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ETHICAL ISSUES IN TRANSGENIC ANIMAL RESEARCH

An Interactive Qualifying Project Report

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in partial fulfillment of the requirements for the

Degree of Bachelor of Science

by

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Date: May 1, 2003

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Dear Professor Shannon,

Attached is one copy of the Interactive Qualifying Project, "Ethical Issues in Transgenic Animal Research," project number 43-TAS-8805.

Sincerely,

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Abstract

This investigative paper will examine the technical, ethical, and legal implications of the transgenic techniques of disease modeling, xenotransplantation, and biopharming. The authors' basis for their discussion is included as a detailed background of past animal usage and animal testing. Specific cases of these transgenic technologies will be studied in depth to demonstrate the applications of the conclusions reached. Through legal and ethical analysis, we have demonstrated the current necessity of continued transgenic animal research.

Executive Summary

This investigative paper will examine the technical, ethical, and legal implications of the transgenic techniques of disease modeling, xenotransplantation, and biopharming. The authors' basis for their discussion is included as a detailed background of past animal usage and animal testing.

The examination of the ethical questions associated with transgenic research has shown that the potential medical gains from the methods discussed outweigh the loss of animal lives that would be needed. Each of the methods has its own balance of gains and losses which made it necessary for them to be evaluated individually. Specific cases were used in the evaluation of each of the methods. A premise for the argument was defined prior to the evaluation.

The legal area is still undefined. Since regulation lags present day technology by one to two decades, the courts have not reached a consensus. As a result, government and sub-government agencies have introduced temporary guidelines in the absence of real regulations to protect consumers. Lack of intellectual property rights is deterring researchers, scientists, and companies from conducting research in this area.

In summary, there is a demonstrated necessity of continued transgenic animal research. It will allow for the continued advancement of medical technology and the more effective treatment or eradication of harmful diseases.

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1. Introduction

1.1. Precedents of Animal Use

Throughout time, humans have used their environment to aid in their development. In their endeavors, they have always used animals, whether it was for food, to make clothes, to build shelter, or to do work. As time progressed humans started to use animals in a new field, medicine. Since the 1790's humans have used animals to produce vaccines to fight diseases. From that time on, animals have become a big part of medical research. They have been used for education as well as for experimentation. Through them, we have gained a great deal of knowledge and have progressed in the medical field.

Along side of this, people have always pursued scientific evolution, always experimenting to learn more. From the early days of alchemy, organized labs and experimentation have been a part of the scientific endeavor. In these experiments, mankind would take its environs and try to alter them to see what would happen, to learn and to harvest. In the sixteenth century, medicine began to develop rapidly, further developing the scientific evolution. By this time, humans had already started to dissect animals to learn more about how living things work. Since this time, mankind has continued to use animals to advance science and medicine in great ways.

Today science and medicine are at a new level that has never been reached before. We now better understand how living things work, right down to the cellular level. Advancements in genetics have brought us to such heights as mapping the human genome. In medicine, humans have successfully transplanted many organs from human to human. In pharmaceuticals mankind has made drugs to fight many of today's diseases and even eradicate some of them completely. Even with all of these advancements, mankind has still not been able to cure every disease, and solve every medical problem. This is why we must find new methods and advancements to continue on with our progress. One of these new areas that should be pursued is transgenics.

Using animals in transgenic research is the next step in the evolution of science and mankind. For close to all of time, people have used animals to accomplish their goals. They are already used in many types of research and other areas of medicine. Using the newfound knowledge of genetics on animals would follow the precedent of using animals that we have already set. This precedent does not necessarily justify our use of animals for any purpose. However, if they are being used in a reasonable, purposeful endeavor such as life-preserving medical research, then the benefits outweigh the collateral losses. One such worthwhile use of animals is in xenotransplantation. Xenotransplantation is not very different than raising animals for food, which we already do. In both cases the animal is raised to be killed for use by people. The

quality of life of the animal is still the same in both cases; just the application of the deceased animal is different. Biopharming is not much different than raising cows for dairy products like we currently do. Raising animals for disease modeling is not very different than the lab animals that we currently use for other types of research. In all of these cases, the life and use of the animal is not radically different. The major difference is that we are now taking the next step in the scientific ladder by using genetics in addition to older, more conventional methods.

1.2. Testing Alternatives

The testing of new pharmaceutical products on animals is a necessary evil that we must undertake to aid in the improvement of the quality of human life. Animal rights activists suggest alternatives to this testing, but none of the current alternatives are viable from the standpoint of safety and accuracy. Proponents encourage testing on cell cultures and tissue cultures, as well as the use of sophisticated computer modeling systems to predict the physiological impact of a foreign substance (http://www.peta.org/mc/facts/fsae4.html). However, none of these alternatives offer an accurate view of the desired information. They are certainly a valuable supplement to the current testing procedures, but it is not safe to rely only on these new methods. Rampant testing of new

compounds on humans is also an unacceptable alternative, for obvious reasons. The consequences of a study of this nature would be vast, both in terms of human life and welfare.

For the initial investigation of the effect of a compound on a subject, cell culture analysis would be valuable. In this method of testing, a culture of cells, either from a human subject or another animal, is grown and exposed to the material in question. A simple analysis of the initial reaction can be obtained, and valuable data can be acquired. Toxicity can be tested, and the reaction of the cells to the substrate can be found. However, only one type of cells is being tested. Because different genes are expressed in different tissue types, a different array of enzymes is present in each different tissue type. Therefore, a chemical compound may have drastically different effects on different cell types. Another problem is the type of cells used in the analysis. For example, a common laboratory culture of human cells are HeLa culture cells. These cells were isolated from a cervical cancer tumor from a patient named Henrietta Lacks, and show the remarkable ability to thrive and divide under laboratory conditions (http://bioresearch.ac.uk/browse/mesh/detail/C0018873L0018873.html). However, a single mutation in these cells, or a single genetic abnormality in Henrietta Lacks, could cause a change in the effects of a compound on the cells, and subsequently an incorrect analysis.

Testing of compounds on tissue culture has similar drawbacks to testing on cell cultures. In tissue cultures, the interaction on only a single tissue type is tested in each culture. This method is slightly better than cell culture, because it uses the actual tissues of a subject instead of synthetic cultures. The effects on a specific type of tissue can be determined with a great deal of accuracy. However, the interaction between various tissue types and organ systems cannot be discovered accurately *in vitro*, in any type of test. *In vivo* tests of some type are needed for this purpose. Again, this analysis would be an extremely valuable initial step to a complete testing procedure. Using it extensively as a primary testing method would expose its shortcomings. Genetic differences between the tissue culture and the general population could become apparent during testing, and could cause unexpected side effects from interaction with enzymes unique to other tissues.

Some opponents of animal testing suggest the use of sophisticated computer systems to mimic the reaction of living animals to the introduction of any chemical. The existence of computer programs to effectively mimic every facet of the chemistry of living beings at our present stage of technology is not possible. We still do not understand much of the complex chemistry which occurs in living cells. Without this knowledge, it is effectively impossible to build an accurate model of the impact of foreign chemicals on the system. If the entire genome of the target animal could be decoded, and the function of each

protein built from it could be deciphered, we may be able to accomplish this feat. Until our knowledge and technology advances a great deal, this is merely a fantasy. These systems, if they could ever be developed and perfected, would be invaluable, and would save many animal and human lives.

Another alternative to animal testing would be the initial and direct testing on human beings. Late-stage testing is already performed on humans, but human subjects could replace animals used in the early stages of testing. This scenario, however it is presented, is not acceptable. The cost in human lives and health would not be acceptable or humane. It would obviously be much too dangerous to test in this fashion. The long-term effects of whatever compound was being tested would also be unknown, and could cause harm later in life, such as the birth defects caused by the mothers who were given the anesthetic thalidomide in the 1950's (http://www.fda.gov/cder/news/thalidomide.htm).

Animal testing at this point in human technology is a necessary evil. No viable alternatives exist which could take its place. Each alternative has its own shortcomings, and if misinterpreted or utilized improperly, could cause serious problems.

1.3. Cost and Benefits

The argument over animal usage today is largely ground in economics. In our free market economy, a major driving force to our ethics is the cost of something. When presented with a more costly, but more humane, process, we look at the differences and decide which marginal utility is greater. What price do we put on life and suffering?

Several applications of these methods have no equal. In biopharming, there is no cost effective alternative. Rare proteins can be too costly or impossible to synthesize. Xenotransplantation eliminates the shortage of organ replacements. This could end donor lists and could save the thousands that die waiting every year. While gruesome to some, disease modeling is crucial to the understanding of many diseases that affect us. Smallpox and polio could be running rampant to this day if it were not for the advances made using disease modeling. These applications are necessary and are required by our society today.

The application of these methods will greatly enhance the quality and quantity of human life at the cost of the lives, qualitative and quantitative, of the animals. This gain is not without cost and for this reason we must take into account the sacrifice of animal life. However, human instinct drives us to survive and continually improve our own quality of life. A balance must be achieved between our desire to constantly better ourselves and our compassion

for the co-inhabitants of our environs. We must prioritize our medical goals and keep in mind the unfortunate sacrifices we make in our quest for an enhanced existence. We must forgo some of our medical goals fore the detrimental effects to animals would be too great.

1.3.1. Disease Modeling

Perhaps the most beneficial of the three methods discussed, disease modeling gives researchers great control over the observation of disease. With the ability to observe the entire lifecycle of a disease, researchers are more able to fully understand how a disease works. Once they are able to comprehend how a disease works, they can engineer methods to nullify its potency and derive a cure. Disease modeling has been used in all aspects of research. This process is responsible for polio and smallpox vaccines.

As the most beneficial to human life, it is also the most costly for animal life. Animals under study are given diseases, original or modified strains, to be examined until death. Possible cures are given with the risk of unknown side effects. Many of the animals that are used live miserable lives. However, the simple fact remains that disease modeling with animals is the reason why so many horrible diseases have cures or effective treatments.

1.3.2. Xenotransplantation

Xenotransplantation is a new area of research that promises replacement organs for all people who need them. Instead of using human donors, replacement organs can be genetically engineered in animals. Prior to birth, the donor animal is genetically modified, by changing genes coding for immune recognition proteins, to make sure that the recipient does not reject the new organ. Upon death of the animal, the organ is given to the human with little chance of rejection. This is an opportunity to eliminate donor waiting lists and save the thousands who die waiting on them.

While the life of the animal is ended in this process, this may actually enhance the quality of life for a species. Presently, we use only some of the animals that we eat. The rest of the animal is thrown away. Xenotransplantation is a method that could increase the usage of an animal. A pig could be slaughtered for its meat but with some prior genetic manipulation, a heart could also be obtained from the pig for transplantation into a human.

1.3.3. Biopharming

Biopharming, or the production of recombinant proteins by modifying an animal to secrete them in its bodily fluids, is conducted for economic reasons or for sheer feasibility. Researchers and scientists have discovered various ways of

using animals to derive human proteins and enzymes. Synthetic processes are often quite expensive, if they are possible at all. Biopharming has created treatments for cystic fibrosis and rare cancers (http://www.transgenics.com/about.html). Without this process, there would no treatments.

For the animals, this has little effect. Needed proteins are secreted into their milk or blood. The milk or blood is collected, reduced and purified. Animals are not harmed in this process in anyway. There is almost no difference between milking an animal for the milk itself or for a biopharming protein secreted in the milk.

2. Thesis Statement

Thorough technical, ethical, and legal analysis demonstrates the necessity of continuing transgenic animal research in the areas of disease modeling, xenotransplantation, and biopharming for the betterment of humankind.

3. Methodology

3.1. Division of Labor

In the interest of becoming familiar with each transgenic method, it is the authors' plan to divide the work by section instead of by major topic. There are three main sections for each method type. There are the legal issues, the ethical issues, and the industry issues. Since the authors share different technical backgrounds, this division is logical and the most equitable.

Common sections will be divided as well. The introduction is made up of three sections: past, present, and future. This corresponds to three authors and they will each write one section. Common tasks such as editing will be shared by the authors.

3.2. Methodology

The purpose for the arguments here are to present a comprehensive ethical and legal analysis of animal testing; specifically the methods relating to biopharming, xenotransplantation, and disease modeling. The analysis presented will inform the reader that these methods may not be desirable in today's society. Instead, research involving these methods is a necessary evil that our society has relied on and continues to rely on to this day. By removing these tools which are currently available to researchers, our society will be prevented from providing even the most basic and expected improvements in modern medical care.

