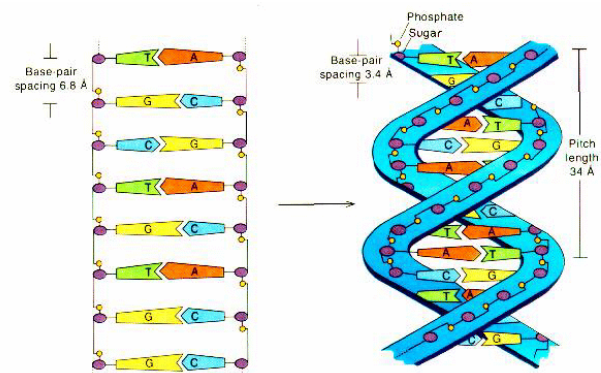


Figure-1: The Structure of DNA.

The four kinds of nucleotides are represented by blue, green, orange, and yellow colors in the center of the diagrams (Hatton, 1995).

Genes are the hereditary factor in DNA because they represent distinct locations on the DNA with a specific array of nucleotides that allow that DNA segment to produce proteins. *Exons* are the regions of a gene that contain the code for directly producing protein. Each exon codes for a specific portion of the complete protein. *Introns* are parts of a gene that are initially transcribed into the primary RNA transcript but are then removed when the exon sequences are spliced together.

RECOMBINANT DNA TECHNOLOGY

DNA genes must be manipulated when transgenic animals are created. For example, a human gene encoding a pharmaceutical enzyme is inserted into the genome of a mouse so the mouse produces that drug. DNA is altered using a genetic engineering method called recombinant DNA technology first developed in the early 1970s by Paul Berg, Herbert Boyer, and Stanley Cohen. Transgenic methodology has already been applied to diverse organisms, including mice, fish, plants, and microorganisms such as fungi and bacteria. This technology involves ligating a DNA fragment encoding the transgene into a vehicle such as a plasmid vector, which is used to amplify the DNA. A plasmid is a small circular piece of DNA found in bacteria which can replicate quickly in the cytoplasm

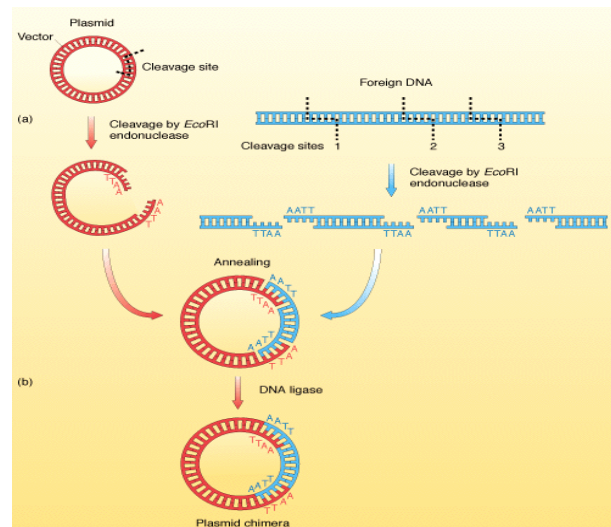


Figure-2: Recombinant DNA Technology. Specific segments of foreign DNA encoding the transgene are selected and placed in a plasmid vector. Foreign DNA that is to be inserted into a host is cut with restriction enzymes to create 'sticky ends'. By making the 'sticky ends' with the restriction enzyme allows the transgene DNA to be oriented into the vector in the right direction. The DNA insert attaches to the vector to become a recombinant DNA molecule. (Introduction to Cloning...2005)

to make a large number of copies of the cloned DNA. The cloned DNA is then purified from the bacteria, and inserted into the host (i.e. mouse) as discussed below.

To excise a piece of human DNA encoding the transgene, restriction enzymes are used which act somewhat like enzymatic scissors (and as bacterial defense against viruses) slicing through the DNA at specific recognized sequences, which allows the DNA insert to be adjusted into the vector in the proper position. Soon after the DNA is excised, a ligase enzyme that acts as a DNA glue is used to seal the two pieces of DNA together creating the recombinant DNA molecule (Introduction to Cloning and Biotechnology, 2005) (Figure-2).

METHODS FOR CREATING TRANSGENIC ANIMALS

Microinjection of DNA into the Male Pronucleus

Of all the available methods for creating a transgenic animal, pronuclear microinjection has been the most successful and most relied upon. DNA microinjection into the male pronucleus was the first the first transgenic technique successful in mammals. It was first successful in mice (Wortman, 2000), and then in other species such as rats, sheep, goats, cows, pigs, birds, chicken, and fish. Later techniques included DNA microinjection into embryonic stem (ES) cells, retrovirus-mediated transgenesis, DNA homologous recombination, and somatic cell nuclear transfer.

A pronucleus is the nucleus of the sperm cell or egg cell before they fuse together to become a fertilized zygote. In this method, scientists clone the DNA into a vector such as a plasmid then harvest newly fertilized eggs before the pronuclei fuse. Next the DNA is injected into the male pronucleus using a microsyringe. After the two pronuclei fuse to become the

nucleus of the new zygote, the cells then begin dividing. After 5 days of growth and division, the embryo is classified as a blastocyst, a hollow ball of cells. The blastocyst is implanted into a pseudopregnant foster mother prepared to receive an embryo by mating with a vasectomized male. After the birth of the pups, they are screened (discussed below) to determine whether the transgene incorporated into the genome.

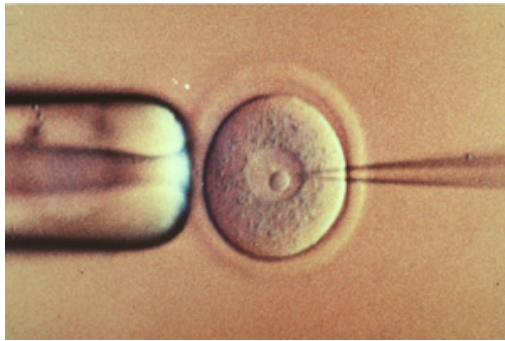


Figure-3: DNA Microinjection into the Male Pronucleus.

On the left, the microtube suction device holds the egg and the micropipette (shown on the right) inserts the transgene into the egg. (UCI, 2007)

Although pronuclear microinjection is the most popular method, it is still a random process, we can not control where the transgene integrates in the host genome, or whether it will integrate at all. Therefore, not all the offspring will have the transgene expressed. This can occur if the gene places itself in an area of the DNA that isn't usually expressed. This method can be used for a variety of species, and is one important reason it is the most popular (Genoway, 2003).

Microinjection of DNA into ES Cells

A second technique for making transgenic animals is the incorporation of the transgene into embryonic stem (ES) cells. ES cells are isolated from the inner cell mass of blastocysts. They are undifferentiated cells, so have not yet generated structures or manufactured protein characteristics of a specialized type. They can renew themselves, and can differentiate to produce almost all major specialized cell types (Stem Cell Basics, 2006).

The process begins by collecting ES cells from the blastocyst of the host (i.e. donor mice). Cells from *in vitro* fertilization are grown for 5 days to the blastocyst stage, then ES cells are taken from the inner cell mass. To help reduce differentiation of the ES cells, they are cultured using an embryonic fibroblast feeder layer that produces a leukemia inhibitory factor. The transgene is inserted into the cultured ES cells using microinjection, electroporation, chemical transfection, or viruses, then the ES cells are injected into a blastocyst. The blastocyst is implanted into a pseudopregnant host, as described before, etc.

The ability to use a variety of techniques for incorporating the transgene into ES cells is one reason this technique has recently become more popular. An efficient process for inserting DNA into ES cells is electroporation. This process uses a pulse of high voltage causing cell membranes to be penetrable which allows the introduction of new DNA. As soon as the DNA is absorbed into the cell, the DNA then repositions into the cytoplasm and incorporates itself into the cellular DNA (Taconic Transgenics 2003). The transfected ES cell lines are then evaluated for transgene insertion. In this way, only those ES cells that actively took up the DNA are reimplanted, which increases the efficiency of the process. If the transgene is present, then ES cells can be microinjected into a blastocyst (Figure-4) and the embryos implanted to make transgenic animals.

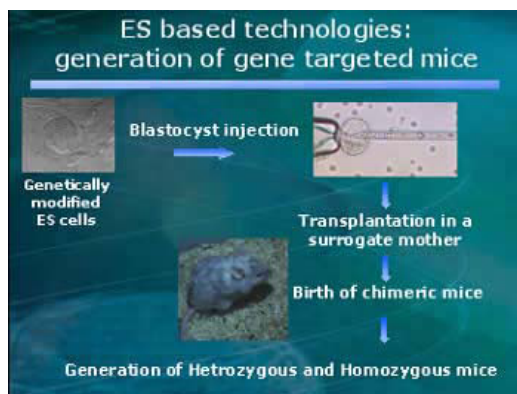


Figure-4: Summary of the ES Method for Making a Transgenic Animal. Cultured ES cells are transfected with the cloned transgene (upper left), then those cells are injected into a blastocyst (upper right). The blastocyst is implanted into a foster mother to produce transgenic pups. This figure was taken from (Genoway...2003).

Though effective, ES cell culturing is very difficult, and the survival rate of ES-injected blastocysts is low. There is no control over where the DNA integrates, or whether the implantation of the embryos into the uterus will be successful. Tests have even shown that no more than one third of the embryos will have successful implantation into the uterus (Transgenic Animals, 2003).

DNA Homologous Recombination

A strong advantage of using ES cells to create a transgenic animal is they allow the use of homologous recombination for inserting the DNA, which can control where it integrates in the host DNA. In homologous recombination or gene targeting, the DNA of the transgene attaches to a known portion of the host's chromosome, and exchanges with it. The transgene is inserted by genetic engineering within a known cloned host gene (Figure-5, middle diagram).

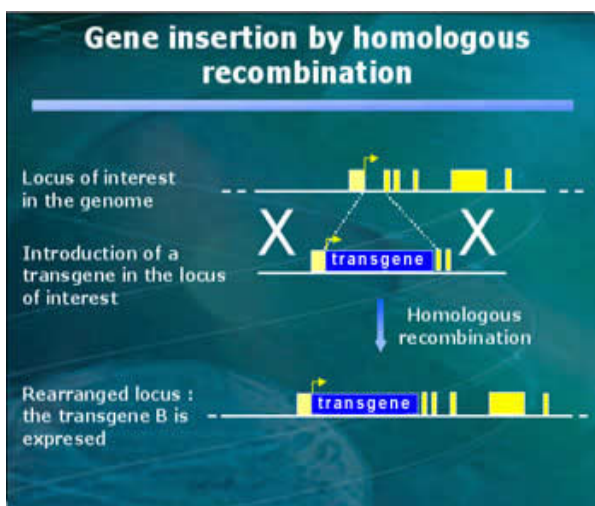


Figure-5: Summary of the Homologous Recombination Method for Making a Transgenic Animal.
The transgene (blue in the diagram) is inserted by genetic engineering within a cloned host gene (middle diagram). When that DNA is introduced into a host ES cell, the DNA flanking the transgene recombines with the host cell DNA to insert the transgene into the specific location (lower diagram). (Genoway, 2003)

Once that DNA incorporates into a host cell, the the flanking host DNA sequences recombines with their homologous sties in the host cell DNA to incorporate the transgene into that location.

