Project Number: 29977 IQP RKS-0203

THE ETHICS ASSOCIATED WITH ENGINEERING ANIMAL MODELS TO STUDY HUMAN DISEASES

An Interactive Qualifying Project Report

submitted to the faculty

of the

Worcester Polytechnic Institute

in partial fulfillment of the requirements for the

Degree of Bachelor of Science

by

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Date: April 25, 2002

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Abstract

The ethics associated with the engineering of animal models to study human diseases is developed and discussed in this report. The tools of molecular biology permit the engineering of mice that mimic, or model, the characteristics of human diseases. Given that many of these diseases severely affect the quality of human life, it is imperative that we consider the ethics associated with designing mice that have these diseases. In this report is first a primer on genetics and recombinant DNA technology, followed by a brief history of animal use in research and testing. Included is a discussion of the evolution of ethics surrounding the use of animals in research. The benefits of animal research, such as a better standard of life and well being for humans and animals, are also considered. Various genetic diseases and the levels of pain caused by those genetic diseases are discussed. Included are details about the genotype, phenotype, history, and treatments for the genetic diseases along with a description of any animal models for those diseases. An analysis is conducted on the benefits of animal research vs. the cost in pain that is endured by the engineered animals. Recommendations are made as to what changes might be made to the current US regulations in order to minimize animal pain. The recommendations focus on reducing the number of animals used for experimentation on genetic diseases, stressing the possibility of research methods that do not require an animal model, and refining the process of experimentation to reduce the number of experiments and the pain caused to each animal.

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Chapter 1: Introduction

The welfare of animals is a very important consideration in today's times of genetic experimentation using animals. The experimentation has already provided many benefits to humans and animals alike, but the "cost" in terms of animal suffering must also be considered. The level of the expected benefit to humans must be weighed against the level of animal suffering required to develop, or even attempt, a cure for a disease. In this document, I will begin with a primer on DNA, modifying DNA, genetically modified animals and genetic diseases. I will then discuss the use of animals for research and testing, its benefits, the groups that oppose it, and the groups that regulate it. I will then list many of the genetic diseases that are currently being researched by the creation of engineered (genetically modified) animals. The focus will remain on the "cost" of potential suffering of the animals that are involved, and whether the research is worth this cost.

Chapter 2: Genes, Genetics and Recombinant DNA Technology

DNA as the primary genetic material

Living beings are composed of cells that translate their genetic instructions to carry out biologic function. These instructions are necessary in order to make a complete living being. The genetic instructions, called genes, are stored in every cell.

Genes are made up of deoxyribonucleic acid (DNA). DNA is the material that stores the genetic code. A DNA molecule is made up of two strands of nucleotides. The nucleotides are made of a five-carbon sugar, deoxyribose, with phosphate group(s) and a nitrogen containing base. The bases are what determine what is encoded in the DNA. The four bases are adenine (A), cytosine (C), guanine (G), and thymine (T). The two strands of nucleotides are complementary which means that in the double-helix formation of the DNA, the nucleotides are bonded to their complementary nucleotide on the other strand. Adenine complements thymine, and cytosine complements guanine. A nucleotide bonded with its complement is called a base pair. The complementary nature of the base pairs lead to a bond between the two strands and makes it easy for errors in the DNA to be self-corrected (Alberts et. al., 1998). For example, if a strand is broken so that there are unpaired nucleotides, the unpaired nucleotides are automatically paired with their complements.

Genes, Transcription and Translation

Genetic information in the cell is *transcribed* from DNA to ribonucleic acid (RNA), and then *translated* from RNA to proteins. Transcription is a process in which the base pairs of <u>one</u> strand of DNA are read and used as a template to generate a single strand of RNA. RNA contains the nucleotides adenine (A), guanine (G), and cytosine (C) just like DNA. However, uracil (U) takes the place of thymine (T) in the strand. One of the major differences between DNA and RNA are that DNA is double stranded while

RNA contains only a single strand of nucleotides. Another difference is in the functions of the molecules. DNA are used to store and replicate (make many copies of) genetic information. RNA are used in an intermediate step in the process that produces proteins. RNA molecules are much shorter than DNA. RNA only carries the information of a portion of the DNA (usually a single gene). After a few more intermediate steps, the RNA is decoded into sets of three nucleotides (a codon) and translated into the amino acid sequence of a protein.

Each gene is transcribed from DNA to RNA, and then translated from RNA to a protein. Each gene encodes one protein. Proteins are made up of long chains of amino acids. The amino acids in the protein determine the structure of the protein. Proteins fold into many different structures. Proteins determine a lot about the living being that they are a part of because they serve so many functions. Proteins can form tiny machines that untangle DNA molecules or channels that pump small molecules in and out of cells. Proteins also form antibodies, hormones, enzymes, and fibers. Proteins relay signals and messages within cells and between cells and serve many other functions (Alberts et. al., 1998). Since proteins serve so many functions and the levels and types of proteins that are produced are determined by the genetic code, the genetic code really determines how a living organism will operate.

Recombinant DNA Technology

In the early 1970s, a method of isolating a specific piece of DNA out of the millions of nucleotide pairs in a nucleus was discovered. This discovery led to the creation of new DNA molecules and the ability to introduce them back into a living creature. This relatively new technology is called recombinant DNA technology. Recombinant DNA technology gives a biologist an ability to modify a gene (or genes) and then insert it back into an organism in order to monitor the effect of that modification on protein and cell function. The knowledge of the expression of genes allows for further understanding of the genes.

Analysis and isolation of genes

The DNA sequence of genes can be analyzed by cutting the genomic DNA into manageable fragments using restriction nucleases originally discovered in bacteria. Each restriction nuclease is designed to separate DNA at a specific nucleotide sequence. DNA fragments are separated according to length using gel electrophoresis. The DNA fragments are placed into one end of a slab of gel to which a voltage is applied. The smaller fragments are drawn to the other side of the slab by the charge because they are not as impeded by the gel. Thus, the DNA fragments are separated along the gel slab by size. Individual fragments from the gel can be isolated and further analyzed. Automatic DNA sequencers have been designed based on the detection of the fluorescent labels. The automatic sequencers eliminate some of the guesswork and possible human error involved in the DNA sequencing procedure.

The sequence of nucleotides of the DNA fragments is determined by copying the DNA and separating the copies based on length using gel electrophoresis. DNA hybridization is used to detect carriers of genetic diseases detecting certain gene sequences and mutations in the gene code. A DNA probe, which is merely a short single strand of DNA, is used to detect a complementary sequence within the DNA of an organism.