From the earliest recorded time and through the present, humans have used animals for various purposes that have drastically improved our society. It is only recently that we have begun to question these practices. However, it is with no small debate that medical researchers continue to rely on animals. Researchers constantly evaluate the costs associated with animal testing and weigh the needs of good with each individual animal life.

There is simply no current technology or system that can replace these methods. Alternatives have been evaluated and shown to be inadequate previously in this paper. Current research involves some of the most heinous diseases and depends on animal testing to save millions of human lives. In

addition, the current legal and ethical climate will show that this trade off is made with compassion and the best spirit and tradition of humankind.

3.3. Statement of Personal Beliefs

We feel that for the betterment of society, certain testing on animals is acceptable and necessary. Throughout time, humanity has used animals where needed. Medical history and past precedent have brought our society into an era were we rely on animal testing to increase the effectiveness of our research. While we wish that this were not the case, regrettably it is our only viable option.

Some would argue that relying on the argument of past animal usage is unfair and that we should re-examine our beliefs toward this subject. However, there are certain applications where animal usage cannot be replaced. We do hope that someday, animal testing can be replaced by sophisticated computer modeling. Until then however, there is no substitute for the integral role that they play in the development of new treatments and medicines.

In the method of biopharming, the animal does not suffer, as any genetic modification does not affect the animal in an adverse way. In normal applications, such as introduction of a hormone into a cow's milk, the cow's quality of life is not impacted in any way. These modifications are harmless to

the animal and beneficial to mankind, therefore there is little argument to support any reason to halt this practice.

Xenotransplantation increases the effectiveness of animals that are currently used. Rather than using an animal for its meat alone, we can modify the often-discarded parts so that they may be used in humans. We feel that this is an ethical step forward as we are using fewer animals and using them more effectively, much like the Native Americans. Like biopharming, there is no reason to halt research in this field.

Disease modeling is an issue that creates a heavy heart, posing a more difficult decision. Unfortunately, the method that causes the most harm out of the three methods that we are examining has the potential to yield the greatest good for humanity. Because of this, we must pursue this process with great respect to the animals that are used. Recent and future advancements in biology can hopefully reduce and eliminate the use of this method.

3.4. History of Animal Usage

Humans have used animals as beasts of burden since the beginning of time. In the early beginnings of recorded history, humans used animals to till the land and as a food source. As societies became more complex, property rights were adapted to include animals. Society has always carried the perception of

animals being inferior and carrying the workload. The development of our current perceptions is based on these past events.

From as early as the Bible, humankind is given dominion over animals. "...and let them have dominion over the fish of the sea, and over the birds of the air, and over the cattle, and over all the wild animals of the earth, and over every creeping thing that creeps upon the earth." (Genesis 1:26).

Archeological findings from 5000 BC to the present have revealed the use of animals by humans for a variety of tasks; some more humane than others. The Chinese used animals' fats for hormones and other medicines (Greacen 2003). The people of South America used animal bones and hide for clothing (PBS 2003). These patterns indicate that humans have used animals for protection and medicine in addition to trade.

Animals have seen other uses in an elevated standpoint. Pets are commonly described in early works. The peoples of many nations such as Egypt and much of western Asia have worshipped cats as gods. China and Babylonia were also countries that prescribed to this model.

During the Roman Era, medical experimentation was first introduced. This method was not held in high regard and not accepted by the mainstream. It was during the Neo-Classical Era when animal experimentation was pursued, after the Church had banned the same practice on human cadavers. Many new and radical thinkers like Leonardo Da Vinci studied human anatomy through the

bodies of other animals or even by defying the Church's prohibition (HSUS 2003).

Jeremy Bentham's late 18th-century utilitarian arguments about the moral significance of animal suffering and Charles Darwin's On the Origin of Species challenged the view that humans were the center of the animal universe. They went on to critique the view that humans have the right to afflict animals with experimentation and work. The social elite heard these arguments and thus gradually changed the commonly held beliefs in Europe during the 1700's.

America experienced a similar social revolt involved the stance of animal testing. While not as strong, several bills were introduced to Congress to regulate the use of research on animals. Several Supreme Court Justices were also involved in these bills. While none of these bills had much merit or created restrictions on medical experimentation, these bills spoke to the uncertainty that Americans had with regard to animal testing (HSUS 2003).

In the early 1900's, animal protection issues were much less noticeable. The American Society for the Prevention of Cruelty to Animals held that medical experimentation was unethical. This belief was dropped shortly after the organization was formed that gave researchers a stamp of approval for future testing.

Later in the 1900s several organizations have been working hard to remove animal experimentation from the public venue. The Animal Welfare

Institute and The Humane Society of the United States were formed with the goal to increase animal experimentation awareness. They have put pressure on governmental bodies to increase and restrict testing. During this time, a small but vocal part the public has grown sympathetic to this cause that continues today.

3.5. Current Animal Usage

Currently, animal testing is used by a variety of companies for a wide range of purposes. These purposes include fundamental and applied biological research, behavioral research, animal use in education and training, production of useful biological and therapeutic materials, and use in product testing. Each of these classifications of testing involves different methods and risks to the subject animals. This testing has led to crucial advances in many fields, and has aided humanity in a variety of ways. The number of creatures used in research, as estimated by federal agencies, is around 17 to 22 million animals, including 50,000 cats, 61,000 primates, 180,000 dogs, 544,000 rabbits, and millions of mice and rats (by far the most abundant subjects) (Williams, 52).

The most basic usage of animals is in fundamental research. The aim of fundamental research is to advance the knowledge of a subject without a specific commercial aim. Most of today's research is not completely fundamental in

nature, as it aims to extract a final product of some kind. A few examples of these types of research are included next. In biomedicine, systems from the cellular to organism level are analyzed and hopefully understood more thoroughly after the research. Interactions between wild animals and their surroundings can also be studied to help understand biological processes or the ecological consequences of certain actions. The field of disease modeling would fall into this category. Some animal species can act as accurate representations of the human body in certain situations, allowing for an in-depth study of the disease without further danger to human life. The animals may be genetically mutated or selected for this purpose to create a more accurate model. This could also include the utilization of animals or animal tissues in vaccine testing or production.

Behavioral research is another important area of current testing. The study of animals should allow researchers to more completely understand human psychology. This includes studies in areas such as depression, drug addiction, aggression, communication, learning, problem solving, and social behavior. These experiments range from the most benign, such as a noninvasive observation of a creature in the wild, to the most harmful, such as the exposure to repeated physical or psychological abuse.

Animals are widely used in educational environments also. The majority of students at the high school level and above have had experience with animal

dissection, simple behavioral studies, or more complicated animal experiments. Again, the scope of these studies will vary widely, from high school students studying the behavioral patterns of small insects to veterinary students receiving vocational training on live subjects. These experiments seek to inform the students, and hopefully aid them in their future careers, not to advance science in any significant way.

A fairly new use of animals is in the production of useful biological and therapeutic materials. Two of our main studies, xenotransplantation and biopharming, would fall into this category. The product can be a naturally occurring substance harvested from the animal, or the production of the substance may be because of purposeful laboratory manipulation, such as through genetic engineering or the introduction of a venom or virus into the animal's system. Many pharmaceuticals, such as protein factors and anti-venom compounds, can be produced much more effectively through this method than any others currently available.

Perhaps the most controversial and publicly disputed method of animal testing is the testing of commercial products on laboratory animals. Certain classes of products, mainly chemicals, pharmaceuticals, and cosmetics, are regulated by the government, and must be tested before being released to the public. These tests include acute toxicity tests, such as the infamous LD50 test (which will be discussed later), biological screening tests designed to determine

the biological effects of a certain compound, carcinogenicity tests, developmental and reproductive toxicity tests, eye and skin irritation tests such as the Draize test (also to be discussed later), mutagenicity tests, neurotoxicity tests, and repeated-dose chronic toxicity tests (Monamy, 57-61).

Two of the currently most debated and most common tests performed on animals are the LD50 test, and the Draize test. The LD50 test was created to standardize toxicity-level measurements of potent compounds. In this test, high doses of a toxin are given to large groups of animals until 50% of the animals die (hence *L*ethal *D*osage *50*%). Since its inception in the 1920's, LD50 has gained wide acceptance as the main measure of the acute toxicity of a compound. However, animal rights activists passionately oppose the use of this test, mainly because of the large numbers of animals sacrificed during the analysis (any surviving subjects are killed and examined two weeks after testing). Despite a number of less cruel alternatives, LD50 testing remains as the main measure of toxicity, and is necessary to standardize some therapeutic agents (Rowan, 203-213).

The Draize test is named after one of its creators, Dr. John Draize. Dr. Draize had been looking for a method to test eye irritants meant for use in chemical warfare, and the test that he created was later adopted by the FDA as a test for eye irritancy after a few examples of harmful cosmetics. In the Draize test, a measured amount of the substance to be tested is introduced onto one eye

of a test animal, usually a rabbit, with the other eye serving as a control. The test does not establish a scale; it is merely pass-fail. Almost any adverse reaction is enough to cause a failing mark, as a way of providing an adequate margin of safety. It has been widely criticized because of its apparent cruelty and probable inaccuracy, due to physiological differences between humans and the animals used in testing. As with the LD50 test, many alternatives have been proposed, but none have gained wide acceptance (Rowan, 216-228).

3.6. Modern Animal Rights Issues

Currently, many people, most vocally animal rights activists, have a variety of issues with vivisection, or the practice of experimenting on animals. Many of the practices that have been widely criticized were brought into the public spotlight by Peter Singer's 1979 book *Animal Liberation*. It inspired a worldwide movement of animal rights activists. Singer made the argument that animals, contrary to popular belief, have basic rights because they have feelings and desires. Much of the book documented various types of abuse caused to animals in medical, psychological, and commercial research. However, the mainstream view towards animal research remains pro-research, mainly because of the distinction that most people have between humans and animals. Moral and ethical arguments have been made towards the quality of life of test animals,

as well as their kinship to humans and the bearing of this on their perceived exploitation. Much of the debate rests on, as stated before, the distinction between animals and humans, and the significant differences that supposedly grant us superiority and the right to use animals for human benefit. The search for an acceptable set of distinctions and the debate over this issue is centuries old, and will not be resolved any time in the near future (Orlans, 20-32).

Perhaps one of the biggest debates today involving laboratory animals is the route through which they are obtained. For many years, the research community has relied on pound animals that were scheduled to be euthanized as a source of test animals. To this day, pound animals still represent a substantial percentage of test animals. However, animal rights activists vehemently opposed this route, saying that it undermines the view of the pound or animal shelter as a sanctuary for lost or abandoned pets. They suggest the alternative of animals bred for the specific purpose of research. These subjects are much more expensive, often prohibitively so, than pound animals. Activists argue that they are in a consistently better condition than pound animals, which may suffer psychological trauma from abandonment or other factors, or which may harbor hidden ailments or diseases. However, without this source of subjects, the research industry could be brought to its knees by skyrocketing costs. Advocates of the use of pound animals argue that the animal is slated for death anyway, and that by participating in an experiment, they can aid humans by their death.

The alternative would be a euthanized animal, and a second dead purpose-bred laboratory animal, doubling the cost in lives. No compromise has been reached in this country as of now, and this likely will be an ongoing debate that will continue to create a large amount of controversy.

3.7. Future animal use

Throughout time mankind has used animals in order to gain more knowledge and in an attempt to better ourselves. Currently animals are being used more than ever before and this usage is increasing every year. There is no doubt that in the near future animals will continue to be sacrificed in the name of research. This does not mean that man should just accept animal sacrifice as the only way, but should continue to strive for new ways of testing. On the other hand, the likelihood of alterative testing on non-living animals in the near future is not a bright prospect. Instead we should try to focus our efforts on finding more efficient and less cruel methods in the meantime. These are the topics of discussion that will continually plague us as we advance.

As time pushes forth, there are always new advances in research, new diseases to cure, new medicines to test, and new methods available. The role of animals in this progression seems to increase proportionally. We always seem to find a new way to use them, or a new reason to test on them. This is a natural

progression. As the demand for testing increases, the need for subjects must increase as well. This does not necessarily justify the use of animals, but it does explain the continual increase. It is important to remember, though, that as we increase the use of animals, in general, the more gain we are able to obtain from their sacrifices.