This method allows for DNA to be targeted to a specific location of the genome (Bronson and Smithies, 1994).

DNA Viral Delivery

Viruses can also be used to deliver cloned transgenes into ES cells. In this method genetic engineering is used to insert the transgene in place of specific viral genes (Figure-6). Next the viral DNA is then enclosed into virions, and the virions used to infect the ES cells. Viral delivery is very effective because it improves the likeliness of transgene expression (Transgenic Animals.... 1997).

Though effective, viral delivery isn't as efficient as desired because the size of the transgene sequence that can be added to the viral genome is limited. Also viral delivery can create mosaic animals, and interference with the expression of the transgene may also occur. Another problem that occurs is the animals that are created will pass the transgene to their offspring only if the germ cells receive a copy of the transgene, which does not always happen with this method.

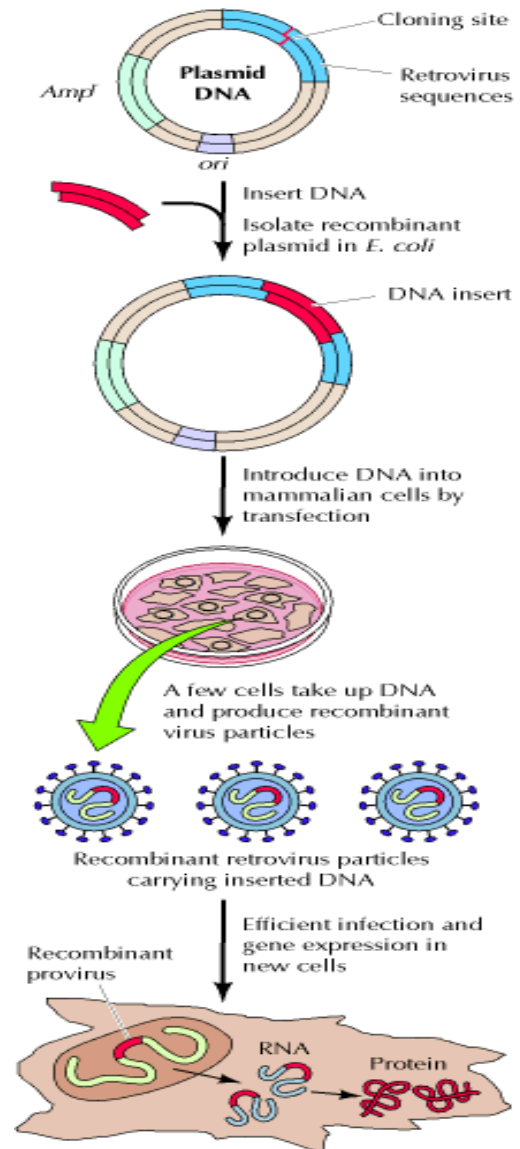


Figure-6: DNA Viral Delivery Method. Viruses can be used to deliver the transgene (Cooper et al., 2000).

Somatic Cell Nuclear Transfer

The nuclear transfer method or somatic cell nuclear transfer (SCNT) is a safer and more efficient way of creating transgenic animals in terms of large numbers of embryos being saved. During SCNT, a nucleus is taken from a somatic cell (such as a skin cell), then inserted with a transgene by microinjection (Figure-7). The nucleus is then reimplanted into an enucleated egg. When the egg develops into a blastocyst it is implanted into a foster mother. This assures the offspring to be 100% transgenic animals because the newly implanted nucleus already has the transgene in its genome.

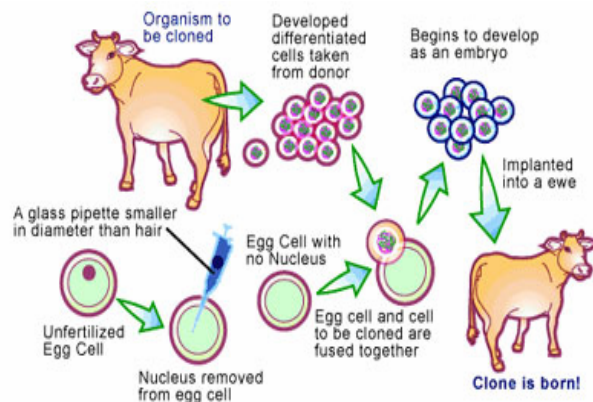


Figure-7: Summary of the SCNT Method for Making a Transgenic Animal.

Skin cells are removed from a donor animal (upper left). A nucleus extracted (center of diagram) and injected with the transgene. The nucleus is then injected into an enucleated egg (lower left) that is implanted into a surrogate mother. (Kae, 2003)

ASSAYS USED FOR SCREENING TRANSGENIC ANIMALS

SOUTHERN BLOT TEST

The Southern Blot is one way to analyze the genetic patterns within DNA. It can be used to determine the number of copies of the transgene integrated, the number of chromosomal sites the transgene was inserted into, to validate the status of the transgene, and to determine if the transgene was intact. This screening technique should be performed before the transgenic offspring have reached 6 weeks old. Selection for intensive breeding is usually dependant on

scientists breeding transgenic animals having at least 5-10 copies of an intact transgene in a single insertion site (Brinton and Lieberman, 2007).

Since transgenes usually insert in a head-to-tail position, scientists usually choose a restriction enzyme that will cut once in a transgene to release DNA fragments that are the same in size as the transgenes from the multicopy concatemers (Answers.com). These DNA fragments are then sorted by size using a process called gel electrophoresis. At this point the DNA is loaded onto a gel, such as agarose, and an electrical charge is applied to the gel, positive charge being at the bottom and the negative charge being at the top. Since DNA is slightly negative in charge it will be attracted towards the bottom of the gel. The smaller pieces of DNA are able to move quicker towards the bottom therefore the different sized pieces of DNA will separate from each other, the bigger pieces at the top and the smaller piece at the bottom. Heating or chemically treating the DNA in the gel denatures the DNA to single stranded form, freeing it to be able to hybridize with a probe DNA. The DNA in the gel is then blotted to a membrane that retains the DNA size pattern while still allowing hybridization to a probe to illuminate the transgene. The presence of a band hybridizing to the transgene probe indicates the transgene incorporated into the host DNA. (Brinton and Lieberman, 2007)

WESTERN BLOT TEST

The Western Blot test is another method of screening transgenic animals. It detects the proteins made from the transgene using antibodies directed against the transgene protein. This method is very similar to the southern blot test except protein extracts are electrophoresed and blotted to membrane. The antibody then detects the trans-protein on the blot. If a band is

present on the gel, it shows that the trans-protein is being made in the cells from which the lysate was made (Western Blot Activity, 1998).

REAL TIME (REVERSE TRANSCRIPTASE) PCR

Real Time RT-PCR is also used to screen for the expression of the transgene. This technique detects mRNA encoding the trans-protein. The tissue of choice, i.e. brain, pancreas, or liver is removed, and the mRNA in the cytoplasm is extracted. Reverse transcriptase, the process of making copies of the RNA into DNA, is carried out, then the remaining mRNA is digested with RNase. Fluorescent primers are used to magnify the mRNA that was previously converted into cDNA. An agarose gel is used to analyze the results of the Real Time PCR then the band is visualized with the aide of UV lights. This method is very useful in proving the expression of the transgene and also proves that this particular DNA sequence of the transgene is being transcribed and expressed (Hunt, 2006).

ENZYME LINKED IMMUNOABSORBENT ASSAY (ELISA)

The ELISA is also another technique used for detecting the presence of the transgenic proteins similar to the Western Blot technique, except it is more quantitative. The amount of trans-protein found in samples of blood, urine, or animal serums are measured in this technique. First, using a plastic tray with wells, the wells are coated with a particular antibody that binds to transprotein present in the added lysate. Then a detecting antibody (usually with alkaline phosphatase or horeradish peroxidase conjugated to it) is added to detect the captured trans-protein. The higher the color formation, the greater the concentration of transprotein present in the lysate (ELISA Activity, 1998).

CHAPTER-2: TRANSGENIC ANIMAL CLASSIFICATIONS AND EXAMPLES

Transgenic animals have been created to benefit mankind. This chapter will discuss background information required to participate in the transgenic ethics discussion contained in Chapter-3, and the legal discussion of Chapter-4. Transgenic animals can be classified into five major types: disease models, transpharmers, xenoplanters, food sources, and biological models.

TRANSGENIC DISEASE MODELS

Disease models are animals modified to mimic the symptoms and development of a particular human disease, so that potential treatments for that disease can be designed and tested on animals prior to humans. Since animals do not normally display the biological equivalent of certain human diseases, a human transgene that induces the disease is inserted so it can be expressed in the animal. This altering allows for pathological characteristics in the animal to be studied. Animal disease models are very useful because they allow us to screen drugs that may be detrimental to humans or have serious side effects. As soon as the therapeutic agents have been discovered and tested in animals, human cells may then be tested *in vitro*, followed by human test subjects in closely monitored clinical trials. Ethical and safety issues prohibit initial tests in humans, thus the need for transgenic animals. Examples include AIDS mouse, Onomouse, Alzheimer's Mouse, and the Parkinson's Fly.

AIDS MOUSE

In the past, chimpanzees were shown to be capable of supporting HIV replication, but no inexpensive animal developed the virus (Bunce and Hunt, 2004). In 2001 at the University of Maryland, a mouse was created to study HIV. The mouse was created by microinjecting the genome of HIV-1 into fertilized mouse eggs. The transgenic genome, which does not include the two genes that cause the virus to become infectious (Reid et al, 2001) makes the animals relatively safe to handle, while permitting the study of HIV biology. Scientists are now able to study early-onset symptoms, and to develop early diagnosis tests. Researchers are also able to track chronic conditions associated with AIDS, and test various treatments in an attempt to cure HIV (Kohn 2001). This is a more cost effective research tool than chimpanzees. The one original female mouse that possessed the modified viral gene was bred to healthy male mice to produce HIV gene-bearing offspring. The AIDS mouse was designed to display symptoms comparable to human AIDS patients such as wasting, atrophic lymphoid organs, atrophic kidneys, and early death. It was observed that during the development of AIDS mouse, skin lesions that resemble Karposi's sarcoma mimicked that seen in AIDS patients, which indicates that HIV may help contribute to that cancer (Vogel et al, 1988). The AIDS mouse provides a huge research opportunity to find treatments to prevent HIV replication, ease the symptoms of, and perhaps even cure the disease.

ALZHEIMER'S MOUSE

Alzheimer's is a neurological disease marked by the loss of cognitive ability, and is associated with the development of abnormal protein deposits in the cerebral cortex and

hippocampus. It is a neurological disease that affects the memory. Since the progression of Alzheimer's has been linked to the synthesis of β -amyloid (and its eventual aggregation to form β -amyloid senile plaques in the brain, the disease model of Alzheimer's mouse is another key example of medical advancement. Alzheimer's mice overproduce highly neurotoxic β -amyloid, as do patients with the early onset type of the disease, and the mice present the symptoms and learning impairments of Alzheimer's disease (Duff et al, 1996).