The location of a gene on DNA or chromosome is found using *in situ* hybridization. *In situ* hybridization is a process of separating the two DNA strands by exposing them to a high pH for a short period of time and then adding the aforementioned DNA probes which have been labeled with radioactive isotopes or florescent probes. The DNA strands are then analyzed for the locations of the radioactivity and/or florescence in order to determine the location of the specific gene on the chromosome.

Creation of rDNA molecules

To create rDNA (recombinant DNA) molecules, DNA molecules must be broken down into very small fragments and joined back together in different combinations. An enzyme called DNA ligase is used to reconnect a DNA strand and match up all of the

bases with their complements. Usually one of the DNA molecules used to create rDNA is a vector, which is DNA acquired from bacteria or a virus, and the other is the DNA sequence (gene) to be modified. The DNA molecule created by the combination of the DNA from the gene and the vector can then be used as a vector for modification of another gene. This process eventually leads to the ability to make DNA sequences that do not occur naturally by modifying the naturally occurring sequences many times. The rDNA molecules can be introduced to animals using *in vitro* and *in vivo* procedures such that the animal demonstrates expression of the gene in every cell. (Alberts et. al., 1998).

Causes of Genetic Diseases

Certain abnormalities in one's genetic code, such as a mutation in the gene, the lack of a certain gene, or the presence of a certain added gene are the primary causes of genetic diseases. Finding the presence or lack of a gene is a relatively simple task for modern science. Discovering the effect of the presence, mutation, or lack of a gene is a much more difficult process. In order to discover the function of a certain gene, an animal model is often created with the specific gene or genes modified in the desired manner in order to study the phenotype (observable effect) of the gene. These mutant animals are created by either adding a gene (transgenic) or taking away a gene (knockout).

Mutant animals that lack a certain protein can demonstrate the primary function of that protein and to some extent, the function of the controlling gene. Some mutants have proteins that are activated or deactivated based on temperature. This allows for easy observation of the effect of the presence of the protein. In the past, mutant animals were either randomly acquired or intentionally bred. With current DNA manipulation techniques, it is possible to intentionally cause a genetic mutation.

Mouse Models

There are three ways that genes can be changed in animals to create mutant animals such as engineered mice. One type of mutation is caused by gene replacement.

Gene replacement is the removal of a normal gene and the insertion of a mutant gene to replace it. Gene replacement is useful for studying the effect of a gene without interference by the normal gene that was previously there. Another type of mutation is called gene knockout. Gene knockout is deactivating or removing a gene. Knockouts are used to study the function of the normal gene. The third type of mutation is called gene addition. Gene addition is randomly adding to the genome without removing the normal gene. Gene addition is often the easiest method to use. Whenever the mutant gene overrides the normal gene, the phenotype of the mutant gene is revealed.

The most common engineered animal is the mouse. Mice are often used for research on human genetic diseases because of their proclivity to reproduce in large numbers, their similarity to humans (mammals), and their size. Transgenic mice created with gene additions are used to study the genetic cause of many diseases and in the testing of treatments for the diseases. Knockout mice have recently led to the identification of the phenotype of certain genes in mammals. Engineered mice created using gene replacement to study diseases that are cause by a mutated gene are more useful because the removal of the normal gene prevents it from interfering with the effect of the mutant gene.

The procedure for making gene replacements in mice is rather complicated. First a gene is mutated *ex vivo* using site-directed mutagenesis. This mutated gene is then reproduced by introducing the gene into embryonic stem cells. A few of the embryonic stem cells will have the normal gene replaced by the altered gene by homologous recombination. Those cells are identified and cultured to produce multitudes of the embryonic stem cells with the mutated gene. A mouse is mated and the early embryo is removed after three days of gestation. The embryonic stem cells with the mutant gene are injected into the three-day-old embryo. The embryo is then introduced into another female mouse that has been hormonally induced into believing that she is pregnant. When the mouse eventually gives birth, the mice produced contain somatic cells that carry the mutated gene and some will contain germ-line cells that contain the mutated gene. Those mice are identified and bred with normal mice to produce mice that contain the mutated gene in all of their cells. These are the transgenic mice that can be used for research on the mutated gene. If two of those mice are then inbred, mice that contain the

two copies of the mutated gene in all of their cells are produced. In many cases, the mutated gene inactivates the function of the gene, called "knocking out" the gene (Alberts et. al., 1998). Thus these mice are called "knockout" mice.

Genetics Primer

Chromosomes and Inheritance

Chromosomes are made up of very long DNA molecules. Human cells have two copies of each chromosome. One of the copies is inherited from the mother and the other is inherited from the father. The copies of the chromosomes inherited from the parents determine the genetic code of offspring.

The likelihood of getting a disease is passed on from generation to generation through the normal process of inheritance. Traits and genetic diseases are inherited from parents to children by inheritance. These traits and diseases are either dominant, recessive, or x-linked. Dominant genes always express themselves meaning that the effect of the gene is seen in a child regardless of the gene given by the other parent. Recessive genes express themselves only if the other parent also has the recessive gene. Recessive genes can be carried from generation to generation without the expression of a phenotype for that gene. An x-linked gene is inheritable only through the x chromosome and is a recessive gene. An x-linked disease is one in which the controlling gene is carried on the x chromosome. X-linked diseases are only commonly seen in males because they have only one x chromosome so the recessive x-linked genes always lead to gene expression in males (Alberts et. al., 1998). Inheritable diseases are of particular interest in modern science because of the possibility of knowing more about a disease by finding its genetic basis. Knowledge of the genetic basis of diseases can help lead to cures and aid in the testing of treatments.

Chapter 3: Use of Animals for Research and Testing

History of animal use in research and testing

In ancient times, live animals and humans were vivisected (cut into while alive) in order to satisfy the curiosity of scientists about the anatomy of the animal. In the third century BCE, the Alexandrian physicians Herophilus and Erisistratus are recorded as having examined functional differences between sensory nerves, motor nerves and tendons (Singer 1957). All procedures were conducted without the use of anaesthetics because anaesthetics were not discovered until the nineteenth century. Throughout the years, humans and especially animals were vivisected without anaesthetics for the purpose of satisfying scientific curiosity.