The most important factor in this testing is to not forget our goals or the losses that must come to reach them. This is why we must continually look for a better way to research and gain our knowledge. It would be ideal if humans could do all testing on highly advanced computer simulations. In this case, there would be no animals lost and we would still gain the knowledge that we desire. The feasibility of this in the near future is not great. Other ideas such as biochemical simulations or synthetic tissues are also being pursued. These ideas have not been developed enough to be used, but are probably more likely to be used before any kind of computer simulation.

With no end for the demand of animal usage in sight, we must focus our efforts on better ways to test on animals. We must try to find a way to improve the quality of life of the animals, use them more efficiently, and basically use them only when necessary. One such example of a method that would better our research is the use of cultured eye tissue in the aforementioned Daize test. When using cultured tissue, you would not be harming an animal but still using living tissue as a sample. In addition you could use a cell count to establish a scale of

irritancy based on the amount of tissue affected by the irritant rather than the current pass/fail method. Another alternative for the same test would be the use of a bovine eye, acquired from a slaughterhouse. In this case the sample would be animal based, but the animal would have died for another purpose and the test is using an otherwise wasted part of the animals body. Either of these cases would lessen the amount of sacrificed animal lives and still be effective (Rowan, 220).

3.8. Premise of Ethical Argument

Ethics is defined as the judgment of the manners, customs and habits and their implications (Dewey, 1). As can be told by the definition, ethics is not a clear science with black and white answers. Being a subjective topic, a premise of argument needs to be defined so that there is a platform for the comparisons to be made. In order to do this a general background on the theories of ethics needs to be discussed so that the basis of the arguments will be known.

There are two main elements that make up ethics. These elements are what is good, and moral duty/obligation. Both of these elements are subjective and have many different theories about each of them. The first of these elements in itself is entirely based on the individual. Some people have defined good as pleasing to God, others as what is beautiful or natural, and still others as what is

pleasurable. In all of these cases the definition of good is just as subjective and would not help in defining what is more ethical. On the other hand, two philosophers of different opinions did come upon methods of quantifying goodness for the comparison on what is more ethical. Jeremy Bentham quantified good based upon that one man's pleasure is just as important as another man's as long as the quantity of the happiness was the same. This philosophy is purely one of quantity, as it can be implied that the method that brings the greatest quantity of good is the more ethical one. On the other hand, Nietzshe believed that certain people had a higher capacity of happiness and that what brought the higher quality of happiness was better. It can be taken from this that he believed that quality is what matters more then the quantity of happiness (Rachels, 21). These quantitative methods are not as subjective because numbers can be implied for quantity and quality, but this still leaves the discrepancy on whether quantity or quality is more important. In this paper a utilitarian ethical analysis will be used with a basis that takes into account both quantity and quality.

In the arguments above, it is assumed that all the subjects are human and therefore of equal importance. In the following situations not all subjects are human, which changes the debate. It is the belief of some that the life of an animal is just as important as the life of a human. More commonly people have the complete opposite view such as one Dr. Spinoza who said:

> It is plain that the law against the slaughtering of animals is founded on vain superstition and womanish pity than on sound reason. The rational quest of what is useful to us further teaches us the necessity of associating ourselves with our fellow-men, but not with beasts, or things, whose nature is different from our own; we have the same rights in respect of them as they have in respect of us. Nay, as everyone's right is defined by his virtue, or power, men have far greater rights over beasts than beasts have over men. Still, I do not deny beasts feel; what I deny is, that we may consult our own advantage and use them as we please, treating them in the way which best suits us; for their nature is not like ours, and their emotions are naturally different from human emotions *(Midgley, 10)*.

Our beliefs are not in either extreme, but stand somewhere between where Dr. Spinoza stands and the view that any one life is equal to another. For the arguments that follow, the life of a human is one that will be weighed more heavily than that of an animal, but the life of an animal will not be considered valueless. This is based on the hierarchy of nature where one animal must use another to survive making it higher in the hierarchy.

When comparing each of the three methods of using transgenic animals, the loss of the animals will be compared to the amount of gain obtained by humans. In this, both quantity and quality of human and animal life will be compared in such a way that the quality of life and number affected will be taken into account. The ideal situation would of course happen if very few animals were affected and those who were affected have little or no change in quality of life, while dramatically improving the quality of life of a large amount of humans. However, this is a very unlikely situation. Unfortunately due to the limitations of science currently, some loss must take place in order to gain knowledge and medical advancement. The bigger question then becomes when the loss is too great for the gain to be ethical. The following arguments will explore to what extent that these losses exist for each case.

3.9. Animal Testing and Usage Regulation

Ever since the formation of the Society for the Prevention of Cruelty to Animals (SPCA), there has been a formal movement against the practices with animals that humans were using. Scientists and researchers have used disease modeling, xenotransplantation, and biopharming in the past 50 years without legal incident but there have been additional laws past to aid in regulation of a

particular process. The more difficult questions remain with the intellectual property rights surrounding modifications.

Not all animal rights groups are the same. Many of these groups were formed in the 1970s. They are broken into welfarists, pragmatists, and fundamentalists. The welfarists accept most of the animal practices and their current usages but continuously work to reduce their pain and suffering. The ASPCA is an example of this organization. The pragmatists, such as Henry Spria, are more radical feeling that only if the outcome of animal usage outweighs their suffering. The most radical type of activists is a fundamentalist group like the Animal Liberation Front. They will use any means possible, including sabotage, to free animals and to break lab equipment.

Disease modeling has the most difficult legal justification. At the same time there is the strongest human justification for this technique. Since its first great success with the Polio vaccine there is been little contest over the recognition of this technique. Laws have not been extended to this area as the courts have not been willing to enter these questions. Most animal rights groups work to reduce the pain and suffering that these animals endure during testing.

Xenotransplantation is one of the most recent additions to transgenic techniques. With conventional techniques, the sale of organs is strictly prohibited. The National Organ Transplantation Act makes sale of organs or tissue a federal felony. The act tries to create a spirit of "promoting a sense of

community through the acts of kindness (Sutton 229)." The Uniform Anatomical Gift Act was written to facilitate the donation of organs and/or tissues for medical purposes. Even with these two acts, there has been a shortage of organs.

The practice of animals donating their organs in a compulsory manner has not currently been questioned. With the first heart transplantation from an animal in 1967, there have only been questions in the legal arena. How are organ shortages handled? Can states sponsor and encourage organ donation and who owns to organs upon a donor's death? With the introduction of genetically modified animals raised for the sole purpose of organ transplantation, only more questions are introduced. Modifying an animal's genetic structure for a single recipient does pose a stronger legal question. This predestines a specific animal for a specific fate. This question is different from raising cattle for the expressed purpose of slaughtering for food, as the law accepts this as a whole population whose fate is predetermined and not an individual.

Biopharming has regulations that are completely different from the other two techniques that have been mentioned. Products of this technique are used in food and pharmaceutical processes, which give the United States Department of Agriculture complete control. They are able to control all of the products that are allowed in the public arena. The public has been vocal about the lack of legal and regulations that oversee the procedures that companies and researchers use.

Intellectual property rights are the main stumbling block for disease modeling, xenotransplantation, and biopharming. The courts have not decided on how best to allow for genetic modifications and whether modifications can even be protected under current patent laws. Until there is a general consensus on the regulations and intellectual property rights, there will be little adoption and further development of these techniques. If businesses are unsure that they will be able to protect and keep their developments then they will invest their research dollars in other areas.

4. Discussion

4.1. Disease Modeling

4.1.1. Background and Theory

For years, humans have been using animal subjects for a variety of scientific tests. They have been utilized in such fields as pharmaceutical research and physical trauma studies. A reasonably new venue of animal research is the use of animals to model human diseases and ailments. These animal models can be studied to analyze symptoms and signs of the progression of the disease. Various novel treatment options can also be analyzed and attempted on the animal subjects. However, until the advent of genetic engineering and transgenic animals, there were very few suitable subjects for testing. The majority of these were randomly mutated strains of small mammals that happened to demonstrate similar symptoms as their human counterparts. Because of this, the field was extremely limited and did not show a great deal of promise. However, as genetic manipulation techniques became more advanced, the possibility of creating extremely accurate models arose. Knocking out or modifying the gene causing the human disease, or the corresponding animal gene, could accomplish this. Transgenic animal models that accurately portrayed many aspects of human diseases began to emerge. Today, these

include mice with Alzheimer's disease, cystic fibrosis, and AIDS susceptibility, pigs with kidney dysfunction and atherosclerosis, and extremely accurate sheep models of cystic fibrosis (Clarke, p. 9-10). Obviously, as our knowledge of the genetic causes of diseases and genetic manipulation increases, this list will grow rapidly and allow us to cure or more effectively treat these ailments.

Perhaps the most common and most widely studied transgenic animal model is the Alzheimer's mouse. To better understand the model, a general understanding of the physiology of Alzheimer's disease is needed. In the beginning of the 20th century, Alois Alzheimer began studying a disease that struck mainly middle-aged people and caused a progressive deterioration of mental and behavioral functions. Seizures were observed in later stages of the disease, and eventually all higher brain functions were highly impaired. The brain itself, particularly the outer layers, appeared decayed and weakened.

Today, Alzheimer's disease has been more thoroughly studied and is characterized by three important pathological changes, diffuse plaques in the brain composed mainly of coagulated β-amyloid (Aβ) protein, intracellular neurofibrillary tangles (NFTs) which consist of hyperphosphorylated tau protein, also in the brain, and loss of neurons in the brain tissue. The most effective animal model of this disease, therefore, would have to accurately portray all three of these features (Chesselet, 51). The most important and prominent change is the appearance of the aforementioned neurofibrils. Their concentration

is especially high in the hippocampus, which helps to control short-term memory. These tangles, which can be readily identified by silver-staining techniques, are normal in older people, but in Alzheimer's patients, the density and concentration of these features is markedly increased. The tangles consist of pairs of filaments intertwined in a helical fashion, narrowing to a width of 100 Angstroms at 800-Angstrom intervals. They form in the neurons, most frequently centered around the nucleus of the cell. These tangles can be caused by a genetic predisposition, as well as trauma or other environmental factors. It is this predisposition which can be programmed into transgenic animal subjects for further study. Another physiological symptom of Alzheimer's disease is the presence of β-amyloid plaques throughout the brain, especially in the same general areas as the neurofibril tangles. These plaques often occur in older individuals, just as the tangles do, and are closely associated with the onset of dementia. The origin of this material is thought to be from the decomposition of certain immune protein chains produced in response to antigens, or foreign proteins in the body. The third symptom, or loss of neurons, causes what is called spongiform encephalopathy, or the degredation of brain tissue. A brain showing this condition would appear full of holes and would resemble a sponge. These pathological changes seem to begin in the hippocampus, the primary center of short-term memory, and eventually affect the amygdala and the cerebral cortex, concerned with emotional and cognitive functioning,

respectively. A combination of these factors appears to cause the typical symptoms of Alzheimer's disease, which include memory deficit, generalized dementia, and increasingly shallow emotional responses (Reisberg, 12-37).

As stated above, to be a perfectly accurate model, an animal would have to faithfully recreate all three of the main physiological changes in Alzheimer's disease. To date, no model truly does this. Different aspects of the disease must be studied in different models, each providing a bit of information and a small piece of the overall puzzle. A few key genetic targets have been identified for further study in Alzheimer's research. Approximately half of the cases of the most common type of early-onset (before age 60) Alzheimer's disease are caused by dominant missense mutations in three genes encoding for transmembrane proteins. Late-onset Alzheimer's is not believed to be caused by a single genetic defect, but one allele of a certain gene on chromosome 19 is believed to cause an increased susceptibility to the disease. The Aβ protein responsible for the plaques in the brains of Alzheimer's patients is another genetic target. This protein can display variability in length, with longer versions being more likely to form the symptomatic plaques. Transgenic models attempting to replicate each of these factors individually have been created. The most common strains replicate the extensive extracellular deposits of Aβ protein, or plaques.

The ultimate goal of these models, as with all disease models, is the development of treatments to improve the conditions of or halt the progression

of a certain disease. These models also provide novel insights into the diseases that they mimic. For example, models have caused researchers to realize that the importance of the Aβ protein is more prominent than first believed. It has also led to the development of novel treatments, such as the vaccination of transgenic subjects with a small amount of Aβ. This essentially prevented the development of the disease in test mice if performed at an early age, and reduced the progression of the disease if performed later. New models consistently appear, mimicking different factors of the disease and providing new insight into its pathology (Chesselet, 51-65).