The first true Alzheimer's mouse was created in 1995 by a joint effort at Worcester Polytechnic Institute and the former Transgenic Sciences, Inc. (which became Athena Diagnostics, then Exemplar Corp, then Elan Pharmaceuticals) (Games et al, 1995). The world's first Alzheimer's vaccine came from this mouse model. The mouse model was used to create a vaccine to clear the plaques once they had formed in the mouse brain, which almost entirely prevented the creation of amyloid plaques in young mice, and reduced the amount of already damaged plaques in older mice (Schenk et al, 1999). The vaccine also restored neurological performance in the mice, and is currently in phase II human clinical trials at Elan Pharmaceuticals. Though the initial phase trials ended in 2001 when a minority of patients exhibited brain inflammation, the phase II trials of a newer Elan vaccine have exhibited no deleterious side effects.

ONCOMOUSE

This famous mouse was originally created by Harvard Medical School in Boston for Dupont, and was the first animal to ever be patented (Stewart and Leder, 1984), though the patent wasn't granted until 1988. A virus tumor promoter / myc oncogene fusion transgene replaced the normal mouse myc gene, thus the oncomouse has been engineered to develop

cancer, the most common type being lymphoma (Harvey et al, 1993). Using this animal model, cancer formation and treatment can be studied. Possible anti-tumor compounds can be tested on the animals to see if the animal has any indication of reduced carcinogenesis. Dupont has allowed researchers with the U.S. National Institute of Health to work on the mouse for non-profit research (Samglik, 2000).

PARKINSON'S FLY

Harvard Medical School in 2000 developed a *Drosophila* fly as a model for Parkinson's disease (PD). Parkinson's fly shows the characteristic loss of motion control and loss of dopamine neurons that humans with the disease show. The fly contains a mutation of the α -synuclein gene linked to PD. The fly's simpler genome serves as an excellent model for learning about PD on a genetic level (Feany and Bender, 2000), and the disease can now be monitored through its different stages. By the time the PD symptoms are visible in humans, it is estimated that 60 to 80 percent of dopamine nerve cells have already died (Vatalaro 2000). This ability to observe the disease in its earliest stages could eventually lead to a cure.

TRANSPHARMERS

Transpharmers are larger, typically farm animals, modified to express a particular protein or group of proteins in their milk. The produced proteins can either be directly consumed in the milk (if they remain active throughout human digestion), or can be purified from the milk for administration. First begun on mice in the late 80's, the process has evolved to scientists utilizing farm animals such as goats and cows for their high milk production. Scientists first

tried this production process using animal blood, but since then the trend has shifted to milk, because it is easier to acquire the drug from milk, and proteins expressed in milk are less likely to affect the animal's physiology than in the blood.

To make sure the transpharmed protein is functional, tests are usually done with mice first before moving onto larger animals, mainly due to the fact that larger animals are more difficult for performing *in vitro* fertilization and surrogate motherhood. The current transgenic success rate is low and very expensive, but the transgenic procedure is promising (Houdebine 1994). Rodents, although useful in the lab because of low cost, do not produce enough milk to be useful for anything other than models. Larger animals like cows, goats, and even sheep are the targets for large-scale transpharming.

In 1991, the first trans-farmer goats were produced at the Tufts University School of Veterinary Medicine. Their purpose was to produce tissue plasminogen activator, a clot-dissolving drug for use in heart attacks and thrombolytic type strokes (Ebert, 1994). In 1997, a team created six transgenic lambs for Roslin Institute, which produced a human blood clotting factor in their milk (Schneike et al, 1997). In 1999 another variation of transpharmer goats was produced using the then new process of somatic cell nuclear transfer (SCNT). The goats not only over-expressed the intended marker gene, but also their offspring acquired the transgene (Baguisi et al, 1999).

Gen Pharm International in California developed the first transgenic cow, dubbed "Herman", and his first transgenic offspring were bred at Gen Pharmaceutical's lab in the Netherlands (Krimpenfort, 1991). Herman produced the human protein lactoferrin under a β -casein promoter, and his female offspring also produced it in their milk (Biotech Notes, 1994), but it was produced at such a low rate that it was never commercialized.

Although this lactoferrin attempt was never brought to market, it was a huge step for science. Now that this has been proven it can be done, research will focus on how to improve it, and someday this procedure could be used to make many medications.

XENOTRANSPLANTERS

Xenotransplanters or xenoplanters are large animals engineered to not express foreign antigens that normally prevent the transplantation of their organs into humans. Pigs are the favored animals for transplant research since their physiological makeup closely resembles humans, and they are more accessible and less expensive than monkeys. This technology is very useful because there is a large backlog of patients needing histocompatible organ transplants, and the body normally rejects animal organs.

When the idea of using animal organs to replace human organs first came about, primates seemed to be the most logical choice. But due to the small size of their organs and the possibility of primate extinction, scientists eventually turned to pigs as the next logical animal for transplants. A pig's physiology is surprisingly similar to a humans (Pearson, 2003). Pig's organs, however, are not a clean transplant. They have molecules on the organ surface that humans do not have, and this variation leads to organ rejection. Four pigs, in 2002, whose sugar transferase genes had been deleted were produced in the Department of Animal Science of the University of Missouri (Lai et al, 2002). The pig's organs were specifically targeted to lack the genes that a baboon's body would reject and a successful transfer into baboons was observed (Logon and Sharma, 1999). Human trials have not begun with xeno-organs because people fear that a virus from the animal organ (zoonotic infection) may be transferred. In England, however, scientists believe clinical trials could begin on humans as early as 2008 (Fabregas, 2006).

TRANSGENIC FOOD SOURCES

These are animals altered to grow larger or quicker to produce a greater amount of food in a shorter amount of time using fewer growth resources than animals of the same species.

Examples of transgenic food sources are Superpig and Superfish.

SUPERPIG

When animals are bred for slaughter it would be better if they grew faster and consumed less food. Since pigs are used very efficiently and are needed in high quantity for ham, bacon, and pork, a pig is a good choice as an animal to grow faster and utilize fewer resources to do so. Transgenic Superpigs were made by microinjection of the transgene for a growth hormone, whether porcine, ovine, bovine, or even rat (all those growth hormones are active in pigs) (Pursel et al, 1997). The famous Beltsville pig was made in Beltsville, Maryland under the supervision of the US Department of Agriculture. These pigs expressed human or bovine growth hormone, and higher levels of insulin-like growth factor (Miller et al, 1989). Animal rights groups also correctly noted that the pig was impotent and had ulcers, heart problems, lameness, kidney disease, and pneumonia (Animal Aid, 2006). Creating this animal in a larger size than the joints were intended did exactly what obesity does to humans, made the pig unhealthy. This topic will be discussed further in Chapter-3 on Transgenic Ethics.

SUPERFISH

Scientists tried to genetically engineer a tilapia to grow faster, but although the fish grew faster it did not grow larger than a regular tilapia (Martinez et al, 1996). Salmon have also been engineered to grow faster (Devlin et al, 2001). The transgenic salmon produce growth hormone

continuously, instead of turning it off depending on the season (Biotechnology Industry, 2006). It took generations of subsequent selective breeding, but the transgenic salmon grew quickly once this was achieved (Devlin et al, 2001). Because of a concern over the escape of these fish into the environment, a very tight control is kept over transgenic fish farms (Stokstad, 2002). To combat the fear that the fish will get into the wild and out compete the natural fish for food, one company raised the salmon on fish pellets so they would not know how to fend for themselves if they escaped (Biotechnology Industry, 2006). These fish have been the most successful attempt at a transgenic food source to date.

Transgenic Biological Models

Transgenic Biological Models are transgenic animals created with the purpose of increasing our knowledge of biology, or understanding the dynamics of natural process. Successes with this type of animal include: ANDi the first transgenic monkey, Smart mouse, and Youth mouse. These transgenic animals helped further human knowledge of the genome, and with this understanding lies the keys to curing a variety of illnesses and diseases that have plagued mankind through history. Transgenic biological models are the cutting edge of transgenic research.

ANDi

ANDi was the world's first transgenic primate. The name ANDi stands for "inserted DNA" spelled backwards (Chan et al, 2001). Scientists engineered a virus that was then used to insert the harmless gene for green fluorescence protein (GFP) into ANDi's rhesus genome. ANDi produced the mRNA for the protein, although he did not actually glow under UV light.

Two of the other monkeys in the project, stillborn twins, glowed under UV light in their eyes and fingernails. The GFP gene was chosen for two reasons: it would have very little effect on the monkey, and it would be very easy to detect whether the transgene had been transmitted properly (Ackerman, 2006). ANDi is the only monkey of 40 fertilized eggs to be born alive expressing the gene. ANDi proves that transgenic primates can be created, and can express a foreign gene delivered into their genome. This opens the doors for creating other primate biological models for research, and eventually this could help cure many diseases that before transgenic research were never thought possible.

SMART MOUSE

In 1999, researchers at Princeton University developed a mouse they dubbed “smart mouse” or “Doogie”. This mouse was engineered to over-express NR2B receptors in synaptic pathways. What this does is make the mice learn much faster through their lifetime, instead of slowing down as they age. The mice did better on tests designed to test learning and memory (Tang et al, 1999). To test the memory of the mice, two objects were presented to them in a cage and then they are given time to explore them. Then researchers replace one object with another, and the mice are again allowed to explore them. If the mice spend more time paying attention to the new object, it is a good sign that they remember the old one as already being explored. If the mice explore each equally, then they have probably forgotten that they already explored the old object. As the smart mice age, they do consistently better on these tests than aged controls. The fact that this gene improves the memory of mice opens the door for research in this area, and eventually could help benefit mankind and society in the next century (Harmon, 1999).

YOUTH MOUSE

Youth mouse was created in 1997 at the Department of Biochemistry, Weizmann Institute of Science, Rehovot, Israel. The mice over-express a gene originally primarily thought to be a clot dissolver. The mice are smaller in size, require fewer resources, and live about twenty percent longer than normal mice of their type (Miskin and Masos, 1997). The youth mouse will help scientists study ageing and diets, and the effects of clot dissolving on the mice. It is believed that one reason the mice lived longer is because they were less likely to have heart attacks than the mice without the transgene.

CHAPTER CONCLUSION

Other transgenic animals are in the concept stage or have been made, which are worth discussing, but this chapter was meant to outline the successes of transgenic research, and provide a base of knowledge for the ethical and legal chapters to follow. These animals are helping cure diseases and solve hunger problems around the world by allowing scientists to study them after their creation. Aid's mouse and Alzheimer's mouse for example have provided priceless information about their diseases and with their help scientists are that much closer to a cure. Without these creatures the leaps and bounds that have been accomplished in medicine and science may never have been possible. Transgenic research is an important part of science today, as long as it is done humanly.

CHAPTER-3: TRANSGENIC ETHICS

While it is true that transgenic animals hold exciting potential for enormous contributions to science and medicine, uncertainty reigns as to whether engineering new life and modifying existing forms is ethical or moral in modern society. Biological technicalities in genetic engineering no longer limit the capability of scientists to manipulate life forms. Knowing how to make such animals, forces us to determine what boundaries we set for this new technology. What are the advantages and benefits of creating transgenic animals? What are the concerns from the public and other sources about these engineered species? How do different religions, cultures, and backgrounds view the morality of this issue, because basic principles may be derived from past values (Curran and Koszarycz, 2004)? 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. Varying levels of animal suffering range from none at all, to life threatening. What are current practices regarding how to proceed with animal transgenesis?