The Christian view on animals was molded by St Thomas Aquinas in his *Summa Theologiae* in 1260 with his belief that humans were unique. Aquinas expressed his belief that all other animals were incapable of rational thinking because they had no mind as humans do. His logic was that since animals were without souls and the ability to reason, animals were merely objects. The Christian church did not believe that any form of cruelty was acceptable because it believed that God had given humans dominion over animals. Since animals were a part of God's creation, they deserved at least some amount of respect. Christians believed that inflicting pain on animals was justified if it was not inflicted merely to be cruel to animals, but for a higher purpose, such as scientific research. (Monamy, 2000)

Descartes followed in the seventeenth century with his Christian-centered ideas on vivisection. Descartes described humans and all other animals as nothing but complex machines. He also stated that humans were the only beings with souls and the only beings capable of rational thought. Early vivisectionists followed Descartes' theory that animals could not feel real pain since they did not have a soul. They wrote off the pain-like reactions of animals that were being vivisected as mechanical reactions of machines (Monamy, 2000).

Evolution of ethics surrounding use of animals

Aquinas and Descartes did not speak without opposition. Professional physiologists expressed their beliefs against vivisection as it became a more common practice. The general public was not opposed to vivisection in the times of Aquinas and Descartes, so those who argued against vivisection were the minority. Professional opposition eventually resulted because of the objection to cruelty to animals. Many also argued that the benefits acquired from research involving vivisection were not worth the cruelty that the animals were required to go through. The majority of the early physiologists did not fully believe that animals did not feel real pain, but they still continued their experiments because they believed that the suffering of the animals was worth the potential benefit to humans that may result from their research. (Monamy, 2000)

Opposition to vivisection began, not due to the public believing that the animals felt pain, but because of the very differences between humans and other animals that Aquinas and Descartes both argued existed. The public was convinced that humans and other animals were so fundamentally different, that research on animals would not help humans in any way. In the mid-eighteenth century, real opposition to cruelty to animals began. Alexander Pope and Samuel Johnson both expressed to the public their beliefs that animals do feel pain and that humans do not necessarily have the right to experiment on animals for the benefit of humans. Over the years, the public opinion became more against animal experimentation.

Three main arguments were set up against animal experimentation. The belief was that animals were not good models of the human condition, so using them for research was pointless. Also, Descartes' idea that animals were nothing but machines without the ability to feel pain was doubted. Thus, the pain of the animals had to be taken into consideration. Those who were against vivisection argued that animals should be given some moral status other than just being machines. (Monamy, 2000)

The statements of Descartes and Aquinas were challenged by those who believed in the emerging philosophy of utilitarianism. Utilitarianism was the belief that pleasure was the only "good" thing and pain was the only "evil" thing. Those who were utilitarian believed that everyone should always try to bring the most pleasure and the least pain

(Bentham, 1789). Since animals could feel pleasure and pain, they deserved to be considered similar according to the utilitarian philosophy. The belief that animals did not have a soul and thus could not feel real pain was replaced by the belief that animals had the capacity to feel pain by Humphry Primatt and Jeremy Bentham in the late eighteenth century.

During the nineteenth century, societies that were against cruelty to animals were focused on eliminating cock fighting, dog fighting, horse baiting, and bull baiting. This led to insistence for consideration of the welfare of animals used for scientific research. Marshall Hall, a neurologist and physiologist from England, pioneered the issue of animal welfare from inside the scientific community. (Hall, 1861) Hall came up with the first set of rules to follow when conducting experiments using animal subjects. The first requirement was that no experimentation should take place if observation was enough to acquire the necessary information. The second requirement was that only experiments with a goal of specifically defined information that was possible to be attained from the experiment should be conducted. The third requirement was that repetition of experiments using animals should be kept to a minimum when it is unnecessary to repeat an experiment. The fourth requirement was that all experiments should be conducted with as little suffering for the animal as possible. The last requirement was that experimentation should be witnessed by peers, thus decreasing the need for repetition of the experiment.

The Society for the Prevention of Cruelty to Animals (SPCA) began in Britain in 1824 with the goal of educating the public about animal cruelty and lobbying for anti-cruelty legislation. In 1871, the British Association for the Advancement of Science was pressured into publishing guidelines for minimizing animal suffering caused by experimentation. These guidelines required anaesthesia whenever possible, banned painful experiments that were only used to show an already known fact, mandated that only qualified scientists with adequate assistance and instruments could perform the experiments to minimize the need to repeat the procedure, and required that operations could not be performed merely to gain the skills in performing later operations.

In 1876, the UK Cruelty to Animals Act was signed into law. The act required that experiments using live vertebrates must be performed only by licensed scientists and

that all experiments on cats, dogs, horses, mules, and asses and those for illustrating lectures would have to be certified by the British Home Secretary. The Victoria Street Society for the Protection of Animals, which was the group that had pushed for legislation against animal cruelty, was disappointed by the permissiveness of the Cruelty to Animals Act. They changed their name to the Victoria Street Society for the Abolition of Vivisection and ultimately the National Anti-Vivisection Society. They pioneered the Animal Rights movement as it is known today.

Henry Bergh, the son of a wealthy shipbuilder from New York, was traveling in Europe and witnessed a horse beating. Based on the horror of that event, Bergh gathered many powerful friends to form the American Society for the Prevention of Cruelty to Animals (ASPCA) in 1866. Extreme members of the ASPCA formed the American Anti-Vivisection Society (AAVS). For years, the National Academy of Sciences (NAS) and the American Medical Association (AMA) have opposed AAVS in their attempts to lobby for a ban on vivisection. In 1966, the Federal Laboratory Animal Welfare Act (AWA) was signed into law. In the years following the AWA, many groups staged protests against animal experimentation, some resorting to criminal acts in an attempt to gain publicity for their cause. Some of the criminal acts have included the burning down of research centers and the theft of animal research subjects. The criminal acts were largely ineffective, but the marches, demonstrations, and company boycotts all led to advancements in the education of the public of a need for animal welfare. Peter Singer, an Australian philosopher, published *Animal Liberation* in 1975. With *Animal Liberation*, Singer gave an intellectual basis for the "equality of consideration" and the capacity for animals to suffer.