Another disease for which transgenic animal models have been developed is Huntington's disease. Huntington's disease is an autosomal dominant progressive neurodegenerative disorder that is generally first manifested in middle age. Onset can range over the entire human life span, from early childhood to greater than seventy years of age. The symptoms are complex and vary from case to case. They include a variety of emotional, motor, and cognitive components. Postmortem studies of the brains of Huntington's patients reveal severe atrophy, up to a loss of 30% of typical brain weight. The HD gene, which is responsible for Huntington's disease, is 180 kbp (kilobase pairs, or thousands of DNA bases) long and has been pinpointed and sequenced. A mutation causing an abnormal chain of glutamine residues causes the production of a defective protein, which is expressed throughout the patient's brain tissue.

Because of the dominant nature of the HD protein, a transgenic model could be produced, in theory, by the direct insertion of a mutated gene without the targeted removal of the wild type genes or selective breeding. A few different methods can accomplish this, but the technical details of these procedures are beyond the scope of this paper. To date, only one published mouse model of Huntington's disease has been produced, expressing a smaller version of the extended protein. These models show a marked decrease in brain mass and some of the symptoms of the disease. Study of the affected mice has caused researchers to rethink the entire proposed mechanism that was believed to cause Huntington's disease, as well as its specific affects on brain tissue. These models have provided researchers with a wealth of valuable data that probably could not have been obtained from other, more conventional, sources (Emerich, et. al, 355-362).

Another application of disease model animals is in the study of tumor suppression genes. These models are among the easiest to produce, because a specified gene can simply be "knocked out" and deactivated. This allows the study of the abnormal phenotype, in the absence of the protein in question, and does not require the insertion of a foreign gene or the production of a novel protein. However, knockout models often produce undesirable, unexpected phenotypes that make it impossible to analyze data obtained from performed experiments. The conservation of a gene's function usually indicates that it is

essential to some process in the life cycle of an organism, and the elimination of that gene will invariably cause problems. Despite their limitations, these models allow the study of genes that have human counterparts, and their effects on the organism. Tumor suppression genes contribute to disease by virtue of their inactivation or improper functioning. In nature, this is usually caused by an acquired somatic mutation or inactivation by viral DNA. Important tumor suppression genes have been identified and studied using this method, including the Rb (retinoblastoma) gene in the Rb deficient mouse, and the vital cell-cycle protein p53. The p53 gene was found to be mutated in the majority of human tumors from a variety of tissues. Therefore, it was obviously a prime candidate for intensive study. The results obtained from p53 knockout mice provided new insight into the function of the gene, but did not exactly match the functioning of the human gene, as expected. As with all models, new information can be gained from exhaustive study, but entire problems cannot be solved with these representations, further study and experimentation is needed (Houdebine, 411-417).

For some time, animal models have been instrumental in AIDS research. However, only recently have researchers begun to use transgenic models. Up until this development, the closest representation of human AIDS was Simian Immunodeficiency Virus (SIV) in macaques, which are prohibitively expensive and rare. Even SIV did not produce a picture of AIDS accurate enough to be of

widespread use to researchers. Biologists have therefore been attempting to produce transgenic animals which are susceptible to infection by the HIV virus and which will demonstrate some, if not all, of the human symptoms of the disease. Regulatory proteins, as well as cellular surface recognition proteins, have been inserted into mouse and rabbit genomes, with varying degrees of success. Vital genes in the development and spread of the AIDS virus have been identified in this way, and researchers are approaching a usable animal model of the AIDS infection in human beings (Houdebine, 427-431).

In addition to the models discussed above, transgenic animals displaying symptoms and phenotypes of other diseases have been created. These include cystic fibrosis mice, rabbits and pigs used in atherosclerosis studies, genetically obese mice, and Drosophila with a variety of neurological diseases, such as Amyotrophic Lateral Sclerosis (ALS, or Lou Gering's disease), Huntington's, Alzheimer's, and Parkinson's (Chesselet, 373-374). In all of these cases, researchers strive to better understand the disease, its symptoms, and its physiology. The eventual goal is a cure or effective treatment for each disease, which would save many lives and improve the quality of life for many people.

4.1.2. Ethics

Disease modeling brings a major ethical argument into question. It has potential to cure or aid in many diseases, but the loss of animal life to get it may be substantial. The diseases that are targeted by diseases modeling though, like Alzheimer's, affect many people making a need for some kind of cure great. Some of these diseases are Alzheimer's, cystic fibrosis, Parkinson's disease, kidney dysfunction, atherosclerosis, and AIDS.

Alzheimer's disease affects about 4 million people in America alone. Currently, that is one out of every ten people over the age of 65 and one out of every two over 85. It is currently estimated that by the year 2050 over 14 million people will have the disease (Alz. Assc.). This is just the number of people affected by one of the diseases that disease modeling targets. For example, as of December 2001, there were 816,149 Americans affected by AIDS (CDC). One other ailment that disease modeling can help to improve is cystic fibrosis, which affects about 30,000 Americans (CFF). As can be seen, the diseases being tackled by disease model research are some of those that affect a great deal of people making the potential for benefit very, very great.

People with diseases like Alzheimer's disease have a highly degraded quality of life. At first, Alzheimer's disease will start to affect cognitive thinking and memory. As it progresses the loss of memory and cognitive thinking ability becomes so great, the person must be under constant care of others. The disease

in its later stages eventually starts the onset of dementia and eventually seizures. Alzheimer's can be a fatal disease, causing vital areas of the brain to stop functioning as the cells in that region die. The rate at which this disease spreads varies anywhere from three to twenty years from early warning signs to its fullest form (Alz. Assc.). HIV is another fatal disease targeted by disease modeling. People with HIV suffer from an extremely impaired immune system. The HIV virus attacks the infected persons immune system, leaving the infected person more susceptible to diseases. People with HIV often are sick with common diseases and are often are sick for longer periods of time than a non-infected person. HIV in its highest form is called AIDS. In addition to the extreme vulnerability of the infected people to disease, they often suffer from coughing and shortness of breath, seizures and lack of coordination, difficult or painful swallowing, mental symptoms such as confusion and forgetfulness, severe and persistent diarrhea, fever, vision loss, nausea, abdominal cramps, and vomiting, weight loss and extreme fatigue, severe headaches, and eventually a comatose state that is often fatal (NAIA). As can be seen, the quality of life of the people infected is highly degraded in both cases of these fatal diseases. This means that the improvement of quality of life for these people would be very high if a cure for these diseases would be found.

As can be seen, there is a great deal of both quantitative and qualitative human suffering caused by the diseases targeted by disease modeling. This is

why there is such a large interest in the scientific and medical community for this area of research. On the other hand, the quantity and quality of life of the animals must also be taken into account as well.

Most diseases cannot be exactly and directly modeled in an animal in which it does not naturally occur. Only a single example or part of the disease can be modeled at one time in one animal giving a small "snapshot" of the disease. This means that many different models must be made in order to get enough data to gain any reasonable amount of knowledge about the disease. Many animals with each of these traits must be bred also. This means that a large amount of animals must be tested in order to achieve the desired scientific goal. This does not account for the number of animals that may have been genetically modified incorrectly, or who didn't respond to the genetic therapy in the desired manner. This brings a total loss of animal life for a single disease model to a very formidable number. The exact number of animals tested on for disease modeling is not known because the government does not require researchers to keep track and/or report a count.

In addition to the number of animals used in disease modeling, the quality of their life becomes a matter of importance as well. This varies greatly with each individual animal and model. In cases like HIV, the animal, normally a pig, is infected with the actual disease. The pig will then suffer from many of the symptoms and problems that a human with the disease would. In this case, the

animal suffers from a very poor quality of life, just like a human with the disease would. In other cases like Alzheimer's the animal, normally a mouse, is only given a certain trait or part of the disease as it cannot be fully transferred to the mouse like HIV to a pig. In this case, the seriousness of the alteration can vary. Some animals will not suffer from any side effects or symptoms at all, but they may show a small physiological change (like growth of a protein). Other mice will show no change or effect at all. With these mice, the quality of there life has not been made worse by the experiment. On the other hand, other mice may show large changes and almost all of the symptoms as if they had the disease. In these mice, the quality of life will be reduced to the same level of that as a human who was infected with the disease. The quality of life of these animals varies greatly from no effect, to severe effects. In general, there is a reduction in quality of life of the animals and often times it is closer to the severe side.

As can be noticed by the amount of mice that are involved and the drop in quality of life of the animals, the animal loss is great. In addition to this, it is general practice to euthanize lab animals when the experiments are done. This means that this method is virtually always fatal to the animal that is used in testing. In addition, it should be noted that the animals that do suffer from this often only suffer a short period of time compared to that of a human with the disease. In any event, there is a very significant loss of animal life both quantitatively as well as qualitatively.

The loss of life (quantity and quality) of both the animals and the humans is great. Both have high mortality as well as have a severe drop in the quality of life. If disease modeling was not studied and the disease was allowed to propagate freely, the loss of human lives would continue to grow but no animals would be harmed. If a cure is found for these diseases through disease modeling, a finite number of animals would be sacrificed, but an uncountable amount of lives would be saved as the disease was fought off by the new cure. In short term comparison, the loss of animal lives would be very close to the loss of human lives since many animals would be tested and have given their life, as humans would have died from the disease. In the long term, the loss of animals lives would be far fewer as the disease was brought to control and fewer and fewer humans died while no more mice would have been sacrificed once a cure was found.

Disease modeling has already contributed greatly to the cure of one disease. An animal model of cystic fibrosis was used in the study of gene therapy as a cure (Upenn). Currently, gene therapy is being used to synthesize healthy proteins in the lungs of people who have cystic fibrosis. This is a temporary aid to the infected person as it helps reduce the number of new infected lung cells, but it does not cure the disease. Research in gene therapy to eliminate the parent gene that causes cystic fibrosis is ongoing and showing promise (CFF).

As a whole, disease modeling shows much potential from an ethical standpoint. The decrease in loss of human life will be large when a cure for a disease is found. Even if the disease cannot be cured, but the intervention increases the quality of life of those infected, there is still a large gain. This has real potential to happen as proven by the progress in cystic fibrosis research and is not just an unproven theory on a way to find a cure. This great gain does not come without any sacrifice though. It is important to remember that there is a large animal loss in order to complete the task. As shown, when disease modeling is used for a widespread disease, the amount of human gain becomes far larger than the loss of animal life. If disease modeling was used for a rare, or non-largely populated disease, the loss for animal life would be much greater in comparison to that of the human gain. For this reason disease modeling should be considered an ethical way to gain medical and scientific research if it is used for a disease that is well spread and poses a large threat to the quality of life of those people infected.

4.1.3. Legal

Disease modeling is not a practice that is questioned. Originally stemming from animal cruelty laws, disease modeling is a legal and accepted process for establishing controlled studies. The more interesting component of

transgenic animals regards the related intellectual property rights. In order for innovation to continue, there must be incentive to researchers to continue their work in that field.

The history for legislation in this area is based on animal cruelty laws. As explained earlier, the Catholic Church was opposed to human testing more so than animals which became the standard. Disease modeling has been questioned in addition to animal testing itself. However, transgenic animals are considered inventions, and as result, there is no jurisdiction. Should laws pass that make disease modeling illegal, transgenic animals would be exempt.

Disease modeling is so commonplace today that courts regular use the results from animals testing in decisions. Many of the cases involving tobacco companies have used modified animals to make stronger arguments about the effects to tobacco products. In "FDA v. BROWN & WILLIAMSON TOBACCO CORP," the Supreme Court upheld research that showed that certain populations were more susceptible to cancers and other diseases.

The problems with intellectual property are well illustrated with a single mouse. Researchers and scientists at Harvard Medical School developed a genetically modified mouse that was used to study the effect of carcinogens by watching the development of tumors. During these tests, the mouse reliably reproduced the characteristics of various human cancers. Using mouse models,

the lifecycle was much faster so researchers could collect massive amounts of data.