Transgenic Medical Advantages

The advantages of using transgenic animals can be divided into three broad categories: medical, scientific, and food benefits. Medical advantages are seen with disease models like Alzheimer's mouse and Oncomouse. These animals teach us how diseases initiate and progress (Lemonick et al, 2001). These models are required for performing experiments not ethical in human beings. The simplicity of these animals, their relatively low cost, and their close physiology with humans, makes them ideal for transgenic research. Medical advantages are also seen in transpharming models that can produce life-saving pharmaceuticals in their milk

(D'Silva, 1998), and xenotransplanters that grow organs for human transplants (Butler, 2002). Scientific benefits are also seen in some animals engineered to over-express a specific protein or to knockout a specific protein to help uncover a newly discovered protein's function. Food benefits are seen in superfish that grow faster and larger than regular fish. This has amazing potential to help to solve the world hunger problem. Agriculturally, farm animals that can produce food or milk more efficiently while consuming less food themselves would be another benefit from this science. Disease resistance and faster production times offer even more incentive to progress transgenic research (Mepham, 1994). Transgenic animals can be used to save millions of lives by curing disease, providing more food, and allowing scientists to learn more about genetics in general. But there is a risk involved in all of this.

Transgenic Negatives

What are the concerns from the public about these engineered species? Some environmentalists believe that simply destroying the integrity of an animal genome is cause for worry (Vorstenbosch, 1993). Would these same people not take something apart to learn how it is made in order to fix it? Human beings have been reverse engineering things for as long as they have been engineering them, isn't that similar to transgenic research, only on a genetic level? Others worry that the animals will simply turn into instruments used for research, and not be considered living things. Then of course there is the argument that someday what is being used on animals will be used on humans (Schroten, 1997). Isn't that the goal however, to save human lives by an animal to human transplant, or to help a sick baby simply by giving the mother a shot?

The most widely and accepted argument against transgenic research is the objection to animal suffering. This argument has to be countered with an argument highlighting the benefits vs. the risks. However, animal rights groups will always side with the animal, and it is up to the scientists and those watching to weigh the risk for the animal vs the benefit for mankind. Mice such as AIDS mouse have helped researchers learn more about a disease that has killed more people than the bubonic plague. No matter what, there will always be people who worry about mutant animals and animal suffering, but they ignore several ways we have of minimizing their suffering. Transgenic research should not be stopped, but simply monitored very closely as the research teeters on the fine moral line.

For centuries farmers have been using selective breeding to enhance the development of larger, healthier farm animals. Modern day horses, cows, sheep and many other species are different and more specialized than their original domesticated species. Selective breeding has given us the broiler chicken, which grows to approximately 2 kg in 40 days, which is half the time it took 30 years ago. The chickens grow muscle faster than the skeletal and cardiovascular systems can support, and they end up with leg problems and heart failure (Christiansen and Sadoe, 2000). But these birds, used to feed millions of people, are bred for just that reason. Is this selective breeding so different than altering a gene of a salmon to make it grow faster? It appears that what has been happening for generations in the farming sector is now what science is refining.

SUPERPIG

Superpig, otherwise known as the Beltsville pig, is one example of how transgenic research can be harmful to the animal. Scientists introduced human growth hormone to the pig

with expectations of a faster growing animal that would produce lean meat. This goal was accomplished, however the pig suffered from arthritis, stomach lesions, gastric ulcers, and a lack of coordination and muscle weakness (D'Silva, 1998). These ailments can all be attributed to the over size of the animal. The hormone worked in creating a bigger pig with leaner meat, but the animal's suffering was too great for the benefit, and the pig was eventually euthanized. In this case it is important to note that most people did not object to the actual transgenic process, rather it was the effects on the pig that were deemed bad. Scientists have since put a *voluntary* moratorium on all growth hormone experiments involving mammals.

ALZHEIMER'S MOUSE

There have been many cases where a transgenic animal has not suffered, but has provided great insight towards the study of disease. Alzheimer mouse is one of those cases. Created, in part, by Professor Dave Adams of WPI, the mouse expresses high levels of a neurotoxic β -amyloid protein in its brain, and it progressively develops many of the symptoms of Alzheimer's disease (Games, Adams et al., 1995). This mouse has helped scientists learn about the onset of the disease first hand, and to design potential vaccines. The mouse has also been used in testing new medications for the disease which could eventually lead to a cure. For example, Elan Pharmaceuticals, a partner in the development of the mouse, has developed an Alzheimer's vaccine that may become a treatment and even help the prevention of the disease (Schenk et al, 1999). Elan is currently in Phase II of human clinical trials for this vaccine; therefore, it is hopefully well on its way into mainstream medicine. This vaccine would not have been possible without Alzheimer's mouse. The mouse does not appear to suffer by any measurable criteria from the genetic change made on it; the mouse lives in an ideal environment from the moment it

is born until the moment it dies, and it reproduces and eats normally. It does learn slower on maze tests, but this does not make the mouse suffer.

TRANSPHARMING ETHICS

As we move into the topic of transpharming, we must look back at the selective breeding that has been taking place for hundreds of years. Farm animals have been forced to mate only with the strong of the species, thus creating a much stronger and healthier animal, with fewer genetic defects. The transgenic research done on cows to insert proteins in their milk or make the cows produce a certain protein valuable for medicines has helped many. The fact that the cow does not undergo any discomfort makes this an acceptable ethical practice. The cows don't even appear to know that their milk is any different. Transpharming is still in its infancy with as little as a 2% success rate, but the benefits, if scientists can improve this, are immense. The only discomfort to the cows in transpharming is put on the mother cow during birth; the genetically engineered cows have been recorded as having higher birth weights, resulting in the need to a caesarian section during birth (Reenan and Blokhuis, 1997).

ONCOMOUSE ETHICS

One of the most discussed and argued transgenic disease models is that of Oncomouse. Oncomouse is Harvard's patented disease model made for the study of how cancer affects human tissues and organs. Breakthroughs in scientific and medical history have been achieved because of the study and examination of this small rodent. Oncomouse has allowed scientists to identify how cancer originates, and thus develop methods for potential cancer prevention.

There have been many legal battles over the concept of patenting this living animal strain, including that of Canada striking down the patent completely. Besides the legal issues which will be discussed in Chapter 4, the ethical and moral battle of whether to create transgenic animals at all is still being debated today. The problem with this cancer mouse is that the animal being studied is going through pain and suffering, the question is whether that animal suffering is worth the human benefit of potentially curing a disease that kills 1,500 Americans every day (Stobbee, 2007).

Although the suffering of animals is against everything that principles of animal rights stand for, it still seems that this experimentation is not only justifiable, but also necessary. It became obvious after Oncomouse, and all of the controversy surrounding it, that suggestions were needed to help weigh this battle of animal suffering versus human benefit. In 1992 David Porter offered an approach of assigning ethical scores to these animal experiments. This suggestion contained scores on animals ranging from 1 to 5, tolerable to not-tolerable, respectively. These ratings would take certain categories into consideration such as animal pain, stress, mental health, immobility, duration of the experiment, and the number of animals per experiment. Although the idea seemed trivial, and a scoring system seemed inappropriate for a matter as involved as moral, ethical and legal struggles, the system seemed to work and proved to be the most logical approach scientists came across at the time (Porter, 1992). As far as Oncomouse is concerned, Porter does not include the score given to that specific animal, however we do know that scientists were able to minimize oncomouse suffering using pain killers while still obtaining extremely useful medical information. After completion of the evaluation of an animal in general, scientists then devise plans to minimize those specific categories high in animal suffering. Methods included the administering of pain killers, limiting

the experimentation time, and putting the animal to sleep before advancing to the severe stages of the diseases. Animal bioethics prove to be one of the most controversial issues in America's culture, society needs the knowledge that comes from animal medical experimentation for survival but cannot forget about the well-being of the creatures surrounding us. The authors of this IQP feel that oncomouse experiments should be allowed to continue, so long as all efforts are made to reduce animal suffering with painkillers and early sacrifice.

TRANSGENIC FISH ETHICS

The purpose of developing a transgenic animal mainly for agricultural use is to increase the productivity (i.e. more meat with less food intake) of the animal, and to minimize disease and maximize nutritional benefit. This has been achieved by over-expressing human growth hormone in transgenic pigs (The Beltsville Pigs; Superpig), or with fish. Superpig indeed grew larger, but also developed severe health problems, so biologists voluntarily ceased creating transgenic mammals expressing growth hormone.

Compared with mammals, fish offer beneficial advantages for the production of transgenic animals because of the large number of eggs laid per female, and also the embryonic development usually takes place outside of the mother. The number of transgenic species is larger for fish than all other transgenic vertebrate animals combined. The problem is that transgenic fish are the most likely species to present an environmental problem if ever accidentally released into nature (Eenennaam, 2006).

A transgenic fish is defined by the technology that is used to create and transfer the DNA sequence, not the species of the donor DNA. There are very specific risks that go along with this quickly reproducing animal that need to be addressed. First is the potential release or escape of transgenic fish. This would raise problems such as interbreeding, and competition for food and

prey species. In determining the ecological risk of the transgenic fish, experts weigh the fitness of the fish. Fitness in this sense is defined as the genetic contribution by an individual's descendents to the future generations of a population (Eenennaam, 2006). In other words, the fitness contains components such as juvenile viability, age at sexual maturity, number of eggs and other characteristics. There are environmental factors that add to the threat of interbreeding such as the containment of the transgenic fish. It is said that the future of transgenic fish likely will be dependent on the development of effective containment. If the fish are successfully contained, they will pose no threat to the environment and native fish populations. Obvious strategies of containment include building facilities to isolate the fish from native populations, or using chemicals that would prove to be lethal to specific life stages of the fish such as gametes or fry (Eenennaam, 2006) that keep the fish from reproducing.

In 2003, a transgenic zebra danio fish became commercially available in most United States pet shops. This fish was a commercial item because it produces a red fluorescent protein which owners of fish thought to look nice. This fish was later called "GloFish," the FDA decided not to formally regulate GloFish based on the rationale that this tropical aquarium fish was not used in any way for food purposes, and therefore posed no threat to the food supply. The FDA underwent much criticism for this decision that was said to be a "dangerous precedent," for the regulation of transgenic animals. GloFish is currently available in pet stores across the nation, with the exception of California (Eenennaam, 2006).

Aqua Bounty is a company based in Waltham (MA) that has been trying to obtain permission to deliver transgenic salmon (that grow twice as fast as regular salmon) into our supermarkets. The FDA has repeatedly rejected their petitions, but they are persistent in their quest for acceptance (Piquepaille, 2006).



Figure 8: Comparison of the Growth of the GM Transgenic Salmon Made by Aqua Bounty and a Non-GM Salmon at the Same Age. Piquepaille, 2006.

The positive benefits for world hunger and abundance of food supply by the use of transgenic animals is far overshadowed by the potential threat of environmental disaster. In December of 2000 a nor'easter struck a fish farm in Machias Bay in Maine, this disaster released 100,000 transgenic fish into the water. The problem was that the transgenic fish that were released were much bigger and more aggressive than that of the native fish (Stokstad, 2002). Figure 9 shows how easily it would be for a disaster such as a nor'easter to move transgenic fish from containment into the open environment. The goal is to ultimately produce 100% sterile fish that will be able to survive in the environment but can not breed, so will not need to be monitored. This could potentially produce ample food supply for over-populated countries and also minimize the over-fishing in certain areas. It is clear to see the obvious benefits of transgenic fish, the authors of this IQP feel that scientists and genetic engineers should continue with the research of genetically modified fish, as long as the fish are safely protected and contained. Based on the nature of their reproduction, even a single transgenic fish escaping could potentially mean the contamination of their whole native environment. Research should continue, but fish farms must be a little safer than the one shown below.