> "…the fundamental common interest between humans and other animals remains the interest in not experiencing pain and suffering. The only acceptable limit to our moral concern is the point at which there is no awareness of pain or pleasure, and no preferences of any kind. That is why the principle of equal consideration of interests has implications for what we may do to rats, but not for what we may do to lettuces. Rats can feel pain, and pleasure. Lettuces can't." (Singer, 1975)

Singer argued that since laboratory animals could feel pain, they must be morally considered by humans. Singer believes that all animals are morally equal. He introduced the concept of the level of animal suffering vs. the level of benefit to humans. Singer stated that experimentation should only be done if the benefit to humans is enough to outweigh the level of animal suffering needed in order to gain the benefit for humans. Singer also challenged anyone to come up with a convincing argument that there is a moral difference between humans and other animals.

While Singer was leading the intellectual argument for animal welfare, animal rights groups were revealing videotapes to the public of gruesome experiments that were being performed. The intellectual arguments, as well as the public outcry caused by the videos, led to a strengthening of the Animal Welfare Act in 1985. The additions to the AWA included clarifications of humane care of animals such as sanitation, housing, and ventilation requirements. It also established the IACUC and the responsibilities of the IACUC to the APHIS. Both the IACUC and APHIS will be discussed in a later chapter.

People for the Ethical Treatment of Animals (PETA) was formed in 1980 with about five members. Today PETA is a powerful lobbying group with nearly a million members. PETA put pressure on companies like Gillette and Mary Kay Cosmetics to stop testing their products on animals and got major support because animal rights became a hot new trend. The gathering of the power of the public has allowed PETA to attempt to lobby legislation against animal testing and experimentation. PETA's main tagline is "Animals are not ours to eat, wear, experiment on, or use for entertainment." PETA pushes for people to become vegetarians and activists campaigning for animal rights. PETA has formed many convincing arguments against believers of animal welfare such as the concept that if one believes that animals should not be put through pain, why does one believe that it is acceptable to put animals through pain as long as it may benefit oneself.

Federal Regulation and Funding Groups

The United States Department of Agriculture (USDA) was founded in order to have a federal department for agencies, services, and programs for agriculture, food, the environment, rural development, research, and regulatory programs. The Animal and Plant Health Inspection Service (APHIS) is one of the regulatory programs of the USDA. The APHIS is for animal dealer registration, animal health, biotechnology inspection, and information for travelers. The APHIS decided that there should be a committee at every institution that does animal experimentation that oversees the research to ensure that it meets a set of guidelines that were designed to prevent unnecessary cruelty to animals. This committee is called the Institutional Animal Care and Use Committee (IACUC). The need for an all institutions that use lab animals for research or educational purposes to have an IACUC was signed into law. (http://iacuc.org)

The IACUC must consist of at least three members. One of the members must be a veterinarian, one must be a non-affiliated person, and one should be the chair of the IACUC. The IACUC must review the program at the institution that they are involved with every 6 months. They must investigate all complaints by the public or faculty to ensure that animal welfare needs are met. The IACUC must make recommendations for changes to be made of programs that do not meet the requirements in the Animal Welfare Act. If the program is not changed accordingly in 15 days, they must report the program and its failure to comply to the APHIS. No study on animals is to be conducted without prior approval by the IACUC. (http://iacuc.org)

There are three basic goals that are being implemented in order to better meet the concerns of animal welfare. These goals are known as the three R's. *Replace* is to replace using animals by using tissue cultures, studies, or other alternative methods in order to gain the same information wherever possible. *Reduce* is to reduce the number of animals used in each experiment and reduce the need for repetition of the same experiment by having it done by a well-renowned scientist originally. *Refinement* is making the procedures less painful by using anesthesia and other methods in order to make the ordeal less painful.

Benefits of animal research

Animals are used in research, teaching and testing because of the benefits they bring to both animals and people. Animal research has led to the improvement of the health and well-being of people. Animal research has also led to the improvement of the health and well-being of animals. Recreational, sport, service, and farm animals have all benefited from animal research. For example, new methods of fixing broken bones were first tested in dogs, thus dogs first got the benefit from the research because for years, only veterinarians were allowed to use the new methods while they underwent further testing and approval. Years before approval is given for using techniques and treatments are approved for humans, they are used on animals by veterinarians. Animal research has led to the protection of endangered species and maintaining an ecologically stable environment so that species do not become extinct. Animal research has also led to better methods of pest control so that the environment and other animals up the food chain are unaffected by pesticides.

Knowledge gained by animal research is passed on through teaching to doctors, nurses, animal care personnel, farmers, veterinarians, and the general public. The research leads to a better understanding of humans and other animals, ecosystems and diseases. This knowledge leads to better care of animals and a better life for animals and humans alike.

Testing on animals is done to insure that products are safe for humans. Testing is also done to see the effect of drugs and to ensure that batches of drugs work correctly. Without animal testing, many people could die from getting either drugs that do not work, or drugs that have adverse effects. The testing ensures the safety of those who buy a product or drug. Animal testing has been heavily debated in recent years. Some products state that they were not tested on animals, to show the understanding for not needing animal testing on the product. All of the three R's have been applied to animal testing. Animals have been *replaced* by tissue cultures for the testing of many products, especially in the early stages of testing. The use of animals for testing has been *reduced* because some products are not tested on animals at all because animal testing is

unnecessary. The procedure of animal testing has gone through so much *refinement* that the animals incur less pain in the testing procedures. (New Zealand, 2001)

Animal research has led to cures for many diseases and treatments for many ailments. Recent animal research in the way of genetic research has led to the curing and treatment of many genetically related diseases. The animals are used for discovering the function of a normal gene and a mutant gene in order better understand what can be done to treat a disease and test the treatment on the animals.

Problems with animal research

Genetic research has its opponents. The general public is against the cloning of a human being, but is accepting of the creation of transgenic mice for research in discovering cures and treatments for diseases. The International Vegetarian Union believes that scientists are "playing God" with mice when they make a transgenic mouse. (IVU, 2001) Some believe that animals have *rights* that cannot be violated, such as the right not to be used in experiments. (PETA.org) Many of these groups use sentimentality, religious, and emotional arguments against the research and often do not have an intellectual basis for their arguments. The International Vegetarian Union advocates that "animals can never serve as models of human disease because they are much too different" (IVU, 2001) even though many treatments for diseases have been found using animal models. Often, animal rights groups are blind to the facts of the actual results and benefits to all humans of animal research.