The Harvard Mouse has been a legal headache. Harvard University sought U.S. patents for the plasmids, the transgenic unicellular material, the mouse itself, and its offspring. The U.S. Patent and Trademark Office granted the patents in 1988. Harvard sought similar protection in Canada in 1985. The university argued that the oncomouse was a "composition of matter" under section 2 of the Patent Act and therefore a patentable "invention." The commissioner of patents approved a patent for the plasmids and the unicellular material in 1993, but not for the oncomouse. Meanwhile, Harvard received patents for its genetically re-engineered mouse in Europe in 1992 and Japan in 1994 Harvard appealed the Canadian commissioner's ruling, but the Federal Court of Appeals' trial division upheld it in 1998. The Federal Court of Appeal, however, reversed the trial judge's decision in August 2000. The Supreme Court of Canada's judgment in December 2002 restored the commissioner's ruling, leaving Canada as the only developed country to refuse the patent (Legal News 28).

As litigation still continues, cases such as these continue to work their way through the legal system. On its own, this is a single case involving two parties. However, there are many others that are being heard, each with its twists. Each

decision is part of a puzzle that when put together brings society into a mutual understanding.

4.1.4. Discussion

Disease modeling offers the hope of cures for many ailments that have plagued humans for centuries. As our understanding of genetics increases, we are becoming better able to deal with common conditions. Researchers are working to develop vaccines and gene therapies to cure these ailments and increase our way of life.

With the ability to remove and interject particular genes into animals, we are able to create controlled experiments to understand how genes and diseases interact. With the ability to isolate certain genes and the corresponding effect, it is quite easy to isolate causes of ailments and conditions.

Research on Alzheimer's disease has demonstrated that transgenic animals are a necessary component of a research's tool. Statistical models have increased the importance of the Aβ protein. Further testing showed that the Aβ protein essentially prevented the development of the disease in test mice if performed at an early age, and reduced the progression of the disease if performed later. This type of discovery is occurring in research for other diseases like AIDS.

From a legal standpoint, disease modeling has become a standard that is used in other cases to substantiate fact. Intellectual property cases regarding modification are a chaotic legal subject. As more of these cases move up to the higher levels of the court system, the Supreme Court will eventually hear a case with these issues. Most likely to occur in the next 5-15 years, that decision will set the precedent on intellectual property regarding these animals.

Studying how contagious diseases affect a large population is vital to understand the dynamics of disease. For full comprehension, mathematical models must be constructed to articulate how a disease behaves. With the numerous complexities of infection and modeling a large population, this is quite a task. However, it is vital to understand what the parameters so that health and government officials fully comprehend all of the factors should and outbreak occur (Hethcote 6).

Disease modeling gives researchers empirical evidence that allow for the creation of a model. The most difficult issue is to find the right combination of data. Preexisting data may exist from previous outbreaks. Unfortunately, it is often incomplete and provides no real insight to how the disease behaves. Since infectious disease models use a formal and controlled environment, disease modeling is the best way to discover the best control procedure.

Transgenic animals are better suited to this task. Researchers can evaluate how a disease based on particular genes. It could be discovered that certain

populations are more susceptible to infection or other immune (Hethcote 6). This information is crucial to doctors and other front line workers.

Transgenic animals used in disease modeling provide researchers their best tool for complete understanding of infections and conditions. With a model of how a disease spreads and possible models to contain infections, health and government officials have the tools that they need to combat infections. Research is continuing that studies dozens of ailments and conditions with the hope that cures can be discovered. Without these methods, medical science would be a less effective.

4.2. Xenotransplantation

4.2.1. Background and Theory

Organ transplantation has become the preferred treatment for the failure of major organs. However, unfortunately, the application of this lifesaving technique has been severely limited by an extreme shortage of donor organs. At any time, there are approximately 80,000 people awaiting an organ transplant in the United States. Unfortunately, many of these people will die before a suitable donor organ is found. According to one estimate, only 10% of people who could use a heart transplant actually receive one. Naturally, interest has arisen in the transplantation of animal organs into human beings, or xenotransplantation.

This has long been a story of science fiction, for reasons discussed below. However, with the advent of genetic engineering, many of the technical hurdles preventing this major medical advance can be overcome (Clarke, 11).

An accurate, concise history of transplantation is difficult to provide, mostly because of the sheer scope and complexity of the field. According to Sanskrit writings, it may have originated as early as 700 B.C., when a successful skin graft was performed as part of nasal reconstruction surgery. Other than this, there are few records of successful transplantations until the Renaissance, where skin grafts were again attempted, with reasonable successes. The field of modern transplantation began to develop in 1728, when John Hunter, the father of experimental surgery, performed a series of very successful autographs, or transplants within the same animal. The roosters used in his experiments survived and the transplanted organs, mostly claws and skin sections, continued to grow and develop. A series of advances were made over the next centuries, leading up to the first human transplantation trials (Keyes, 79-86). These experiments almost always failed miserably, mainly due to the lack of knowledge of the human body and immune system.

In 1932, a Kansas City doctor began using skin grafts regularly to treat patients with severe burns. He observed that skin grafts from family members seemed to fare better and last longer than those from unrelated donors. However, it was still impossible to accurately predict the longevity of a given

sample in a transplant patient. In 1937, J.B. Brown, a St. Louis doctor, achieved permanent survival of skin grafts between identical twins. This provided hope for the field of organ transplantation and demonstrated the viability of the methods being developed. Later, T. Gibson and P. B. Medawar discovered that a second graft from the same donor was rejected more rapidly than the first graft. This showed that the process of rejection was governed by an allergic or immunological mechanism which could potentially be blocked. A series of experiments with specific breeds of cattle eventually led to the discovery of acquired immunological tolerance. This development allowed a recipient mouse to successfully accept a skin graft from a donor which they had been previously prepared for. However, it was not of direct use to human organ transplantation, because conditioning for the new genetic material had to take place before birth. This development did bring hope and optimism to experimenters, because the immunological barrier between transplant donor and acceptor had been breached, paving the way for further research into the field.

In 1954, an opportunity arose for transplant researchers which could not be passed up. A patient with severe renal disease was referred to the famous Brigham transplant program. It was discovered that he had a healthy identical twin brother who would be able to donate one of his kidneys. There had been a series of human-to-human kidney transplants before this, but they had all been unsuccessful in one way or another. These experiments usually ended in failure

with the rejection of the organ. However it had been proven that, in animals, all immunological barriers were surpassed if a transplant was performed between identical twins. The surgical skills of two teams, one for the donor and one for the recipient, were put to the test. In the end, the transplant began functioning perfectly as soon as it was implanted. Both brothers recovered and the focus of further transplantation research became the issue of biological incompatibility and immunosuppression, since most other barriers had been overcome with this experiment (Murray, 1411-1413).

Research into the field of immunosuppression, a relatively new field before these developments, was increased in an effort to find a method to deal with the problem of organ rejection. When a foreign organ is transplanted into the human body, our immune system recognizes it as an antigen and begins to attack it. The organ is soon rejected, and ceases to function. The first means of immunosuppression was full-body irradiation, but this dangerous process was soon replaced by a series of potent drugs which accomplished the goal of suppressing the immune system much more safely and effectively. The first of these compounds was a derivative of 6-mercaptopurine, tested in the early 1960's. Today, a drug cocktail including a series of immunosuppressive drugs and steroids is used in transplant patients (Keyes, 79-86).

After medical science had overcome the problems of extracting and successfully transplanting organs between species, the issue of rejection came to

the forefront of clinical research. Immunosuppressive drugs cannot control certain types of severe rejection mechanisms, either at all or without severely harming the patient. These mechanisms had not been observed with human-to-human transplants, due to the nearly identical biochemical makeup of the donor and patient (other factors control rejection in these cases). The first of these to be encountered is a process called hyperacute rejection. Hyperacute rejection, if left unchecked, causes the complete rejection of a foreign organ within minutes. It is caused by the binding of natural antibodies in the blood of the organ recipient to the foreign organ. The attachment of these antibodies causes activation of compliment proteins in surrounding blood vessels. This leads to hemorrhage, edema, and thrombosis, causing the destruction of the organ (Houdebine 455).

If the first hurdle of hyperacute rejection can be overcome, there still exists a series of other mechanisms that can reject the transplanted organ. Over a period of days or weeks, a process called acute vascular xenograph rejection leads to the inflammation of transplant blood vessels and the coagulation of the blood inside. The cause of this process is not completely understood, but it is believed that many of the same xenoreactive antibodies and compliment proteins as hyperacute rejection are involved. Beyond acute vascular rejection, other types of tissue rejection are observed, such as cellular rejection and attack by the T-cells of the transplant patient. However, it is believed that these immune responses can be safely and effectively controlled using current

immunosuppressive methods (Houdebine 455-456) and further genetic modification of transgenic donor tissue.

Several attempts have been made at genetically altering a host animal to produce a transgenic organ donor whose organs will not be subject to hyperacute rejection. Most of these tests have been focused on pigs, whose organs are comparable in size and structure to human organs. Pigs are also less expensive and more readily available than higher primates which are genetically closer to humans. However, by using a non-primate mammal, the issue of hyperacute rejection becomes more severe. The major biochemical problem is the presence of the carbohydrate Galα1-3Galβ1-4GlcNAc-R, or αGal. This sugar residue is present in all mammals except for humans and some higher primates. In pig organs, it is found mainly on glycoproteins on the cell walls of organ tissues. Humans and higher primates possess an inactive form of the enzyme which produces αGal and xenoreactive antibodies which attack this carbohydrate. The binding of these antibodies leads to a cascade of compliment protein attack, and eventually to the loss of barrier function, hemorrhage, thrombosis, and ischemia of the cells of the foreign organ (Cozzi, et. al, 1).

Two potential strategies to overcome hyperacute rejection are currently being studied. The first of these involves the blockage of the compliment protein cascade that would lead to the rejection of the organ. The second would be to reduce the levels of αGal on the transplanted organ. Two lines of transgenic pigs

have been established which encode for two of the three main regulator proteins of human compliment activation. These are human decay accelerating factor (hDAF) and human CD59. In tests, the genetically modified hearts are protected from the attack of human compliment proteins (Clarke 11-12). The transplantation of these hearts into cynomolgus monkeys, which act as a very accurate model of the human immune system, yields promising results, with a marked increase in survival rates and corresponding decrease in rejection. In one test, one quarter of non-modified transplants in cynomolgus monkeys immediately underwent hyperacute rejection, while none of the experimental group of transgenic xenotransplants had this problem (Schmidtko, et. al, 1).

In a second route, the offending carbohydrate, αGal, could be eliminated from the surface of the cells in a transgenic pig. Unfortunately, knockout technology in pigs will not allow the elimination of the gene that produces αGal. If this was possible, this problem could have been solved very easily. Instead, researchers have inserted a gene into the pig genome that encodes for the enzyme α-1,2-fucosyltransferase. This enzyme competes with the enzyme that produces αGal, effectively reducing the concentration of the sugar. This development appears to be very promising, but actual transplantation of these engineered organs has yet to be attempted. With the advent of these studies, it appears that the hurdle of hyperacute rejection between pig organs and humans has been effectively overcome (Cozzi, et. al, 1). Other immune problems can be

addressed similarly as they arise, and solved with the same transgenic technology or with immunosuppressive therapy.

One of the biggest concerns with interspecies transplants is the introduction of zoonoses into the human population. Zoonoses are human diseases caused by infectious agents transferred from animals. Xenotransplantation creates a new environment for microorganisms, in which animal tissue is brought into direct contact with human tissue in a patient with a weakened immune system. This lowers many critical barriers for cross-species infection, creating a vital health hazard. If these infections are limited to the immediate transplant patient, the effects could be treated and may be negligible compared to the alternative of no transplant. However, there exists a remote possibility of the introduction of a new, transmittable epidemic that could be spread throughout the human population. Serious infections such as Ebola, which could be contracted from lower primates, could easily be detected, contained, and treated in patients. However, long-term persistent infections could go undetected in a patient and in the human population until it was too late. It is now believed that the HIV epidemic that we are facing today originated from the chimpanzee equivalent of the virus, SIV (Simian Immunodeficiency Virus). The main concern of researchers is a class of retroviruses that include porcine endogenous retroviruses (PERV). These retroviruses are endogenous, or not harmful to their hosts. However, their

effects on humans, especially in an immunocompromised environment, are unknown. Retroviruses contain RNA and use the enzyme reverse transcriptase to convert this to DNA. Because of this, the genomic sequence of these retroviruses is native to a normal pig genome. With the inclusion of this DNA, some porcine cells spontaneously produce virus particles, and there is no effective way to decontaminate a tissue sample. In Vitro, human tissue samples have been infected by PERV. However, it is not known exactly which types of pig cells spontaneously produce virus particles, which types of human tissue are vulnerable to infection, and what effect a PERV infection would have on a human being. Testing is currently proceeding, but results have not currently been published (Takeuchi, 1-3).