Figure 9: A Transgenic Fish Containment Area. This is used to show the easy possibility of the transgenic fish escaping into the environment in the event of a natural disaster (Procean.com).

RELIGIOUS BIOETHICS

Throughout history, some of the most renowned and respected theologians and thinkers had little regard for animals. German philosopher Immanuel Kant in the mid 19th century said that “As far as animals are concerned we have no direct duties...animals are not self-conscious...they are merely a means to an end. That end is man” (Curran and Koszarycz, 2004). Famous thinker Descartes was a big advocator for the superiority of humans over animals or “lower life forms” as he worded it. Descartes dismissed animals as merely machines; he was a staunch promoter of animal experimentation.

Richard Wade, a theologian from the Aquinas campus of Australian Catholic University in Victoria recently summed up the Christian ethic on animals in the following way: “avoid cruelty to animals and treat them with kindness; animal lives are not considered sacred and hence they have no significant right to life; as they lack reason, animals may be reasonably used for human benefit; food, companionship, transport, work, recreation and so on” (Wade, 2004). As some Christians may not agree with this quote, a generalization of their faith, and this ethical standpoint on the issue of animal experimentation, it gives one an understanding on how Christian thought about this subject may be interpreted. The animal has a special significance to Christians, as the shepherd would tend to his flock. It is said that helpful interventions are welcomed as God’s will to alleviate the pain and suffering caused by sin. Support for these

transgenic animals relies on our value for common good (Loma Linda University, 1997). St. Thomas Aquinas opposed animal cruelty immensely; he stated that if we are cruel to animals or slaves, karma will return the cruelty back to the human race. The current Roman Catholic Catechism states nothing of significance in relation to animal welfare, however does state the purpose of animals as a whole; “Non-human animals, like plants and inanimate things, are by nature destined for the common good of the past, present and the future community”(Curran and Koszarycz, 2004).

Most of the Judaic and Christian beliefs and thoughts that people have today stem from the Old Testament Genesis 1:1-2:4 where God bestowed human dominion over animals, and contradicts the thoughts and feelings of these previous theologians. Throughout the Bible we see animals share in glorification with humans, such as fulfilling God’s will as in the story of Jonah and the whale. Other examples in the Catholic Bible are in Isaiah 11:6-8 it states that “all life is sacred”, and in Ecclesiastes 3: 18-20 states that humans and animals all draw the same breath.

The Muslim religion is one of the most represented in the world; they are guided by the Qur’an and also ethically guided by the Hadith, the narration about the life of the prophet. Islamic religion welcomes genetic engineering and transgenic animal studies just as it welcomes all new discoveries to ease human suffering. Although many people think that the Muslim religion is inflexible and scripted, there is much openness as well. Animal sacrifice is that of the Muslim culture and tradition, although many other religions react very strongly against it, such as Jainism and Buddhism (Curran and Koszarycz, 2004).

Hinduism is one of the strongest believers in the sanctity of animals. Hindus are said to see divinity in all living creatures, and therefore have an important place in Hindu Dharma. Many of the Hindu Gods have specific animals that are associated with them and express their

being. Examples of this would be Garuda, the bird deity with the head and wings of an eagle, he is said to be the vehicle of the Lord Vishnu. This image of Garuda is placed at the entrance of all Vishnu Temples. Another example would be the Kaamdhenu (Figure 10), which is the sacred cow of gods, who is said to fulfill desires and wishes.



Figure 10: Kaamdhenu, The Hindu Sacred Cow of Gods. This animal is said to fulfill all desires and wishes. Curran and Koszarycz, 2004.

CHAPTER CONCLUSION

In describing some of the ethical dilemmas involved with these different transgenic examples, sometimes one may miss the potential positive benefits as well. This chapter discussed many negative impacts that are caused by transgenic animals, one must also realize that at any given moment a scientific breakthrough may happen that could potentially cure a disease that kills thousands of human lives. Society's perception on the use of transgenic animals for human benefit is slowly gaining more respect and understanding, there will always be protesters and activists that think we should leave all animals alone and they are not ours to use as experiments. However, one must measure and weigh out the actual suffering versus the benefit; many religious ideals teach their students that as long as the animal does not undergo significant suffering it is acceptable to proceed with experimentation. Scientists and engineers have developed different strategies for the reducing of pain, such as pain killers and restrictions to the time period of experiments such that sacrifice occurs prior to advanced disease.

There are also different levels of ethics for different transgenic models. There are obvious cases where the pain of the animal is minimal and the potential benefit is high, which makes the ethical question an easy one, such as the case of the transgenic Alzheimer's mouse. Another case where one side outweighs the opposite would be that of the Beltsville Pig, engineered to produce human growth hormone (HGH), the pigs were expected to grow faster and produce leaner meat with far less fat. The thought of more meat per pig and helping in some of our global hunger problems was great; however the scientists didn't predict the suffering of arthritis, stomach lesions and gastric ulcers that the pigs would undergo. The result of this was that the animal suffering outweighed the potential benefit, because one may always just breed more pigs. Now comes the grey area of transgenic animal ethics, this resides in cases such as the Oncomouse and other cases where the animal suffers, but the potential benefit could be incredible. Oncomouse, the cancer model that was previously discussed and will be discussed more in detail in Chapter-4, is one of the most controversial issues in today's scientific society. The possible curing of cancer would obviously alleviate an amazing amount of societal problems (suffering and economic problems), however the pain and suffering of the mouse makes it a tricky case to argue with. The authors of this IQP believe that the Oncomouse experimentation should proceed as long as scientists take every possible precaution to alleviate the animal suffering by use of pain killers and avoiding painful levels of tumors by any means necessary.

The authors of this IQP feel that transgenic animal models are a major breakthrough in the progress of technology and science. These animals are beneficial to our lives and survival, and the purpose of the experimentation should always focus on saving human lives while minimizing the suffering of our beloved animal creatures. The focus should never be that of a

capitalistic mentality, that the exclusive rights to a transgenic animal will increase revenue or benefit a company financially.

If people did not ask these difficult ethical questions, society would not be educated in the field of transgenic experimentation, and that is what is needed to gain support. Widespread education of this subject is what is lacking, with opinions too often based on partial information. Transgenic experiments may cure a disease to save a person's life or that of their loved ones. Taking advantage of these experiments and using them for personal benefit is also wrong, so there needs to be a soundboard on which people can know what is legally acceptable. There needs to be legal monitoring to ensure the virtues of transgenic animal experimentations continue to be that of human life saving, and not corrupt money making incentives.

CHAPTER 4: LEGAL ISSUES REGARDING TRANSGENIC ANIMALS

As mentioned in the previous chapters, the topic of Transgenic Animals is one of the most influential and significant breakthroughs in scientific, technological, and medical history. Due to the means by which these animals are created, and the effects on their health in the case of disease models, it has also become one of the most controversial. With any major economic or societal change throughout the history of the world, it has always been necessary to have corresponding legal guidelines. This not only ensures safety and well being of the common person and their environment, but gives scientists and engineers an understanding of what is acceptable and what is not. One of the major concerns of patenting transgenic animals lies in the intellectual property battle of what is considered patentable. One argument is that these patents offer incentives to attempt to help or even cure illnesses, diseases and major problems that face our society, such as AIDS, Alzheimer's and starvation. Conversely, there are many social and religious activists that have a strong moral and ethical problem with the patenting of man-made and genetically altered animals, stating that they are not ours to have as "intellectual property." In this chapter both sides will be discussed in detail to better establish the justifications for the disagreement in our society. These opposite sides will be referenced with groundbreaking Supreme Court cases, also discussing the variation of interpretation between specific nations on Transgenic Animals.

General Issues Regarding Patents

Article I, Section 8 Clause 8 of the United States Constitution, recognizes the value of patents as a catalyst in developing the country. The clause not only provides the authority for the patent system, but also spells out its purpose. “The Congress shall have the power to promote the Progress of Science and useful Arts, by securing for limited Times to Authors and Inventors the exclusive Right to their respective Writings and Discoveries.” The drafters of the Constitution were saying that the purpose of U.S. patent laws is not to simply protect the rights of inventors, but to also promote the progress of technology. In this article of the Constitution, it does not mention that the object being patented must be lifeless.

The purpose of a patent was recently described as almost identical to that in the 1997 case of *Warner Jenkinson v. Hilton Davis Company*; the federal court described patent law as: “The patent law is directed to the public purposes of fostering technological progress, investment in research and development, capital formation, entrepreneurship, innovation, natural strength and international competitiveness.” (“Warner Jenkinson Co...”, 1997). As you can see, from the beginning, the purpose of patents is to have society and science as a whole benefit from the entitlements, animate or not.

Animate Patenting and Legalities

“A Patentable object is any new and useful art, machine, manufacture or composition of matter and any new and useful improvement on any art, machine, manufacture, or composition of matter” (“A Brief History of the Patent Law...”, 2003). When Thomas Jefferson wrote this in 1793, he probably did not think that over 200 years later historians would be arguing the context of the “composition of matter.” This term is somewhat ambiguous because much of

biotechnology falls under the umbrella term composition of matter, however for many years judicial doctrine would limit “composition of matter” to exclude that of living beings as patentable.

This products-of-nature doctrine throughout the mid-1900’s prevented the issuing of patents to inventors who *discovered* their new “composition of matter.” Discoveries were not inventions, which led the Patent and Trademark Organization (PTO) to strike down all that which was discovered and not invented. This doctrine acted as a pedestal for many scientists because they knew no matter how beneficial to society and science their discoveries were, they would not be able to obtain a patent, which deterred many people from coming out with their ideas. (Walter, 1997)

In 1941, the Supreme Court case of *Cuno Engineering v. Automatic Devices Corporation* faced off over an electrical heating unit. Cuno Engineering was pushing for a patent, but the Supreme Court ruled against them saying “an invention or discovery must reveal the flash of creative genius not merely the skill of calling.” This changed the way people viewed an invention, it was no longer just an invention, it could now be categorized as a discovery, but must include a flash of creative genius. After that case, applications for patents suffered a loss in 1941 since creative genius was hard to achieve, but the term patent and everything it stood for had become broader (“Cuno Engineering v...”, 1941).

It wasn’t until 1980 that the historical groundbreaking case of *Diamond v. Chakrabarty* overturned the past precedent of patenting living organisms. With the discovery of a bacterium that was capable of breaking down crude oil, Chakrabarty changed the way we look at science and technology today.