Chapter 4: Genetic Diseases

In this chapter, I will highlight a collection of genetic diseases. For inclusion in this category, a disease must fulfill a number of criteria: 1) it must have a genetic basis, 2) research must be ongoing in an attempt to cure the disease or research has already been done to cure the disease, 3) there should be some element of pain associated with the disease (there are a few that do not meet this criteria in order to have counterexamples), and 4) there must exist an engineered animal model for the disease. Some of the diseases are monogenetic, meaning that a single mutated gene is what causes the disease. Monogenetic diseases are often the easiest to research and thus are often the easiest to cure or treat. Some other diseases are known to have a genetic basis due to analysis of the inheritability of the disease, but the exact genes involved are unknown. For each disease there is a description of the genotype (gene that causes the disease) and the phenotype (effect of the disease). There is also a description of the history of the disease and how common the disease is. Animal models and treatments for diseases are also stated whenever applicable. Each disease is organized in a table on the pages to follow.

Table 1

Name:	**Cystic Fibrosis**
Gene Count:	Monogenetic
Occurrence:	1 / 2500 babies born have CF
Genotype:	Faulty gene that interferes with the normal passage of salt and water through the body's cells. CF is often caused by the deletion of three nucleotides in phenylaline F508 of the cystic fibrosis transmembrane regulator (CFTR) gene. (Riordan et. al., 1989)
Phenotype:	Called "thief of breath", CF slowly destroys the lungs through recurrent infections. CF ultimately leads to death. Mucus in the lungs that is normally secreted to trap dust and bacteria is moved upward towards the mouth by ciliated cells. (Pier et. al., 1996) Pancreas becomes clogged and fibrous, thus it does not secrete the required amount of digestive enzymes to the intestine. The lack of the digestive enzymes to dissolve fats results in undernourishment and excess fats in the stool. (Corey et. al., 1989) Reproductive ducts become clogged in males which leads to sterility. (Oppenheimer et. al., 1970) Salt from sweat is not absorbed back into the skin, thus salt residue forms on the skin and the body has a lack of salt. (Corey et. al. 1989)
History:	In the middle ages, children with salty sweat were recognized as dying in early childhood (Scully et. al., 1977). In 1938, the term "cystic fibrosis" was used by Dr. Dorothy Anderson to describe "disease of respiratory and GI deficiency". In 1989, the CFTR gene and protein were identified. (Riordan et. al., 1989)
Animal Models:	A mouse model called the delta F508 mouse was created that shows the phenotype in its severe form. The mice models have effectively be used for testing of drugs and research on Cystic Fibrosis. (Colledge et. al., 1995) A knockout mouse model was created that shows the phenotype of Cystic Fibrosis in its severe form. (O'Neal et. al., 1993)
Pain:	Cystic Fibrosis is a painful disease. Mouse models created for research on CF go through pain in that their lungs gradually are destroyed and clog up while they become undernourished because of the lack of digestive enzymes. (Corey et. al. 1989)
Treatment:	Adenoviruses as a vector. Recombinant Adeno-Associated Viral (AAV) vector. (Crystal et. al., 1994)

Table 2

Name:	**Sickle-Cell Anemia**
Gene Count:	Monogenetic
Occurrence:	Mainly African Americans (1 / 400)
Genotype:	A defect in the gene encoding the b-globin strand of hemoglobin. A variant globin causes hemoglobin to polymerize under low oxygen tension, damaging the red blood cell. (Ingram, 1956)
Phenotype:	The faulty gene codes for a defective hemoglobin molecule that forms long helical polymers when deoxygenated. These polymers result in red blood cells with abnormal sickle-like shapes, and these cells then clog capillaries all over the body. The sickle shape prohibits the cell from adequately performing its designated role of carrying oxygen to the body's organs and tissues. (Platt, 1997) This leads to pain episodes, strokes, increased infections, leg ulcers, bone damage, jaundice, early gallstones, lung blockage, kidney damage, painful erections in men (priapism), blood blockage in spleen or liver, eye damage, low red blood cell counts (anemia), and delayed growth. (Platt, 1997)
History:	James B. Herrick discovered in 1910 that a patient of his from the West Indies had sickle shaped red blood cells. (Herrick, 1910) In 1956, Vernan Ingram discovered the molecular basis of sickle cell anemia. (Ingram, 1956) In 1995, Hydroxyurea was created as the first drug proven to prevent complications of sickle cell disease. (MSH)
Animal Model:	Knockout mouse created by mating transgenic mice that expressed human sickle hemoglobin with alpha and beta-globin knockout mice and are useful for trial of drug and genetic therapies. (Ryan et. al., 1997)
Pain:	Mouse models created for research on Sickle-Cell Anemia endure pain episodes, strokes, organ and bone damage, and many other painful aspects of the phenotype. (Ryan et. al., 1997)
Treatment:	Adequate hydration, oxygenation, bone marrow stimulation, and blood transfusion are commonly used to treat sickle cell crisis.

Table 3

Name:	**Alzheimer's Disease**
Gene Count:	Multi - More than one gene mutation can cause AD, and genes on multiple chromosomes are involved.
Occurrence:	10-12% of people get AD at some point in their life
Genotype:	Familial AD (FAD) is a rare form of AD, affecting fewer than 10 percent of AD patients. It is associated with gene mutations on chromosomes 1, 14, and 21. Sporadic AD (much more common) is believed to be related to the apolipoprotein E (apoE) gene on chromosome 19. (Delabar et. al., 1986)
Phenotype:	AD is a progressive neurodegenerative disease characterized by memory loss, language deterioration, impaired visuospatial skills, poor judgment, indifferent attitude, but preserved motor function.
History:	Dr. Alois Alzheimer identified brain abnormalities in 1906 that were later referred to as Alzheimer's Disease. Dr. Alzheimer noticed dense deposits on the brain of a deceased patient of his that had AD. (Alzheimer, 1907) In 1993, Cognex was approved as the first drug for treatment of AD. Cognex slows the degenerative process in the brain, thus slowing the development of AD.
Animal Model:	PDAPP transgenic mouse expresses mutant form of APP and develops AD pathology (Schenk et. al., 1999)
Pain:	Alzheimer's disease is not a painful disease. Those afflicted with Alzheimer's disease merely have memory loss and impaired judgment. Mouse models exhibit memory loss and impaired judgment, but do not endure any pain.
Treatment:	Gingko Extract, acetylcholinesterase (AChE) inhibitors, neural stem cells. Huperzine A can lower neuronal cell death attributed to glutamate. Anti-amyloid vaccines are currently being tested in hopes of having Alzheimer's Disease vaccines. (Morgan D. et. al., 2000)