4.2.2. Ethics

Because of the scientific issues involved, xenotransplantation brings a very special ethical case to the table. It has potential to be argued against merely for the fact that it involves vivisection, or the fact that we are genetically altering an animal, or even still that it involves the transplantation of organs from another species to humans. Even through all of this, xenotransplantation is accepted by society. Major religions of the world have already made statements on their standpoint. The Vatican released a statement on September 26, 2001 stating that

they do not reject the use of xenotransplantation saying it is 'morally acceptable' and will have benefits for mankind (TRENDS). Also, the Jewish religion stands behind xenotransplantation saying, "The preservation of human life is of infinite and supreme value (Veatch, 61)." These two religions are only two of thirty religions that have spoken out in support xenotransplantation. Ethical arguments should not be ignored because of acceptance by religious groups, but they are indicators of the feelings of the general public, particularly those who would have some specialized objections.

As of this year, there are approximately 80,000 people on the organ and tissue waiting list. A little more than one third of them will die before the organ they are waiting for is available to them. Since the year 2000, the donor list has grown by about 1,000 people per month. This demand of people who need organs is constantly growing and is already much higher than what can be supplied. This means that thousands of people who could be saved by an organ transplant are dying. Of the people on the list currently, it is estimated that over 26,000 of them will die before they see the organ they need (GSDS). This is a fairly large number of people who will die that could have potentially been saved if organs were available.

The quality of life of a person on the organ and tissue waiting list varies greatly. The quality of their life is completely dependant on what their medical problem is. Quality of life ranges from non-life threatening symptoms like

blindness to highly life threatening and extremely painful symptoms. People waiting for heart transplants often have a quality of life that is highly degraded, often not being able to engage in many physical activities. They are eventually hospitalized or under constant medical watch for the rest of their life or until a transplant is received. People waiting for tissue to aid in the repulsion of cancers like lymphoma and leukemia are often in severe amounts of pain. Burn victims often need skin grafts in their recovery. All of these people's quality of life is affected by a situation that could be corrected if they can get the tissue that they need.

In xenotransplantation, the use of animals to save the lives of humans is directly proportional to the amount of people who need organs. In this situation, the loss of animal life is kept to a number directly proportional to the amount of humans on the donor list. For every one human that is saved, only one animal will have to be sacrificed. In some cases, it is even possible that many organs could be salvaged and used to save multiple lives. If this were to happen, the loss of animal life could be highly minimized. If human organs were first searched for instead of first reaching for xenotransplant organs, the number of animals sacrificed could be further minimized. A second method that could reduce the number of animal lives being lost is the use of one animal for multiple purposes. For example, thousands of pigs are killed every year for food. If a transgenic animal was grown and was used to donate organs, the remains of the

animal could still possibly be used by the meat industry, effectively reducing the number of animal lives lost for use by the humans.

The quality of life of an animal that was raised for organ donation would be about the same of a farm animal being raised for food, if not better. In this case, the animal suffers no loss of quality of life in any manner. Xenotransplantation animals would be kept in a sanitary environment, and would be fed and treated well. Many farm animals would live under much worse conditions than these. Beyond this, the actual euthanasia of the animal would probably be even more humane than those of the farm animals who are slaughtered. In the case of xenotransplantation, the animal would be put under using an anesthetic until the organs were taken, in order to keep the organs alive. The animal would be put to sleep without ever feeling any pain.

As can be seen, the potential of gain compared to the possible losses for xenotransplantation is very favorable. The number of animal lives lost can only be as high as the amount of humans saved. It is even possible that the number of humans saved could be higher than the amount of animals lost. Beyond the numbers, the quality of life of an animal that is being used for xenotransplantation is not negatively affected at all, while the quality of life gained by the recipient human is great. This balance of gain and loss is also immediate, and does not take time to become positive like disease modeling.

In addition to the traditional arguments about xenotransplantation, there are three specific arguments that come into play. These three arguments are the Natural Law problem, the Nontherapeutic Interventions problem, and the Resource Allocation problem. Each of these problems is specific to xenotransplantation and in addition to the animal rights problem that was discussed earlier.

The Natural Law problem is probably the biggest ethical problem of xenotransplantation. The Natural Law problem is the ethical question concerned with whether it is right or not to take organs from one species and transplant them to another. Some people believe that the human is completely unique and far superior to any other animal on earth and by transplanting organs from other species; the divine order of nature is being violated. At a genetic level, the human body is very similar to that of many animals. However, each individual's body is completely unique, which would imply that any transplantation at all would violate the divine order of nature. In addition, genetically altered xenotransplantation organs would differ from the one being replaced only in the fact is was grown in an animal, and not a human. The physical nature of the organ would be nearly identical. Although there are some people who still view the topic this way, the use of xenotransplanted organs is gaining acceptance from most religions (Veatch, 261).

The second specialized problem with xenotransplantation is the Nontherapeutic Intervention problem. This problem states that it is not believed to be moral to do any medical procedure purely for medical research and not for any kind of therapeutic reason that the patient may benefit. This mainly applies to the period of time in which xenotransplantations would be very experimental. It is the belief of some people that experimenting with the xenotransplantations would be purely for research and that the patient would never stand a chance, or have any medical gain. This argument, assuming it is unethical to do experimental procedures as described, restricts the testing of xenotransplantation, but does not make it immoral. In most cases, the experimentation of xenotransplantation would be done so that the recipient of the organs would have a possibility of medical gain if the procedure were successful. If this were to happen, the Nontherapeutic Intervention argument would be null and void regardless of whether experimental medical procedures are unethical or not (Veatch, 263-4).

The third and final specialized problem of xenotransplantation is the Resource Allocation problem. Many people believe that the exorbitant amount of resources it would take to develop such an exotic and risky procedure could be much better spent on researching how to prevent the problems that put the person in need of an organ transplant before they are even in the position. This argument is a formidable one that goes beyond the ethics of xenotransplantation

into the entire society and socio-economical structure. This argument can be countered in a scientific manner. If xenotransplantation is perfected, the procedure could be used to cure future diseases that we have not even seen yet. If we used the resources to eradicate the disease that caused damage to the organ, nothing is to say that another disease will not soon attack the organ in the same manner and mankind would again have to expend resources to find a cure or preventative measure for the disease. In addition, there are birth defects that require transplantation to save the patient's life. Unfortunately, there is no preventative measure for these people. Beyond this, even if the money was spent of the research of a cure or preventative measure for the diseases, there is nothing to say this research will be any less exotic, risky, or resource inefficient. In many cases, the resources would be spent on disease modeling, a method that is nearly as exotic but with much higher animal life losses (Veatch 265-267).

As a whole, it can be seen that xenotransplantation has very great potential to help mankind. The loss of animal life would be minimal compared to the gain of human life, almost making a one to one ratio. The animals that would give their lives would not suffer from any loss of quality of life either, but the human recipient would gain leaps and bounds in the quality of life. In addition, it would be possible that the very same animals that gave their life to save a humans could be used for food, something we already raise them to slaughter for. Beyond the comparative of loss of animals to human gain, the

three specialized arguments against xenotransplantation can easily be countered. Assuming that no procedure is done for research only, the patients do not object to having animal organs transplanted to them, and they are indeed in great pain and suffering, the specialized arguments would not make the procedure unethical.

4.2.3. Legal

Only since 1967 has transplantation, when the first heart transplant was completed, has there been any legal discussion centered on transplantation. Xenotransplantation, an even more recent technique, has faced even less jurisdiction. Even so, it's possible to predict how courts will respond to this issue. No has denied that there is a shortage of human organs for transplantation and most believe that xenotransplantation is a logical extension. Therefore, it is likely that xenotransplantation has a bright future.

The most formal rules that are in place today are from medical institutions. Institutions that serve as the brokers for organs listen and field organ requests based on chances of survival and wait time. They have strict rules about receiving organs, transport, and usage.

Xenotransplantation has faced some discussion in UK. In 1997, the UK Xenotransplantation Interim Regulatory Authority (UKXIRA) met for the first

time. Their intention was to fulfill certain requirements as stated in a 1995 report from the Advisory Group on the Ethics of Xenotransplantation. Their basic conclusion was that xenotransplantation was acceptable provided that some requirements were met. The terms of reference are as follows (UKXIRA):

> "To advise the Secretaries of State for Health, Northern Ireland, Scotland and Wales on the action necessary to regulate xenotransplantation, taking into account the principles outlined in "Animal Tissues into Humans", and worldwide developments in xenotransplantation. In particular to advise:
>
> (a) on safety, efficacy and considerations of animal welfare in liaison with the Home Office, and any other pre-conditions for xenotransplantation for human use, and whether these have been met;
>
> (b) on research required to assess safety and efficacy factors in xenotransplantation procedures;
>
> (c) on the acceptability of specific applications to proceed with xenotransplantation in humans; and
>
> (d) to provide a focal point on xenotransplantation issues within Government."

Like transplantation, there has been little discussion in the legal community. One difference between genetically modified organs is that there

are intellectual property rights. To this day, there have been no cases that have discussed or challenged the usage of xeno-organs. This will be an area to watch as it develops. The conventional wisdom is that transplants of any kind have become a routine operation and do not need any legal regulations.

4.2.4. Discussion

Xenotransplantation is becoming the most viable method of organ transplantation. There is a well-documented shortage of readily available organs for human recipients. There are some moral and technical issues that exist but the majority of the population and scientific community dismiss these issues. Xenotransplantation is a technique widely accepted and is growing in practice

As there aren't enough human donors, scientists have developed the process of modifying animal DNA so that a human recipient will accept the transplant. To reduce animal loss, new procedures are being developed so that a human can use an organ but the rest of the animal can be used for food. This would increase the loss of animal life by a statistically insignificant amount while saving thousands of human lives.

Some believe that vivisection is an immoral process and should not be allowed. This group feels that the humans and animals should not be "violated." The majority of the population does not agree with this position. Their primary

reason is that the lack of available organs makes this process necessary. Even religious groups have cited their favor for xenotransplantation process saying that it is morally acceptable.

Zoonoses are one of the only problematic issues that remain. As there is a chance of infection that is introduced from the transplantation, critics have latched on to this fear as a last attempted to dismiss this procedure. The biggest fear is that one of an infection could cause an epidemic in humans. Since this ailment would have been previously unseen in humans, humans would have almost no immunity. This is a valid concern but it is an extremely remote risk. Unfortunately, an ailment of this nature would be exceedingly difficult to treat.

There are no legal barriers to a more widespread application of this procedure. Some researchers and companies have expressed concern as to the intellectual property related to their research and developments. Most researchers identify the need for development to continue. Therefore, they have been unwilling to wait for the courts to reach a consensus as to the application of intellectual property rights.

Xenotransplantation is a process that will only increase in frequency. The technical and social issues are all but overcome. While there are some lingering issues relating to intellectual property rights, these will be decided by the legal system in the next five to ten years. At that time, a surge of xenotransplantation will be seen.

4.3. Biopharming

4.3.1. Background

With the advent of transgenesis and current genetic engineering developments, it has become possible to insert genes for foreign proteins into the genome of a target animal. If the inserted gene codes for a useful protein, the animal can be used as a biological factory to produce a desired product. Many of these target proteins are high-demand, high-priced pharmaceutical products which are difficult or impossible to synthesize artificially. Before the advent of genetic engineering, these compounds had to be carefully extracted and purified from either living donors or cadavers. This slow, painstaking process leads to the exorbitantly high prices of some rare pharmaceutical products. However, using new genetic engineering techniques and transgenic animals, the process of biopharming was developed. Biopharming is the commercial production of pharmaceutical products from the body fluids of transgenic animals. The fluid that is of the most interest is milk. Milk is usually the most easily obtained fluid, and can be procured in fairly large quantities without serious harm to the donor animal. However, urine and blood have also been used in certain cases. Each fluid and target compound poses unique challenges in obtainment and purification (Clarke, p. 6-7).

Milk is usually the fluid of choice for many biopharming researchers. It is a less complex fluid than blood, allowing for easier and less expensive purification of the desired protein. The proteins secreted in milk are present in the transgenic animal's circulatory system at only very low levels. This minimizes the health risks to the animal due to the presence of a biologically active foreign protein in their body. One main problem with the production of complex proteins in processes such as bacterial fermentation or artificial chemical synthesis is the absence of posttranslational modifications that activate or complete the protein. When produced in the mammary gland of a transgenic animal, the compound is posttranslationally modified in a manner which is almost identical to that of a human. This allows for a better therapeutic product that is usually more stable, more active, and less likely to cause a negative immune response in a human patient (Clarke, p. 7).