Diamond v. Chakrabarty

Contrary to popular belief, the patenting of living organisms did not start with animals, in fact it started with "a bacterium from the genus *Pseudomonas* containing therein at least two stable energy-generating plasmids. Each of these said plasmids providing a separate hydrocarbon degradative pathway" (Diamond v. Chakrabarty, 1980). In other words, Dr. Chakrabarty created a new *Pseudomonas* strain of bacteria containing two plasmid DNAs with the genes for breaking down crude oil while creating energy for the bacterium. In 1972, microbiologist and genetic engineer Ananda M. Chakrabarty (Figure-11) filed a patent application that was assigned to the General Electric Company. This specific application had 36 claims that were all associated with Chakrabarty's invention of genetically altered bacterium. The reason this invention was so potentially beneficial to society was because at that time, there were no naturally occurring bacteria capable of the property of breaking down crude oil. This would serve as a major benefactor in disasters such as oil spills.



Figure 11: Ananda M. Chakrabarty. A picture of the microbiologist famous for patenting the first ever living organism. Influential proponent in the emergence of transgenic animal patenting (Diamond v. Chakrabarty, 1980).

Chakrabarty's patent application had three specific types of claims; the first claim was for the method of producing bacteria, the second was for an inoculum that was made up of a carrier material floating on water, an example of this would be straw and the new bacteria. The third and final type of claim was to the bacteria themselves, a patent on the living organism of created bacteria, this is where he ran into trouble (Edwards, 2001).

The patent examiner accepted Chakrabarty's first two claims of the method and the inoculum of bacteria, but rejected the third claim for the patenting of "products of nature." The decision was based on the grounds of micro-organisms are products of nature, not ours to patent, living beings are not patentable subject matter under 35 U.S.C. § 101. Chakrabarty was not surprised by the rejection of his patent on a living organism, the past precedent set by the United States Court of Customs and Patent Appeals (C.C.P.A.) on the case of *In re Merat* on the patenting of a dwarf chicken in 1975 was rejected under the same pretenses, 35 U.S.C § 101. The courts said that the dwarf chicken was that of selective breeding and not manufacture or composition of matter (Edwards, 2001).

Chakrabarty was ready for the rejection of his patent, and was also prepared to fight for it. He immediately appealed the rejection to the Patent Office Board of Appeals (POBA), where he was ready to use past precedent of the Plant Act of 1930. The Plant Act on 1930 extended patent protection to certain asexually reproduced plants, thus giving patent rights to living organisms. However once again Chakrabarty was struck down, the POBA stated that 35 U.S.C. § 101 was not originally intended to cover living organisms such as these laboratory created micro-bacteria. However the Plant Act of 1930 was not the only reference point that Chakrabarty had to his advantage, he needed something more recent than a patent that was accepted in 1930, it could have been said to be outdated by 50 years. In 1977 *In re Bergy* was taken to the C.C.P.A.

and a patent was granted to Malcolm E. Bergy for a micro-organism that was used in the creation of an antibiotic. The C.C.P.A. stated in the case of *Malcolm Bergy v. Lutrelle Parker* (The Acting Commissioner of Patents and Trademarks) that “ the fact that microorganisms are alive is without legal significance” (Diamond v. Chakrabarty, 1980). This was a major resource for Chakrabarty to use, however even after citing the pertinent rationale of *In re Bergy*, the C.C.P.A. still reversed their past precedent and again rejected the patent.

Chakrabarty still did not give up; he applied first for a remand, which is when an appellate court sends a case back to the trial court or lower appellate court for action (Dictionary.com, 2007). After this was granted he then applied for a *writ of certiorari*, which is when a superior court requires a lower court to submit the full record of a case for review (Dictionary.com, 2007). This was Chakrabarty’s last chance; he had appealed his patent application all the way up to the United States Supreme Court. One thing that was not in Chakrabarty’s favor was his resource of the past rulings. *In re Bergy* was dismissed as moot in the Supreme Court case, meaning no further legal proceedings with regard to that case may be permitted (Edwards, 2001). During the case much precedence was discussed, many quotes were interpreted including that of Thomas Jefferson and even Shakespeare (Diamond v. Chakrabarty, 1980). In the end the Supreme Court affirmed the case, giving Chakrabarty patent rights to the method, the inoculum, and also the patent on the micro bacteria itself. This opened the doors for animate patenting and genetic altering as we know it today.

Seven years after the landmark case of *Diamond v. Chakrabarty* on April 21, 1987, The Patent and Trademark Office (PTO) revised their past views on what is to be considered patentable subject matter. “The Patent and Trademark Office now considers non-naturally occurring non-human multicellular living organisms, including animals, to be patentable subject

matter within the scope of 35 U.S.C. 101” (Patent and Trademark Office Notice, 1987). The gates are now open for animal patents; this is the first time in history of patent law that we have seen the approval from the PTO to patent animals. From that day on experimentation and patent applications drastically increased, and so did the criticism and protests.

Events Leading to the First Animal Patent

On April 3, 1987 the Patent Office Board of Appeals refused the case of *Ex parte Allen*, which dealt with an attempt to patent the process of creating more edible oysters by putting the shellfish under pressure. The board struck this patent down for reasons that it was too obvious, and also said the mere fact that “a multicellular animal was involved was not a bar to patentability” (Woessner, 1999).

Days after the *Allen* decision, on April 21, 1987, the aforementioned statement was released by the PTO stating that they would accept “nonnaturally occurring nonhuman multicellular living organisms, including animals.” This created quite a stir, the *Allen* case was rejected partly on the basis that it was a multicellular organism in question, then days later the PTO contradicts itself. However the PTO went on to also state that a patentable animal must be “given new form, quality, properties or a combination not present in the original article existing in nature in accordance with existing law (Woessner, 1999). Although the *Allen* case was rejected, it still stood as the last big step before a patent on an animal was made. The PTO made it clear to the nation that *Allen* was not struck down because he was trying to patent a multicellular animal, he was rejected because of its obviousness, and the fact that his oyster was not genetically altered or given new form. It didn’t even take a year until the first patent on an

animal was successfully affirmed; the first animal patent was on April 12, 1988 for the Harvard Oncomouse.

Harvard and DuPont's Oncomouse

On April 12, 1988 the United States Patent and Trademark Office granted Patent # 4,736,866 to Harvard University for property rights over the Oncomouse and other transgenic mice and mammals. The patent was awarded to Harvard University geneticist Philip Leder (Figure-2) and Timothy Stewart of the

University of California, San Francisco. On January 19, 2000, DuPont (Figure-3) made an agreement with the National Institute of Health and Harvard University for the exclusive license of Oncomouse in exchange for providing funding to Harvard University.

The original Oncomouse patent signed in 1988 also came with much controversy and debate. This was the first time in history that an institution had gained property rights on a living breathing animal. The patent included a wide claim about the accessibility of the mouse. The claim is: (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. (Woessner, 1999) This claim had two main components; first it covered all mammals and not just mice, and second it addressed the ancestry of the mouse, which DuPont has license to, and is able to sell to research institutions. DuPont later set up distribution through a company called Taconic, a company founded in 1952 that has grown from



Figure 12: World famous geneticist Philip Leder of the University of California. Most commonly associated with Harvard University for the first ever animal patent on Oncomouse, pictured above (Stern, 2000).

a family-owned business to one of the largest laboratory rodent providers in the world (“A Tradition of Quality”, 2007). Taconic took advantage of this opportunity to work with DuPont in hope that it may elevate the company to a new level of access to researchers.

In Chapter-3 we discussed the ethics and moral issues regarding cases like Oncomouse and Superpig, however in this particular case more than just ethical questions were raised; the United States patent system was questioned with the emergence of Oncomouse. Two key issues were raised in regards to this rodent. One question raised was how moral implications should be addressed in court with respect to animal suffering. Another detailed question regarding the patent system was should patents be granted for animals, particularly high-order animals such as mammals, even if they do not meet patentability criteria i.e. novelty, nonobviousness, and utility (“The Case of Oncomouse”, 2006).

In Indiana University’s Carrie Walter’s paper on Current Patent Practice regarding Oncomouse she discusses the criteria of what makes a patent, and how Oncomouse fulfils them. In today’s patent law, to obtain such entitlement one’s invention must pass three requirements; the invention must satisfy the criteria of novelty, utility, and nonobviousness. (Walter, 1997) Because animals and genetic sequences are created naturally, many argue that it is not possible for living matter to be novel. However Walter argues that many people fail to recognize the ways in which biotechnology alters naturally existing organisms so they differ dramatically from naturally created organisms, thus being novel. As touched upon earlier in the case of *Diamond v. Chakrabarty* it is precedent that “nonnaturally” occurring organisms that have been man-altered satisfy this novelty requirement.

The next patent requirement is utility requirement, which pertains to the usefulness of the subject matter. It is obvious how a transgenic animal may be useful in the field of medicine or

science to develop disease models, pharmaceuticals, and improve food products. However, the standard of usefulness is raised in the case of biotechnology considering the fact that many transgenic animals can be said to be useful, but in reality may be unrealistic. The guidelines now require the PTO examiner to clearly address why an invention may be rejected for lack of utility.

The last requirement of a patent is the nonobvious condition of the patentable subject matter. However in the case of biotechnology, the nonobvious requirement can get confusing. In 1995 an amendment was made to 35 U.S.C. § 103 which revamped the nonobvious requirement in order to include biotechnology. The amendment added the fact that in order to ascertain the obviousness of an invention, the invention must be viewed in light of other inventions in the prior art (Walter, 1997).

As one may infer, Oncomouse raised quite a stir in American Society. It was awarded “Product of the Year” by *Fortune* Magazine. And as soon as DuPont got their entitlement rights they placed a full page ad in *Science* Magazine announcing the arrival of a “potentially disruptive and radical” new research tool (Murray, 2006).

However the United States was not the only nation to have moral and ethical battles with this animal patent, the European Patent Office (EPO) studied the Oncomouse at length and did not resolve its decision until 2004. The EPO applied the standards of the European Patent Convention which contained two main relevant revisions; Article 53(a) excluded patents for inventions “the publication or exploitation of which would be contrary to *ordre public* or morality.” The next provision was Article 53(b) which excluded patents on “animal varieties or essentially biological processes for the production of animals” (Bioethics and Patent Law, 2006). The EPO later concluded that Oncomouse was not included in “animal variety” and did not fall in the exclusion of Article 53(b). What the EPO meant by *ordre public* was morality, they

developed a utilitarian balancing test which aimed to assess the potential benefits of Oncomouse against the negative aspects. The EPO concluded that the usefulness in the advancement of cancer research outweighed the moral concerns in the suffering caused to the animal. However the EPO did make a small adjustment, in the original application the claim referred to animals in general, but they amended it to claims limited to mice.

This same utilitarian approach was used in 1992 by the EPO in the moral issue of the Upjohn mouse. The patent subject in review was filed by the Upjohn pharmaceutical company and was on a transgenic mouse in which a hair-loss gene was present. The mouse's objective was to test hair-loss products to possibly treat human baldness. Although there were many people who would have loved for this to have passed, the EPO weighed the positive and the negatives of the case and decided that the suffering of the mice outweighed the possibility of hair-loss cures. (Bioethics and Patent Law, 2006)

Transgenic Animals Since Oncomouse

Since 1988 there have been approximately 660 animal patents, with one-third of those patents belonging to foreign companies for use with Biochemical and Medical research. In addition to Harvard's Oncomouse there have been numerous other mice patented, examples of this include an Alzheimer's mouse and an HIV mouse. Besides mice, animals such as Beagle dogs, cats, sheep, pigs, cows, Macaque monkeys, fish, chimpanzees, birds, rabbits and many others have all been patented (American Anti-Vivisection Society, 2007). Some recent examples of patented animals include the transgenic mouse that comprises of a genomic human Tau transgene which received a patent on January 9, 2007 (Davies and Duff, 2007). An example of a non-mouse patented animal would be the patent of a transgenic cow that secretes foreign proteins

into its milk (Gordon, 2006). Lastly on February 7, 2006 there was a patent given to transgenic mammals that express mutant GP IIIa ($\beta 3$) protein (Law and Phillips, 2006). As time goes on more and more specific and useful patents are evolving, science is expanding, and we are learning more about different diseases. However it is important to know the history of the original animal patent, the trials and tribulations that were associated and how one small mouse sparked one of the largest advances of technology as we know it today.