Table 4

Name:	**Down Syndrome (Trisomy 21)**
Gene Count:	One extra chromosome
Occurrence:	1 / 900 births
Genotype:	Caused by having an extra copy of the 21st chromosome (Lejeune et. al., 1959).
Phenotype:	Moderate to severe mental retardation, physical deformities, congenital heart defects, increased susceptibility to infection, respiratory problems, obstructed digestive tracts, Alzheimer's disease and Childhood Leukemia.
History:	John Langdon Down published an essay in England in which he described a set of children with common features who were distinct from other children with mental retardation. (Down, 1866) Jerome Lejeune first determined the cause to be trisomy (triplication) of the 21st chromosome (Lejeune et. al., 1959).
Animal Model:	Chromosome transfer used to create chimeric mice containing human chromosome 21. Impairment in learning and emotional behavior was observed, which are the typical phenotype of Down Syndrome. (Shinohara et. al., 2001)
Pain:	Down Syndrome is not a painful disease, rather it is a disease that causes physical deformities and mental retardation. Mouse models that are created to research Down Syndrome do not endure pain related to the disease.
Treatment:	Modifying the genes on chromosome 21 to lessen effects (Fuentes, Juan et. al., 2000)

Table 5

Name:	**Rheumatoid Arthritis**
Gene Count:	Multi?
Occurrence:	2Mil Americans
Genotype:	Gene unknown - HLA complex genetic marker (Hasstedt et. al., 1994)
Phenotype:	The body's immune system mistakenly attacks the lining of joints and other internal organs. Causes pain, stiffness, swelling and loss of function in the joints and inflammation in other body organs. RA leads to osteoporosis.
History:	In 1591 Guillaume de Baillou, the French physician and Dean of the University of Paris medical faculty writes one of the first books on arthritis. In this book he uses the term rheumatism to describe a condition characterized by inflammation, soreness, stiffness in the muscles, and pain in and around the joints. The first known appearance of RA is in the remains of Native Americans in Tennessee from 4500 BC. (Burch et. al. 1964)
Animal Model:	Transgenic mice were created for HLA-DQ6 which is an allele associated with a nonsusceptible haplotype. The DQ6 mice were resistant to collagen-induced arthritis. Those mice were also investigated as to how these resistances can be used for development of treatments for Arthritis. (Bradley et. al., 1997)
Pain:	Arthritis can be an extremely painful disease. In order to test some treatments for arthritis, mouse models are created and allowed to develop severe cases of arthritis. (Bradley et. al., 1997)
Treatment:	Symptomatic medications, such as NSAIDs and aspirin, analgesics, and glucocorticoids, help reduce joint pain, stiffness and swelling. These drugs may be used in combination. Disease-modifying medications include low doses of methotrexate and biologic agents.

Table 6

Name:	**Cancer (various types)**
Gene Count:	Different for each type of cancer
Occurrence:	500,000 deaths / year
Genotype:	Cancer is caused by mutations in the DNA of somatic cells. Can be inherited as genes that behave as tumor suppressor genes. In effect, all the cells of the body have taken one step towards malignancy, so the chances of accumulating enough mutations to cause cancer are increased. (Coles et. al., 1990)
Phenotype:	Severe pain, destruction of organs, death.
History:	Cancer has afflicted humans throughout history. The first known cases of cancer occurred about 1600 B.C. from Egyptian writings of tumors on the breasts. Greek physician Hippocrates (460-370 B.C.) wrote about "carcinoma" which is where the name cancer began. As autopsies began as a practice in the eighteenth century, the details as to the phenotype of cancer were discovered. (cancer.org, 1999)
Animal Model:	A breast cancer mouse model was created by transfection of NIH 3T3 mouse cells. The mouse displays the transforming gene in a human mammary tumor cell line MCF-7 which is similar to human breast cancer. (Lane et. al., 1981)
Pain:	Cancer is a painful disease. The destruction of organs is a painful and drawn-out process. Some mouse models are allowed to develop tumors that are larger than the original size of the mouse for research on cancer.
Treatment:	Drugs, Surgery to remove tumors, Chemotherapy

Table 7

Name:	**Familial Hypercholesterolemia**
Gene Count:	Monogenetic
Occurrence:	1/500 (moderate), 1/1000000 (severe)
Genotype:	A defective gene that encodes the LDL "bad cholesterol" receptor. People with FH have too few functioning receptor molecules and cannot remove LDL from their blood. (Grossman et. al., 1994)
Phenotype:	Very High Choloesterol leads to the possible development of xanthomas, corneal arcus, and coronary artery disease. (Goldstein et. al., 1973)
History:	In 1964, the relationship between the levels of lipoprotein(a) levels and the occurrence of coronary heart disease was studied. It was found that those with high levels of lipoprotein(a) had a much higher risk of coronary heart disease. This discovery led to the ability to asses the risk factors of a patient for getting heart disease. (Khachadurian, 1964) In 1973, it was found that FH is caused by a defect in the cell membrane receptor for LDL. (Goldstein et. al., 1973)
Animal Models:	Four types of Chinese hamsters with defective LDL receptor function. Hamsters exhibit moderate phenotype. (Kingsley et. al., 1984). The Watanabe heritable hyperlipidemic (WHHL) rabbit is deficient in LDL receptors due to a defective gene. WHHL rabbits exhibit moderate phenotype. (Hornick et. al., 1983)
Pain:	Familial Hypercholesterolemia is not a particularly painful disease. FH leads to heart disease, which causes a fairly sudden death, but is not associated with long-term pain. Rabbits used as animal models did not show signs of being in pain because FH does not cause much pain. (Hornick et. al., 1983)
Treatment:	Testing has shown success when corrective copies of the receptor gene are transferred into liver cells via a retroviral vector. (Agnello et. al. 1999)