Biopharming is the most advanced and most widely used of the three techniques discussed in this study. The first recombinant human protein was expressed in the milk of a sheep in 1988. In the next 10 years, 17 human proteins were reported to be expressed in the milk of five livestock species. Eleven of these were at commercially viable levels which could be useful in industry. In the same time period, three of these products entered clinical trials, anti-thrombin-III from transgenic goats, α1-antitrypsin from sheep, and α-glucosidase from rabbits (Rudolph, p. 367).

The transgenesis methods used in biopharming need to be more precise than those used in other applications. The desired gene must not only be effectively inserted into the genome of the subject, it must also be expressed as a milk protein, not in the bloodstream or another location. Before insertion, the gene must be combined with the promoter from a milk-specific protein so that it will be secreted by the mammary gland. These hybrid genes are microinjected into an embryo using a very fine glass needle. After being cultured for a short period of time, the embryos are transferred to the wombs of foster mothers. First generation transgenic animals are identified using a tissue biopsy. These first generation transgenic animals are called "founders." If the founder is female, it can be milked almost immediately, using hormones to induce premature lactation. If the founder is male, it must first be bred to produce female offspring which will yield milk. These methods yield a low ratio of viable transgenic offspring. New, more effective methods are currently being developed which make the process much more efficient. Besides the obvious dairy producers, cows, sheep, and goats, other animals have been used to produce transgenic biopharmers. These include pigs and rabbits, which were chosen for their larger litters and shorter generation times (Rudolph, p.367).

Despite the promising results obtained from experimentation, a variety of technological hurdles still exist before the use of biopharming will become commonplace. The isolation of simple, easily handled milk protein promoter

regions is a difficult task, and progress has been slow in this area. Many desired genes are also too large to be used with current gene insertion technologies. These genes must either be truncated without loss of function, or new gene insertion methods must be developed which allow for longer sequences. The location of the insertion also partially dictates the level of expression of the target gene. Different sections of different chromosomes permit higher rates of transcription than others, due to a variety of regulatory factors. The insertion made by current techniques is random, so it is just as likely that the gene will appear in an area of low activity as in a high activity area. A targeted insertion or a method to reduce the positional dependence of transcription of the selected gene would greatly improve the output of future transgenic biopharming animals (Rudolph, p. 369-371).

Once a transgenic milk-producing animal has been created, its milk must be harvested and purified. A variety of techniques have been studied to obtain the maximum amount of milk from each animal, from automated milking machines to varying the timing of milkings. However, the purification of the desired product from this milk is still an important technical hurdle. Milk, because it is meant to have a high nutritional value, is rich in proteins, vitamins, lipids, and many other complex organic compounds. The separation of a single one of these compounds, especially at the relatively low concentrations encountered in this process, is extremely difficult, expensive, and time-

consuming. The first concern is usually the presence of any infectious particles in the milk. The animals cannot be kept completely pathogen-free, but a series of steps can help to minimize the concern of infectious diseases. All new animals must be held to a strictly defined health status and maintained in quarantine-like conditions. They must also be routinely inspected visually for ill health symptoms and autopsied post-mortem if any ailments are suspected. Milk must only be collected from healthy animals. Bodily fluids can be routinely screened for bacterial and viral infections, and conditions that promote growth of disease particles can be avoided. However, the issue of molecule-sized infectious prion proteins, such as scrapie and BSE (Bovine Spongiform Encephalopathy, or Mad Cow Disease) is more difficult to control. Even though none of these animal forms of prion diseases have been observed to be transferable to humans, proper precautions should be taken to prevent any contact. These would include constant monitoring of transgenic flocks and periodic sacrifice of select animals which would be examined for any signs of the disease. Also, sources of feed for the biopharming animals must be closely monitored. The most common avenue of infection for prion diseases seems to be through rendered animal feed containing "recycled" nervous tissue from infected individuals. Because it presents such an excellent growth media, the milk may also become contaminated during milking or subsequent storage. Therefore, milking

methods and storage before processing are important issues to consider (Houdebine, p. 469-470).

The milk of sheep is very similar to that of cows, so these facts and methods apply nearly identically to both species. Calcium is the most abundant mineral, present at a concentration of approximately 2g/l. Lactose is the major carbohydrate at approximately 40 g/l. These two components are fairly easy to remove, and tend to be removed in steps aimed at other compounds. Lipids are present at approximately 50-70 g/l in sheep and 40 g/l in cows, and proteins, mostly caseins, exist in a concentration of approximately 50 g/l in both species. The number of other proteins in milk is hard to determine, but it is known to be extremely large, with many different types of proteins meant for a variety of purposes. To be economically feasible, a transgenic target protein must be at a concentration of approximately 1 g/l or more. Any less than this and product purity suffers and extraction costs grow exponentially.

The first step in the purification of milk for biopharmaceutical purposes is usually the removal of the lipid content. This can be accomplished with a 95-98% efficiency using a skimming centrifuge. This process will also remove any solid particles, and many of the bacteria present will be removed with this solid mass. If other methods are used to remove more of the lipid molecules, product loss becomes a problem. Lipid levels after skimming can remain as high as 1-4 g/l, but as much of this material must be removed as possible, or more expensive and

complex filtration steps used later in the process will become clogged or damaged. Casein, the most common protein in sheep and cow milk, tends to aggregate and block filters and chromatography columns. Therefore, an interest has arisen in the removal of this material before these steps are taken. Ultrafiltration using ceramic and organic membranes shows promise in this area. The traditional method involves the addition of renin, obtained from the stomach of a calf. This causes the casein to precipitate out of solution and allows it to be easily recovered. However, the addition of any biological substance such as renin can invite further contamination and introduction of pathogens. Another promising route involves the lowering of the pH of the milk to 4.5. At this pH, casein will precipitate out of solution, but the target protein may also precipitate out or be damaged and lose their activity. A variety of other compounds can be added to precipitate out the casein, but each presents its own problems, and must be carefully added at a suitable concentration which will not remove or damage the product protein. The solution is next passed through a microfilter to remove any remaining contaminants and bacteria. After these steps are taken, further purification steps become dependant on the properties of the desired product rather than the generic properties of the milk it resides in. At this point, we are left with an aqueous solution of many different proteins, and unique properties of the target protein must be identified to allow for its isolation. Chromatography is usually the process of choice, but the type of

chromatography must be chosen. For example, ion-exchange chromatography is used in proteins with acidic iso-electric points. The most specific type is immunoaffinity chromatography, which uses targeted antibodies and allows for extremely high specificity. This process is unfortunately expensive and can be damaged by harsh cleaning chemicals. The isolation and purification of biopharmaceutical products from the milk of a transgenic biopharming animal should be possible for almost any product using a series of steps first generic to milk, then highly targeted at the desired protein (Houdebine, p. 470-471).

4.3.2. Ethics

From an ethical standpoint, biopharming is a relatively dormant topic. The methods and applications leave little to be considered unethical or cruel. Unlike disease modeling and xenotransplantation, biopharming poses few health risks to the animal but still can save lives of humans. The only main oppositions against biopharming are those that are not specific to disease modeling alone, such as the belief that milking animals is cruel and that genetically modifying animals is unethical. Because of this there is little to no strong opposition to biopharming as a medical process.

Biopharming is sometimes the only way to produce certain proteins and other times it is much more efficient that current methods. These proteins can

make a major impact in the fight against a disease. One example of this is diabetes. Diabetics are dependant upon insulin shots to survive. Currently, insulin is produced using fermentation in bacteria. This method is effective in producing the insulin, but it lacks efficiency and completeness. In the bacterial fermentation method, the insulin is produced but the post-production modifications that would occur in the human body are not performed. When produced by biopharming, insulin would go through the modifications that would make it more effective and more natural. This would make for a more effective treatment of diabetes. From a numerical standpoint, this means that this application of biopharming alone could save the lives of 17 million people (ADA).

People who suffer from diabetes suffer from a disease that can be fatal if not treated. The quality of their lives without treatment is not very high, as they will constantly be sick and eventually suffer organ damage do to the imbalance of glucose in their body. People with treatment, however, have next to no loss in quality of life, although they must constantly monitor their disease. The difference in quality of life for these people with insulin compared to those without the necessary medical treatment is extremely large. For this reason, the need for production of insulin can be seen.

Animals that are used for biopharming do not suffer from the degraded quality of life that other animals used for medical research do. Their lives in

most cases are identical to those of everyday farm animals. Their use in the medical community does not even cause their lives to be sacrificed in order for the gain to be made. Because of the fact that the animals do not suffer from a loss in quality of life, the amount of animals needed for the technique becomes a relatively moot point. This does not matter because the animals are not sacrificing anything for the gain of human beings.

Overall biopharming tends to be the most ethical procedure for medical gain compared to the xenotransplantation and disease modeling. This is so because the humans gain in quality of life and has the potential to save a large quantity of lives while the animals do not loose any quality of life or loose their lives at all. The downside to biopharming is not an ethical matter, but one of applicability. The uses of biopharming are limited to those in which the diseases targeted are cured or fought by a single protein. This is the trade off in biopharming as opposed to the trade off of the loss of animal lives or animal suffering.

4.3.3. Legal

Biopharming has an interesting background. Since the animal that is used in this processed is not harmed, the laws and regulations that are attacked to biopharming are quite different.

Researchers have been saying that this process is safe and better than using conventional methods. Other groups say that there is evidence that traceable components of drugs are found in the derivative works. They cite that these components are a new risk to consumers. Research in this field is still only preliminary and inconclusive.

Since it is possible that animals process proteins differently than humans, unknown risks could exist. A certain protein could perceived as foreign by the body and elicit an allergic reaction, including life-threatening anaphylactic shock.

The United States Department of Agriculture (USDA) has the largest clout with regards to this process. (Wisconsin). As most of the derived products in biopharming are ultimately used in products that are used by humans, the USDA has the authority to set policy regarding the use of biopharming.

Currently, the USDA is overseeing experimental trials involving transgenic animals. In many states, field tests are being performed to observe the effects of the large-scale use of genetically altered animals. The USDA keeps all drug and chemical crop sites secret from the public. This anonymity has been extended to biopharming. Farmers and researchers prefer this process. The general consensus at present is that theft is a major concern and the solution is not to treat the crops any differently.

Pharmaceutical companies have been using animals for the production of proteins. As a business enterprise, they are not forced to publish many of the

techniques that are used. As a result, they are not subject to the USDA. As most of their products go through the Food and Drug Administration, their legislation is through a different channel. This setup has made current legislation not applicable to companies.

The FDA has a yet to be determined role in regulatory actions. When contacted by telephone, FDA representatives were unwilling to speak about the agency's possible involvement in the review of biopharm trials conducted thus far, citing confidentiality claims by business. An FDA spokesperson recently was quoted saying, "And I think to be honest, the FDA is used to applying regulations to manufacturing plants, but not to plants used for manufacturing. So a lot of this is new to us as well, and that's why I won't be able to answer any questions at the end."

This criticism is being met with additional regulation. In March of 2003, the USDA created strict rules for those who employ biopharming (Times 2003). Included in this regulation are laws that require different feeding and housing units for modified and non-modified animals. Also included in this law is the requirement that a USDA official must inspect facilities five times a year. There had been no previous requirement for field inspections.

Michael Pauly of Epicyte, a biotechnology firm involved in biopharming experimentation, praised the Agriculture Department's decision to impose stricter regulations instead of an outright ban on growing engineered drugs in

food crops, which some biotech opponents are urging. "Some fallacious and emotional arguments are being waged in the public debate," said Pauly, Epicyte's executive director of biotechnology, "but the bottom line is millions of patients around the world are fighting cancer, HIV, Alzheimer's and other diseases." (http://www.signonsandiego.com/news/uniontrib/tue/business/news_mz1b11biofar.html)

As with all recent biotechnology advances, intellectual property is a question that remains. Companies see large enterprises for biopharming. This process can be used to enhance the production of current drugs at a lower cost or have the potential to manufacture drugs at a large scale that were previously impossible.

Since most companies do not disclose the details of genetic modifications, these changes have been kept formally secret under trade secret status. Trade secrets are not disclosed in the same fashion as patents or trademarks. Instead of filing full disclosure of the technological invention with a patent, trade secrets are not filed but simply kept. Also, provisions prevent employees from disclosing details surrounding the trade secret. This way companies can protect their modifications while still keeping them out of the public eye.