Nations Differing on Transgenic Animals

Although it may seem like most courts would agree that advancement of cancer research far outweighs the animal's suffering, that is not the case. Many nations to this day still oppose not just Oncomouse, but the patenting of any transgenic animal. Canada for example rejected the claims to transgenic animals on the basis that they were not an invention, but passed the claim on to the process for obtaining one. In 2002 the Supreme Court of Canada ruled that higher life forms were not patentable because they were not "manufacture or composition of matter within the meaning of invention" of the Patent Act of *Harvard College v. Canada* in 2002. Canada considered manufacture as a non-living substance, and "composition of matter" was interpreted as substances that had been combined or mixed by a person. This being said, the oncogene-injected egg may be a mixture of ingredients capable of being patented, however the body of the mouse is not. (Bioethics and Patent Law, 2006)

Canada is not the only nation in the world that looks down on mouse patenting, Belarus, Brazil, China, Denmark, India, Ireland, Netherlands, Norway, the Philippines, Russia, and Thailand have prohibited any transgenic animal patenting. Countries along with the United

States that also patent transgenic mice such as Oncomouse are the United Kingdom, European Union (EU), Australia, and Japan (American Anti-Vivisection Society, 2007).

Current Legislation and Clarification

With so many recent legislative changes and Supreme Court cases it isn't hard to get lost in the mix of what is allowed to be patented and what is not. After the April 1987 PTO statement, the use of the term "non-human" had many scientists and laypeople wondering how far that could be taken. Jeremy Rifkin and Dr. Stuart Newman from New York Medical College asked if the term "non human" would apply to a human-animal chimera. In 1997 Rifkin-Newman applied for a patent that "covers the production of human-animal chimeras that could be up to 50% human" (Edwards, 2001). The patent has been struck down many times, however Rifkin and Newman continue to amend the application and resubmit it. Their reasoning for this could be many things from actually receiving the patent and preventing others from using it, to raising enough public debate that the PTO must come out and make exact guidelines on the human percentage that can be patented, which has yet to be done.

Another topic that is currently a part of the transgenic animal law debate is the Farmers' exemption, which was proposed in the Transgenic Animal Patent Reform of 1989. This legislation allows farmers to breed transgenic animals without having to pay any royalty fee for their offspring. However this exemption also prevents company-owned farms from patenting specific animals which would put most family-owned farms out of business (Walter, 1997).

Transgenic animal patenting is not an exact science; it takes much thought and deliberation to make and enforce rules and guidelines. As long as technology continues to grow,

legislation and law will have to expand with it. It is in the hands of the leaders of our country to make the right decisions to balance animal suffering and benefit of the good of mankind.

Negatives for Animal Patents

Although it may be obvious to see the vast benefits of biomedical studies from transgenic animals, there are still a large number of activists that make it a point to speak out on this “unethical” practice. Recently in March of 2007, the American Antivivisection Society filed for re-examination of allowed transgenic patents on the basis that animal patents provide incentive to harm animals for economic gain. The main piece of legislature that these activists continue to revert to is the Animal Welfare Act. The AWA prides itself on its protection of "any live or dead dog, cat, nonhuman primate, guinea pig, hamster, rabbit, or other warm-blooded animal, which is being used, or is intended for use, in research, teaching, testing, experimentation, or exhibition purposes, or as a pet" (Perzigian, 2003). This act conveniently leaves out “birds, rats...mice...bred for use in research, and horses not used for research purposes, and other farm animals, such as, but not limited to livestock or poultry.”

In a recent study done by Opinion Research Corporation 1,008 adults were asked questions about Animal Patenting and the ethics behind it. Studies showed that 672 of the 1,008 people consider animal patenting to be unethical and immoral, and 857 of those 1,008 were not even aware animal patenting was legal (Letterman, 2007). Many people may be shocked by the high percentages represented in this study, however you must take into effect different variables in this study. The question itself could have been biased, they could have asked in a way that one may feel uncomfortable saying that they agree with animal patenting, one must also consider the geographical and socioeconomic levels of this study. 1,008 people in a city certainly do not

represent the opinions of the 301,139,947 citizens of the United States (Statistics of the United States, 2007).

Another example of this bias of information by these animal rights' activists is in Andrew B. Perzigian's article on Genetic Engineering and Animals rights. He states that a smart alternative to transgenic animal food sources is culturing muscle meat *in vitro*. This is a good idea, however cannot be used until perfected. Also Perzigian speaks of the suffering that cows undergo and how this idea would alleviate that, society must see through the bias that the suffering by the cow only happens in its death, not because it is transgenic (Perzigian, 2003).

Positives for Animal Patents

Having the stroke of genius to be able to obtain a patent on a living breathing organism is no easy feat, it takes years of practice, failure, and determination. If there were no entitlement rights on all of the experimentation that scientists perform day in and day out, science and technology as a whole would suffer.

Until 1995 the United States was one of the only nations without a committee in the field of bioethics, but with the creation of the National Bioethics Advisory Committee, America started paying more attention to the morals of these animal patents. In 1995 President Bill Clinton instructed the NBAC to address human gene patenting as one of its first topics of discussion. To this day no discussion has taken place, leaving America in the dark on some issues that should be well discussed by now. (Walter, 1997)

The authors of this IQP agree with Carrie Walter when she states that "the economic and social benefits of patenting transgenic animals and gene sequences far outweigh the possible social costs" (Walter, 1997). The key to biotechnology is in the field of transgenic animals, they

have the potential to cure diseases such as cancer, Alzheimer's, improve quality of food, provide organs for transplant, and make our pharmaceuticals more cost efficient. However we also feel that the issues and concerns of people should not be overlooked, there are strong ethical and moral considerations that the government should take into consideration. However, it should not be the job of the PTO to discuss morality, their job is deciding what is legally patentable. We feel as though there should be a branch or section of the US PTO that can weigh the possible benefits and negatives, similar to the utilitarian test that the EPO underwent. They took into consideration both legal and moral standpoints and made decisions based on both aspects.

CONCLUSIONS

This project explored numerous key issues dealing with transgenic animals, including explaining what a transgenic animal is, describing how one is created, classifying the different kinds of transgenic animals created to date, and discussing both the ethical and legal issues that surround their use. In regards to the main ways of creating transgenic animals, though the earlier techniques were highly inefficient and produced dead embryos, more recent techniques have improved the efficiency significantly, although there is still a long way to go among larger farm animals. With respect to issues with transgenic ethics, we agree that some experiments, for example those involving mammalian growth factor transgenics, should be put to an end due to considerable animal suffering. Nonetheless we believe that most transgenic experiments producing major benefits to society with minimal (or highly controlled) animal suffering should be continued. With regards to transgenic legalities, we concur that strong legislative supervision should be carried out to ensure animal suffering is reduced, and that laws should be passed regulating transgenic experiments with no apparent benefit to society.

BIBLIOGRAPHY

"A Brief History of the Patent Law of the United States." Ladas & Parry Intellectual Property Law. (September 2003), <http://www.ladas.com/Patents/USPatentHistory.html>

"A Tradition of Quality." (2007) Taconic.com <http://www.taconic.com/wmspage.cfm?parm1=308>

Ackerman S (2006) "ANDi: The First Genetically Engineered Monkey." Division of Comparative Medicine of the National Center for Research Resources and by the National Institute of Child Health and Human Development. <http://www.ncrr.nih.gov/newspub/apr01rpt/ANDi.asp>

American Anti-Vivisection Society (2007) "Examples"
<http://www.stopanimalpatents.org/faq.php>

Animal Aid Youth Group (2006) "Animal Experiments."
<http://www.animalaid.org.uk/youth/topics/experiments/genetics.htm>

Baguisi, A, et al. (1999) "Production of Goats by Somatic Cell Nuclear Transfer." *Nature Biotechnology* **17**: 456-461.

"Bioethics and Patent Law: The Case of the Oncomouse" (2006) WIPO Magazine.
http://www.wipo.int/wipo_magazine/en/2006/03/article_0006.html

Biotechnology Industry Organization (2006) Five Myths About Transgenic Salmon.
<http://www.bio.org/animals/salmonmyths.asp>

Biotech Notes (1994) Herman Becomes a Father. U.S. Department of Agriculture.
http://www.accessexcellence.org/AB/BA/Herman_the_Bull.html

Brinton, Kate and Lieberman, Kim-An (2007) "Southern Blot" Basics of DNA Fingerprinting.
<<http://protist.biology.washington.edu/fingerprint/blot.html>>

Bronson SK, Smithies O (1994) Altering mice by homologous recombination using embryonic stem cells. *J. Biol. Chem.* **269**: 27155-27158.

Bunce N and Hunt J (2004) "The AIDS Mouse". College of Physical Science University of Guelph. The Science Corner. <http://www.physics.uoguelph.ca/summer/scor/articles/scor206.htm>

Butler, D (2002) Xenotransplant Experts Express Caution Over Knockout Piglets. *Nature* **415**: 103-104.

Chan AW, Chong KY, Martinovich CC, Simerly C, Schatten G (2001) Transgenic Monkeys Produced by Retroviral Gene transfer into Mature Oocytes. *Science* **291**: 309-312.

Christiansen SB, Sadoe P (2000) Bioethics: Limits to the Interference With Life. *Animal Reproductive Science* **60**: 15-29.

Cooper, Geoffrey M (2000) *The Cell - A Molecular Approach*. 2nd ed. Sunderland (MA): Sinauer Associates, Inc.

Curran G, and Koszarycz Y (2004) Animal Transgenesis and Cloning: Scientific, Religious, and Ethical Considerations. http://dlibrary.acu.edu.au/research/theology/ejournal/aejt_3/Curran_Koszarycz.html

Davies, Peter and Duff, Karen (2007) "Transgenic Mice Comprising a Genomic Human Tau Transgene." US Patent and Trademark Office. Patent # 7,161,060.

Devlin RH, Biagi CA, Yesaki TY, Smailus DE, Byatt JC (2001) Growth of Domesticated Transgenic Fish. *Nature* **409**: 781-782.

Diamond v. Chakrabarty (1980) 447 US 303-322, 1980. Dictionary.com, 2007

“DNA” World Encyclopedia (2005)
<<http://www.encyclopedia.com/doc/1O142-DNA.html>>

D'Silva J (1998) Campaigning Against Transgenic Technology. In: Holland, A. and Johnson, A., Editors, 1998. *Animal Biotechnology and Ethics*, Chapman & Hall, London, pp. 92-102.

Duff K, et al (1996) Increased Amyloid-Beta-1-42(43) in the Brains of Mice Expressing Mutant Presenilin-1. *Nature* **383**: 710-713.