Table 8

Name:	**Fragile X**
Gene Count:	Monogenetic
Occurrence:	1/6000
Genotype:	Fragile X syndrome is generally related to a mutation of the FMR-1 gene on the X chromosome. The trinucleotide CGG is repeated a number of times on the FMR-1 gene. The sequence is transcribed, but not translated into protein because it is not on the part of the gene that is translated. Usually, there are around 30 repeats of CGG. Fragile X usually occurs when people have 200 or more CGG repeats. This prevents the transcription of the FMR-1 gene so that none of the protein is made. (McCabe et. al., 1999)
Phenotype:	The major expressions of Fragile X are learning disability of varying severity, behavioral problems such as hyperactivity and autistic tendencies, and physical characteristics including long face, protruding ears, and lax joints.
History:	In 1969, the marker X chromosome was discovered. (Lubs, 1969) In 1988, studies were done on 113 Fragile X Females and found that Fragile X is related to drastically lower nonverbal IQ scores. (Loesch et. al. 1988) In 1990, it was found that of 150 males that had Fragile X, 75 had flat feet, 85 had excessive laxity of joints, and 10 had scoliosis. (Davids et. al. 1990)
Animal Models:	Transgenic (Faust et. al., 1992) and knockout mice (Dutch, 1994) used to discover the location of the gene on the X chromosome and observe the phenotype. The knockout mice displayed a severe phenotype.
Pain:	Fragile X syndrome does involve pain. Animal models of Fragile X do not endure pain related to Fragile X.
Treatment:	Medications for behavior problems and speech therapy, no treatment of the disease.

Table 9

Name:	**Huntington's Disease**
Gene Count:	Monogenetic
Occurrence:	1/3500
Genotype:	The genetic defect responsible for HD is a small sequence of DNA on chromosome 4 in which several base pairs are repeated many, many times. The normal gene has three DNA bases, composed of the sequence CAG. In people with HD, the sequence abnormally repeats itself dozens of times. Over time—and with each successive generation—the number of CAG repeats may expand further. (Horton et. al., 1995)
Phenotype:	HD is a degenerative brain disorder that affects cognitive ability or mobility causes depression, mood swings, forgetfulness, clumsiness, involuntary twitching and lack of coordination. Death follows from complications such as choking, infection or heart failure.
History:	In 1994, an important study was conducted on Huntington's Disease patients and a control group. The result of the study was the possible implication that neither depression nor psychiatric disorders are related to HD. (Shiwach et. al., 1994)
Animal Model:	Transgenic mice were made that expressed cDNA encoding of an N-Terminal Fragment. The mice developed behavioral abnormalities including tremors, loss of coordination, hypokinesis, and abnormal gait before dying prematurely. (Schilling et. al., 1999)
Pain:	Huntington's Disease is a somewhat painful and distressing disease. Transgenic mice for HD had tremors and loss of coordination, altogether not awfully painful.
Treatment:	Medications to control emotional and movement problems. No treatment for curing the disease.

Table 10

Name:	**Muscular Dystrophy**
Gene Count:	Monogenetic
Occurrence:	1/3500
Genotype:	Mutations in the dystrophin gene that codes for the dystrophin protein, which is crucial for the strength and movement of normal muscle tissue. (Boland et. al. 1996)
Phenotype:	Generalized weakness and muscle wasting affecting limb and trunk muscles first. Calves often enlarged. All voluntary muscles are eventually affected. MD often leads to death before the late twenties.
History:	In 1935, Haldane predicted that 1/3 of all cases of X-linked recessive will be the consequence of a new mutation. (Haldane, 1935) In 1956, he suggested that the mutation rate for Duchenne MD was probably higher in males. (Haldane, 1956)
Animal Models:	Knockout mice were made to find that the deletion of the dystrophen muscle promoter in a male short-haired cat led to muscle hyperthrophy, stiffness and histopathologic dystrophy. The phenotype was expressed in the skeletal muscle, but not in the heart. (Winand, 1994)
Pain:	Muscular Dystrophy is a painful disease. The enlarging of the calves and the weakening of the muscles lead to a poor standard of living. Animal models, including mice and cats, have exhibited the phenotype of MD including the swelling and stiffness.
Treatment:	Treat symptoms w/ physical therapy, corrective surgery, and pacemakers. Viral vectors and direct injection of dystrophin have been used to treat MD. (Wang et. al., 2000) (Rondo et. al. 2000)

Table 11

Name:	**Hemophilia**
Gene Count:	Monogenetic
Occurrence:	1/9000
Genotype:	Defects in the genes for factor VIII and factor IX.
Phenotype:	Prevents proper clotting of the blood. Wounds bleed for a long time. Internal hemorrhages result in the knees, ankles, elbows, and into tissues and muscles. Bleeding in an internal organ can lead to death.
History:	In 1976, Biggs and Rizza conducted a study on 41 mothers of cases of hemophilia A and observed that 39 were carriers. (Biggs et. al. 1976) This study showed that hemophilia is an X-linked recessive disorder. In 1977, it was found that the location of the cause of hemophilia A is on the long arm of the X chromosome. (Samama et. al., 1977)
Animal Model:	A natural mutant homozygous female dog expressed the hemophilia geneas studied by Brinkhous and Graham. Using the research, they discovered that the hemophilia gene is not lethal in homozygous females. (Brinkhous et. al., 1950).
Pain:	Hemophilia is not a particularly painful disease until it is allowed to develop to the point where there is internal bleeding due to hemorrhages. Studies with animal models for hemophilia have not needed to allow the disease to develop to such an extreme level, thus minimal pain is endured by the animal.
Treatments:	Moloney retrovirus-mediated gene transfer (Dwarki et. al., 1995)

Table 12

Name:	**Type 1 Diabetes**
Gene Count:	At least 18
Occurrence:	800,000 Americans
Genotype:	Many different combinations of genetic mutations (Concannon et. al., 1998)
Phenotype:	The beta-cells in the pancreas that produce insulin are gradually destroyed. Without insulin to move glucose into cells, blood sugar levels become excessively high. This causes weight loss, weakness, hunger and thirst. If the diabetes is not treated with insulin, death can result.
History:	In 1977, it was found that diabetic siblings shared the HLA genes, leading to the understanding that diabetes is caused by a recessive gene linked to HLA. (Rubinstein et. al., 1977) In 1998, the results of a genome screen were used to show that Type 1 Diabetes (insulin dependent) in HLA region at 6p21.3 and only one other region (1q). (Concannon et. al., 1998)
Animal Model:	Mouse models were first made with virus-induced diabetes in 1978. The mice exhibited a moderate form of the phenotype including excess blood sugar and weight loss. (Onodera et. al., 1978)
Pain:	Diabetes is not a particularly painful disease. As long as insulin injections are used to supply the needed insulin, the effects of the disease are avoided. Mouse models were shown to have extreme cases of diabetes in order to prove that they accurately met the phenotype of diabetes.
Treatment:	Insulin Injections

Chapter 6: Ethics Associated with Engineering Animal Models of Devastating Human Diseases

Many of the diseases described in Chapter 5 are very painful, both to the humans afflicted with them and the animals that are used for research. For example, people endure severe pain when they have extreme cases of Rheumatoid Arthritis (RA). Research on arthritis often involves the creation of animal models to learn more about arthritis and to test different treatments. For many of the animal experiments, the arthritis in genetically engineered mice is allowed to progress to a point where the mice are crippled. In order to test drugs for cancer, sometimes the tumors are allowed to grow to a size larger than the animal. These extreme cases are thought necessary to perform some of the experiments to learn more about the diseases and test treatments, but they present an interesting ethical quandary. The issue is whether the results of the experiments are "worth" the pain caused to the animals. Different diseases cause different levels of pain in those that are afflicted with the disease.