5. Conclusions

5.1. Ethical Conclusion

The three methods of transgenic animal research discussed in this paper are shown to be ethically viable medical methods. Each of them comes at its own cost, but each has its own set of gains. By comparing each of them to the others, we can see their individual costs and benefits. Even though one may call for less loss than another, it should be known that all of them are important and all have their own individual importance in the medical field.

Of the three methods, biopharming would affect the most people by a fair margin. The largest target of disease modeling is Alzheimer's disease, which affects 4 million people. This figure is significantly lower than the 17 million people with diabetes, the principle target of biopharming. Both of these numbers are far greater than the 80 thousand people that could benefit from xenotransplantation. This does not necessarily mean that biopharming is the best of the three methods, but it does mean that it has the greatest potential to save human lives.

As far as quality of life is concerned, all three methods are approximately equal when compared to each other. The people with the diseases targeted by all of the methods experience a severe degradation in the quality of their life. The exact quantification and comparison of these could only be done on a case-by-

case basis because of the great fluctuation in each area. For this reason it should be assumed that all three methods are about equal in the loss of quality of life for the people who are affected. It is important to note that in general, the loss of quality of life for all of these people is great. This implies that all three methods have great potential for increase in the quality of life for these patients.

When comparing the amount of animal lives that would be lost in each method, it would be seen that disease modeling would cause the greatest loss. The actual number of animal sacrifices is unknown, but it would be greater than the 80 thousand that would be needed to transplant organs to every person on the current organ donor list. Both of these methods cause a large loss of animal lives to accomplish the gains that they can offer. Unlike these two methods, biopharming would not cause such a large loss. Assuming that only minimal numbers of animals died in the development stages of biopharming, the amount of animal lives lost would be negligible fore the method does not cause the animal to die in order to make the medical gains.

The method that would cause the greatest loss in quality of life for the animals would be disease modeling. In this method the animal would be used in actual lab experiments that may cause the animal to be in pain or suffer. In the other two methods, the animal would be raised and would suffer from no loss in quality of life compared to a regular farm animal. This does not necessarily make disease modeling a cruel method of scientific gain. If done with an

expressed purpose and done in a manner that would minimize the animals suffering, it would not be a torturous or cruel event. This does not mean that the animal would not be in pain, but its suffering would be a necessary loss needed for medical gain.

As can be seen, biopharming is the most ethically viable of the three methods, as it saves the most lives, has the lowest cost in terms of animal lives, and has little or no affect on the quality of life of the animals that are used. The least ethical of the methods by this comparison would be disease modeling as it causes a severe degradation in the quality of life of the animals, causes the highest loss of animal lives, and is in the middle in the amount of human lives saved. This does not mean that disease modeling is not ethical; it just shows that it is the least ethically acceptable of the three methods. Xenotransplantation is in the middle of the two with having no affect on the quality of life of the animals involved, being in the middle with the amount of animal lives lost, and saving the least amount of human lives. Xenotransplantation also has a special situation that contributes to it being in the middle. In this method, the number of animal lives lost is directly proportional to the amount of human lives saved.

It is important to note that one method is not necessarily better than another just because it is more ethical than another. The potential for each of these methods to save human lives as evaluated above is only relative to the current medical situation of the world. For example, biopharming currently has

the potential to affect the most humans that are currently infected by a disease. This does not take into account the fact that the insulin produced by biopharming does not cure the disease, but only allows the patient to continue to live a more normal life. It also does not prevent anyone from contracting the disease. Disease modeling, on the other hand, is used to find a cure for the disease, which could eventually lead to its eradication. If a given disease is eradicated, there is no telling the amount of lives that would be saved. When compared in this manner, disease modeling would be a more beneficial method. Each of these methods has its own individual importance, and no one is more important than another. For this reason we can only judge which is more ethical and not what is the best method.

5.2. Legal Conclusion

It has been said that that the law is always lagging the current world by at least a decade. Biotechnology is no exception and the courts lag behind by an even greater amount. Disease modeling, xenotransplantation, and biopharming are specific examples where the legal system has not reached a consensus. As more disputes arise involving biotechnology, courts will have more cases to shape and model the legal landscape regarding these techniques. Until this time, there is only ambiguity in regulations.

Law is a formal representation of the will of the people that it serves to protect. That is why norms and traditions find their way into common law. Legal systems work by hearing two parties in disagreement. During this display, the court determines a ruling that represents what the reasonable majority would decide. This is based primary on the principle of past precedent. When precedent is combined with new facts and questions, a decision is made and new precedent is formed.

Transgenic animals that have been created for the three techniques that are being discussed deal with two separate branches of past precedent. The first branch is the regulations that deal with the use of animals in medial applications. There is less than a hundred years of animal testing regulation, which is a light background. The second branch of law is the intellectual property rights. As these animals with modified genetic material are the work of others, it stands to reason that they are afforded some protection. This protection is from the Constitution in the form of "the protection of the individual artist or thinker or inventor, to the encouragement of science and the arts." (U.S. Const. art. I, § 8, cl. 8) Property rights in the medical and pharmaceutical company are so important that their companies develop products lifecycles around the length of their patents. Without a concrete intellectual property protection incentive in place, researchers and scientists are less willing to develop works in this industry.

Despite the lack of legal protection, research has continued. Each of the techniques has too much potential for researchers to wait. As a result, scientists have continued to develop techniques. This has lead to problems as developed techniques are deem public domain years after their creation.

Each of these techniques has their own legal puzzles and issues. Disease modeling has the question of pain and suffer. To what extent is allowable and to what extent is justifiable? Xenotransplantation and biopharming have almost no legal issues with the exception about intellectual property. Researchers are quite reluctant to invest large research dollars if they are unsure about the protection that the law will afford them when other competitors try to use their works.

Intellectual property is the last legal key issue remaining for researchers and scientists in the states. While there are many plans to eliminate the need of human donors, many of these plans are on hold until there is a general ruling regarding the intellectual property. As previously mentioned, researchers and scientists do want to invest resources until they know what type of protection their works will receive.

The international community is still undecided as to the extent of transgenic animals in commonplace applications. Northern Europe is the main hot spot supports transgenic research. Americans have also been one of the leading research centers for these techniques. Popular opinion is questioning the extent of embryonic research. Future techniques could heavily rely on those

methods that would make future development more difficult. Europe, with the exception already mentioned, has strong views against transgenic animals. As a result, they have past strong legislation that prohibits imports of any food products from genetically altered animals. There is also a strong animal right movement there which works strongly to reduce experimentation with transgenic animals.

The general population and the legal community have no disagreements about the importance of these procedures. The questions arise in determining how far legal protection can go. Also unknown is a legal standard for the amount of pain and suffering that is "reasonable" for experimentation. Those are both difficult issues that have yet to be fully explored with conventional experimentation. Transgenic animals only make these questions more difficult.

5.3. Final Discussion

In conclusion, we feel that these three methods are legally and ethically sound. They are needed to further advance our current society and to improve the quality of life of many suffering individuals. Humankind has always used animals for their own gain, and will continue to do so. Animals have always been used as beasts of burden, and are now being used for medical testing. This is merely the natural progression, and is widely accepted by society today.

Guidelines must be set to define reasonable medical usage of animals. These must be implemented to prevent harmful or extraneous testing which needlessly sacrifices animal lives without sufficient benefit to human beings. A cost-benefit analysis reveals that the suffering of a relatively small number of animals is acceptable for the benefit of a large number of human beings through the cure for a widespread disease or a source of donor organs. For example, the one-time cost of the life of a transgenic animal to develop a vaccine or treatment method for a disease is outweighed by the benefit of the control or eradication of a continuously spreading harmful disease. In the processes involved in xenotransplantation, there is no radical change in the life of the donor animal, but human beings are offered the huge benefit of a large and reliable supply of compatible transgenic donor organs for transplant patients. The same is true of biopharming. The transgenic animal is not harmed in any appreciable way, and the benefits to humans are immeasurable. Protein products which previously could not be produced, either technically or economically, could be offered at a much lower cost and higher quality. This could aid many people who previously were denied treatment for financial reasons or for lack of supply. At the present time, there are no current viable alternatives to animal testing. Many of the proposed alternatives can be used in conjunction with testing procedures, and can help to reduce the loss of animal lives and their suffering. However

these methods cannot completely replace the three techniques that have been explored.

We feel that transgenic animal testing is justified and correct, and that it should continue for the advancement of society. The methods that we have discussed above each benefit human beings in immeasurable ways. While it is unfortunate that animals do suffer as a result, some cost must be paid for every gain. If compassion is used in the implementation of these methods, the loss of animal life and degradation of quality of life can be minimized. We can only hope that future methods will be as beneficial and will replace the need for these unfortunate sacrifices.

6. Appendix

6.1. International Animal Care and Use Committee Guidelines (IACUC) for Ethical Treatment of Transgenic Animals

- The attending veterinarian (AV) should be consulted in planning studies involving discomfort, distress, or pain to the animals. The investigator should state how pain and distress will be evaluated and whether analgesics, anesthetics, or sedatives will be administered. If treatment for pain is to be withheld, the investigator must provide scientific justification in writing and obtain approval from the IACUC.

- The investigator must consider alternatives to animal use in studies involving pain or distress, and must provide a written narrative description of methods and sources used to determine that alternatives are not available. The investigator should report any alternative methods employed in the study, including measures to reduce the numbers of animals, use of less sentient animals, and performance of preliminary work in nonanimal models. Evaluation of the alternative methods in IACUC reviews

can produce dilemmas, such as deciding whether it is preferable to develop a transgenic rodent model of AIDS, which would require many animals, or to continue to use the nonhuman primate, which is more sentient.

- The investigator must provide assurance that the proposed study is not unnecessarily duplicative of previous work.

- Animals that would experience severe or chronic pain should be humanely eunthanized at the end of the procedure.

- Appropriate living conditions should be provided. Many genetic studies involve immunocompromised animals, and the IACUC should ensure that isolator caging systems, autoclaved food, and sterile water are provided, as necessary.

- Personnel must be trained and qualified to perform the procedures proposed. Assessment of personnel qualifications should consider not only knowledge and adherence to accepted methods, but also outcome-based assessments such as percent survival of injected embryos in the generation of transgenic mice. There is an art to

pronucleus injection, and the ability to answer questions about the procedure does not guarantee expertise in the laboratory.

- Adequate surgical facilities must be available. Personnel must employ aseptic surgical techniques and provide adequate perioperative care. When reviewing protocols involving generation of transgenic animals in larger species, IACUCs may be asked to approve multiple major surgical procedures. One operation is for embryo collection and the second is to transfer injected embryos. The IACUC must weigh the merits of two major survival surgeries on one animal against using two animals. Another option is to have a donor remain in a prolonged single surgery while the embryos are injected and then reimplanted.

- The investigator should describe the criteria and process for timely evaluation, intervention, removal, or euthanasia of animals if painful or stressful outcomes are anticipated.

- Pre- and post-procedure care should meet accepted criteria.

- Provisions to ensure the safety of personnel involved in the proposed study must be in place. It is helpful for the IACUC if IBC recommendations are made available.

Procedures to be performed on the animals must be described by the investigator and approved by the IACUC. Reviewers of genetic engineering protocols can be facilitated by initially delineating acceptable policies (e.g., for procedures to obtain tail biopsies to determine if offspring are transgenic). These policies should include what anesthesia is acceptable, age of animals at the time of biopsy, and biopsy size requirements. Some institutions allow a sample up to 5 mm in size if the biopsy is taken before the animal is four weeks old; if animals are older, the size of the biopsy must be reduced. If a second biopsy is needed, the interval after the first sampling, such as one week, should be stated. The federal guidelines state that all genetically engineered neonates or their containers should be permanently marked within 72 hours of their birth. The IACUC should review and approve the identification method to be used. If little is known regarding outcomes of a new procedure, limited pilot studies designed to assess the effects and conducted under IACUC oversight might be appropriate. The IACUC should ensure proper monitoring of such studies and be provided with results for review before granting final approval. Appropriate

containment should be required for animals used in studies involving potential hazards.

Research and animal care personnel should be enrolled in an occupational health program that includes assessment of risks and consideration of serum banking. Adequate veterinary care must be provided. Surveillance programs for important pathogens should be in place.

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