Ebert KM, Selgrath JP, DiTullio P, Denman J, Smith TE, Memon MA, Schindler JE, Monastersky GM, Vitale JA, and Gordon K (1991) Transgenic Production of a Variant of Human Tissue-Type Plasminogen Activator in Goat Milk: Generation of Transgenic Goats and Analysis of Expression. *Bio/Technology*, **9**: 835-838.

Eenennaam AL (2006) Careful Risk Assessment Needed to Evaluate Transgenic Fish.
<http://calag.ucop.edu/0603JAS/pdfs/BiotechFish.pdf>

“ELISA Activity” The Biology Project/Immunology (Jan. 1998)
<http://www.biology.arizona.edu/IMMUNOLOGY/activities/western_blot/west3.html>

Fabregas L (2006), “‘Million-dollar pigs’ are Medical Marvels.” Pittsburgh Tribune-Review April 9, 2006.
http://www.pittsburghlive.com/x/pittsburghtrib/s_441762.html

Feany MB and Bender WW (2000) A Drosophila Model of Parkinson's Disease. *Nature* **404**: 394-398.

Games, Dora, David Adams, et al (1995) Alzheimer-Type Neuropathology in Transgenic Mice Overexpressing V717F Beta-Amyloid Precursor Protein. *Nature* **373**: 523-527.

Genoway: “A Tool Provider in Transgenesis” (2003)
<<http://www.genoway.com>>

Gordon, Katherine (2006) "Transgenic Animals Secreting Proteins Into Milk." US Patent and Trademark Office. Patent #7045676.

Harmon J (1999) “Scientists Create Smart Mouse”. September 1, 1999.
<http://www.princeton.edu/pr/news/99/q3/0902-smart.htm>

Harvey M, et al (1993) Spontaneous and Carcinogen-Induced Tumorigenesis in p53-Deficient Mice. *Nature Genetics* **5**: 225-229.

Hatton, Greg H. (1995) “The Life Spectrum Hypothesis”
<<http://mypage.uniserve.com/~ghatton/lifespec.html>>

Houdebine LM (1994) Production of Pharmaceutical Proteins From Transgenic Animals. *Journal of Biotechnology* **34**: 269-287.

Hunt, Dr. Margaret. “Real Time PCR” Microbiology and Immunology (Sept. 2006)
<<http://pathmicro.med.sc.edu/pcr/realtime-home.htm>>

“Introduction to Cloning and Biotechnology” (2005)
<www.bio.miami.edu/dana/104/104F02_24.html>

Kae, Helmut (2003) "The New MacDonald Pharm" The Science Creative Quarterly
<<http://www.scq.ubc.ca/the-new-macdonald-pharm/>>

Kohn C (2001) "First HIV Rat Seen as Best Model for Human Studies". *Science Daily*, August 2, 2001. pg 5.
<http://www.sciencedaily.com/print.php?url=/release/2001/08/010806074655.htm>

Krimpenfort P, Rademakers A, Eyestone W, van der Schans A, van den Broek S, Kooiman P, Kootwijk E, Platenburg G, Pieper F, Strijker R, and Herman de Boer (1991) Generation of transgenic dairy cattle using 'in vitro' embryo production. *Biotechnology* (N Y). Sep; **9**(9): 844-847. Department of Embryology, Gene Pharming Europe B.V., Leiden, The Netherlands.

Lai L, Kolber-Simonds D, Park KW, Cheong HT, Greenstein JL et al (2002) Production of Alpha-1,3-Galactosyltransferase Knockout Pigs by Nuclear Transfer Cloning. *Science* **295**: 1089-1092

Law, Deborah and Phillips, David. (2006) "Transgenic Mammals Expressing Mutant GP IIIa." US Patent and Trademark Office. Patent # 6995298.

Lemonick, M. et al. (2001) "Monkey Business". *Science*, Vol. **157** Issue 3, pg 50. January 22, 2001.

Logan J, Sharma A (1999) "Potential Use of Genetically Modified Pigs as Organ Donors for Transplantations into Humans". *Clinical & Experimental Pharmacology & Physiology*, December 1999, Vol. 26, Issue 12, pg 1020.
<http://www.blackwell-synergy.com/links/doi/10.1046/j.1440-1681.1999.03185.x/abs/>

Loma Linda University (1997) Seventh-day Adventist Guidelines on Genetic Engineering.

Martinez R, et al (1996) Growth Enhancement in Transgenic Tilapia by Ectopic Expression of Tilapia Growth Hormone. *Mol. Mar. Biol. Biotechnol.* **5**: 62-70.

Mepham TB (1994) Transgenesis in Farm Animals: Ethical Implications for Public Policy. *Pol. Life Sci.* **13**: 195-203.

Miller K, Bolt D, Pursel V, Hammer R, Pinkert C, Palmiter R, Brinster R (1989) Expression of human or bovine growth hormone gene with a mouse metallothionein-1 promoter in transgenic swine alters the secretion of porcine growth hormone and insulin-like growth factor-I. *J Endocrinol* **120**(3): 481-488.

Miskina R, Masos T (1997) Transgenic Mice Overexpressing Urokinase-Type Plasminogen Activator in the Brain Exhibit Reduced Food Consumption, Body Weight and Size, and Increased Longevity *Journal of Gerontology* **52A**: B118-B124.

Murray, Fiona (2006) "The Oncomouse that Roared: Resistance & Accomodation to Patenting in Academic Science." Massachusetts Institute of Technology. (pg. 5-7)

Patent and Trademark Office Notice: Animals-Patentability, (April 21, 1987) Official Gazette U.S. Pat. & Trademark Off. 24

"Patent Requirements." (2007). Stevens Institute of Technology.
http://www.stevens.edu/engineering/research/inventors_handbook/patents.html

Pearson, Helen (2003), Engineered pig organs survive in monkeys.
<http://cmbi.bjmu.edu.cn/news/0312/52.htm>

Perzigian, Andrew (2003) "Genetic Engineering and Animal Rights: The Legal Terrain and Ethical Underpinnings"
<http://www.animallaw.info/articles/ddusgeneticengin.htm#IIB>

Piquepaille, Roland (2006) GM salmon on the market in 2008? <http://www.primidi.com/2006/01/11.html>

Porter, David (1992) Ethical Scores for Animal Experiments. *Nature Magazine* **356**: 101-102.

Procean.com (2007) "Fish farm image."

Pursel VG, Wall RJ, Solomon MB, Bolt DJ, Murray JD, and Ward KA (1997) Transfer of Ovine Metallothionein-Ovine Growth Hormone Fusion Gene into Swine. *J. Anim. Sci.* **75**: 2208-2214.
<http://jas.fass.org/cgi/reprint/75/8/2208.pdf>

Reenen CG, Blokhuis HJ (1997) Evaluation of Welfare of Transgenic Farm Animals: Lessons from a Case Study in Cattle. In: Nilsson A, Editor. *Transgenic Animals and Food Production: Proceedings from an International Workshop in Stockholm*, KSLA, Stockholm, pp. 99-105.

Reid W, et al. (2001) "An HIV-1 Transgenic Rat that Develops HIV-related Pathology and Immunology Dysfunction" *PNAS* July 31, 2001. Vol. **98**, No. 16, pg 9271-9276.

Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, et al (1999) Immunization with Amyloid-Beta Attenuates Alzheimer-Disease-Like Pathology in the PDAPP Mouse. *Nature* **400**: 173-177.

Schnieke AE, et al (1997) Human Factor IX Transgenic Sheep Produced by Transfer of Nuclei From Transfected Fetal Fibroblasts. *Science* **278**: 2130-2133.

Schroten E (1997) Animal Biotechnology: Public Perception and Public Policy from a Moral Point of View. In: Nilsson A, Editor, 1997. *Transgenic Animals and Food Production: Proceedings from an International Workshop in Stockholm*, KSLA, Stockholm, pp. 151-156.

Smaglik, Paul (2000) NIH Cancer Researchers to get Free Access to Oncomouse. *Nature* **403**: 350.

"Statistics of the United States." (2007). Nationmaster.com

"Stem Cell Basics: What are Embryonic Stem Cells?" Stem Cell Information (2006)
<<http://stemcells.nih.gov/info/basics/basics3.asp>>

Stern, Marc. (2000). "NIH and E.I. DuPont Sign OncoMouse[®] Agreement." National Institute of Health.
<http://www.nih.gov/news/pr/jan2000/od-19.htm>

Stewart TA, Pattengale PK, and Leder P (1984) Spontaneous Mammary Adenocarcinomas in Transgenic Mice That Carry and Express MTV/myc Fusion Genes. *Cell* **38**: 627-637.

Stobbee, Mike (2007) "Cancer Deaths Decline for Second Year in a Row."
<http://www.cleveland.com/news/plaindealer/index.ssf?/base/news/1169123607298930.xml&coll=2>

Stokstad, Erik (2002) Engineering Fish: Friend or Foe of the Environment? *Science* **297**: 1797-1799.

Taconic Transgenics (2003) "Embryonic Stem Cells." <http://www.taconic.com/emerging/ESCells/ES_WEB.htm>

Tang YP, Shimizu E, Dube GR, Rampon C, Kerchner GA, Zhuo M, Liu G, Tsien JZ (1999) Genetic Enhancement of Learning and Memory in Mice. *Nature* **401**: 63-69.

"Transgenic Animals" (2003)
<<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/T/TransgenicAnimals.html>>

"Transgenic Animals" (2007)
<<http://www.ucalgary.ca/~browder/transgenic.html>>

UCI (2007) "Transgenic Mouse Facility"
<<http://www.research.uci.edu/tmf/dnaMicro.htm>>

Vatalaro, M. (2000) "Fly Model of Parkinson's Offers Hope of Simpler, Faster Research." NIH Record June 13, 2000. http://www.nih.gov/news/NIH-Record/06_13_2000/story05.html

Vogel J, et al., (1988) The HIV tat gene induces dermal lesions resembling Kaposi's sarcoma in transgenic mice. *Nature* **335**: 606-611.

Vorstenbosch J (1993) The Concept of Integrity: Its Significance for the Ethical Discussion on Biotechnology and Animals. *Livest. Prod. Sci.* **36**: 109-112.

Wade, R (2004) "Animal Theology and Ethical Concerns."
http://dlibrary.acu.edu.au/research/theology/ejournal/aejt_2/Wade.htm

Walter, Carrie (1997) Beyond the Harvard Mouse: Current Patent Practice and the Necessity of Clear Guidelines in Biotechnology Patent Law. <http://www.law.indiana.edu/ilj/volumes/v73/no3/walter.html>

"Warner Jenkinson Co. V Hilton Davis Chemical." Supreme Court Collection. 1997. Cornell University. <http://www.law.cornell.edu/supct/html/95-728.ZS.html>

"Western Blot Activity" (1998) The Biology Project/Immunology
<http://www.biology.arizona.edu/IMMUNOLOGY/activities/western_blot/west3.html>

Woessner, Warren "Patenting Transgenic Animals- From Oncomouse to Hello Dolly"
<http://www.slwk.com/CM/IPPapars/IPPapars12.asp>

Wortman, Marc (2000) Yale Alumni Magazine: "The Magical Medical Mouse". (March 2000) <http://www.yalealumnimagazine.com/issues/00_03/mouse.html>