Some diseases do not pose as much of an ethical dilemma because they don't involve much, if any pain. An example would be Alzheimer's disease. A person or animal with Alzheimer's disease is often afflicted with memory loss, language deterioration, impaired visuospatial skills, poor judgment, and has an indifferent attitude. The individual suffering from Alzheimer's disease is probably not suffering, and likely feels no pain related to the disease. Motor skills are retained with Alzheimer's. The indifferent attitude exemplifies the fact that they are not bothered by pain. For people, it is not usually the person with Alzheimer's disease who suffers, but the family and friends of those who are afflicted. They get frustrated by the afflicted person's inability to remember. Do the other mice feel pain? Possibly, but not likely to any major extent. In this author's opinion, diseases like Alzheimer's make the decision to engineer animal models of the disease easy. Since there is very minimal, if any, pain at all and there is a potential benefit to the well-being of humans, experimentation with animal models of Alzheimer's disease is likely acceptable.

A central theme addressed in this paper is the notion that animals are genetically engineered specifically to have a specific disease and may feel pain as a result of this

genetic manipulation. These animals do not have the disease by chance; they are intentionally engineered to have the disease. This adds an additional ethical dilemma in addition to those involved with standard animal testing.

Scientific research has led to a better standard of living and cures for diseases. Many treatments for genetic diseases have been found by experimentation with engineered animals. According to the American Medical Association, "Biomedical advances depend on research with animals, and not using them would be unethical because it would deprive humans and animals of the benefits of the research."(American Medical Association, 1989) Animals as well as humans, benefit from the research. Those that believe that animals are of equal value as humans are often presented with the raft example in which one can save a dog or a man but not both. Animal rightists usually concede that in that case, one must save the man. Thus, animals and humans are not seen of being equal value by even many animal rightists. According to those responses, human life is more valuable than animal life. However, animals do have some value, and thus should not be subjected to unnecessary pain for the betterment of the well being of animals and humans.

With animal welfare being a high priority, it is important to balance the benefit to science and our well-being against the pain that the animals might endure. It should be addressed on a case-by-case basis as to whether an experiment meets certain guidelines, such as those of the Animal Welfare Act (AWA), and has appropriate expected benefit for the suffering that is caused for the animals. The IACUC is a good starting point because it is designed to prevent extreme cases of suffering to animals without justification and forces checks on researchers that have complaints against them. However, this system could be modified to require a strict following of the three R's (Replacement, Reduction, and Refinement). Focusing on minimizing the suffering of the animals is necessary in order to make the decision as to whether an experiment is ethically justified considerably easier.

The government of New Zealand requires an Animal Ethics Committee similar to the IACUC that reviews any plan for experimentation with animals before the experiment can begin. The ethical principle on which experiments involving animals are required to follow is, "Using animals for scientific purposes is acceptable only when any harm done

to the animals is very greatly outweighed by the benefits of their use." The harm done to animals must be made as low as possible in order to make the benefits outweigh the "costs" as much as possible. The benefits of the work must not only be much greater than the suffering, but they must also be achievable goals. (New Zealand Gvt, 2001) Animal ethics committees such as IACUC should ensure that the plan of action of researchers has as large as possible of a gap between the benefits and the "costs" in animal suffering.

Chapter 7: Recommendations

Using methods of research other than designing animal models can lead to the desired results in some cases. Replacement calls for using methods other than experiments using animal models whenever possible. Animals should not be used at all if the research can be done without the use of animals. Replacement makes it so animals that might suffer are only used when necessary.

It is important to reduce the number of animals that suffer in order to achieve the desired result. Reduction calls for using as few animals as absolutely needed to effectively conduct the experiment. It is important that the experiment be conducted to insure meaningful statistical judgments. If the experiment must be repeated because of poor experimental design, more animals end up feeling pain.

It is necessary that minimal pain is endured by the animals. Refining the process of experimentation and taking extra care not inflict as little pain as possible on the animals while still being able to gain the desired results. Anesthesia should be used wherever possible in order to minimize pain. The studies should be reviewed by a committee such as the IACUC to ensure that procedures used are minimally painful.

Enforcing the three R's is a good way to force researchers to evaluate the unnecessary harm that could end up being inflicted on an animal, to minimize the number of animals used, and only use animals for research when necessary. It is also important to ensure that the goal of the research is truly beneficial and attainable.

The guidelines used in New Zealand and their ethics committee are good in concept, but can be seen as being too restricting. If the ethics committees are too restricting, many people will suffer even though they could be cured. Whenever an experiment that may lead to a cure to a disease that is causing hundreds of thousands of people to suffer is denied because the animal pain "cost" is determined to be too high, the people afflicted with the disease are forced to suffer longer. Future generations and people that possess the genotype but have yet to express the phenotype may also suffer even though the experiment could have prevented their suffering altogether. Thus, the decision to disallow an experiment is a tough one.

Chapter 8: Conclusions

The pain that engineered animals must endure must be validated by the hope of leading to a cure for a devastating disease. Ethics review committees, such as the IACUC, should have stringent requirements that the researchers demonstrate that they will be performing the experiment with minimal animal pain. If every step is taken to ensure minimal animal pain, the minimum number of animals is used, animals are shown to be necessary, and the expected benefit is great and achievable, then there is no reason not to allow an experiment to be conducted. This is true even if the genetically engineered animal is inflicted with a disease that causes severe pain. I came to these conclusions through a process of analyzing the painfulness of genetic diseases and the moral value of humans and animals. I also researched other sources of solutions such as the ethics committees of the New Zealand government.

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