eISSN 2255-0569

ARTICLE ESPECIAL

Bioethical analysis of transgenic animals and genetically modified organisms (GMO)

Análisis bioético de los animales transgénicos y de los organismos genéticamente modificados (OGM)

Miguel Capó Martí[1], Ricardo Roa-Castellanos[2], María José Anadón Baselga[2], James Drane[3]

1. Dpto. Toxicología y Farmacología. Facultad de Veterinaria. Universidad Complutense de Madrid 2. Dpto. Toxicología y Legislación. Facultad de Medicina. Universidad Complutense de Madrid 3. Dr. Drane Bioethical Institute. Edinboro University. Edinboro, PA, USA.

Correspondència
M. A. Capó
Dpto. Toxicología y Farmacología. Facultad de Veterinaria.
Universidad Complutense de Madrid.
Avda. Puerta de Hierro S/N. 28040-Madrid. España.
E-mail: capo@ucm.es

Rebut: 6 – IX – 2014
Acceptat: 26 – IX - 2014

doi: 10.3306/MEDICINABALEAR.29.03.41

Abstract

The important technological advance in genetic manipulation has led to the development of genetically modified animals. The resulting being is called a transgenic animal. This term refers to an animal, whose genome has been deliberately modified by transferring an exogenous DNA into all its cells, including the germinal ones. In 1981, GORDON and RUDDLE coined the term transgenic as an animal variant, result of the introduction of a gene, or genes, into its genome. More recently, there exists a tendency to use the term GMA (Genetically Modified Animal) to refer to transgenic animals.

Some arguments against the use of transgenic animals in research are related to the fact that during the creation of a transgenic animal its genetic integrity is not respected because of the recombination of genetic material from different species and even different kingdoms, as for example, animals and plants. Some people consider that this recombination of genetic material between species, or the creation of chimeras, which in occasions is a part of the technical strategy for the obtaining of a transgenic animal, alters the concept of *"species"*. In addition, they consider it as an unnatural intervention that might interfere in the conception of what makes that an animal is such. There is argued that the direct genetic modification is a mere extension of the traditional technologies of crossing. The genetic modifications of animals provide arguments for accusations as how to treat animals as things or merchandises.

Keywords: Transgenic animals, Biodiversity, Genetically modified organisms, Bioethics, Biotechnology, Recombinant DNA

Resumen

El gran avance en tecnología para la manipulación genética, ha conducido al desarrollo de modelos animales modificados genéticamente. Al ser resultante se le llama animal transgénico. Este término, se refiere a un animal cuyo genoma ha sido deliberadamente modificado, mediante transferencia de un DNA exógeno, en todas sus células, incluidas las germinales. En 1981, GORDON y RUDDLE acuñaron la palabra transgénico como una variante animal originada tras la introducción de un gen, o genes, en su genoma. Más recientemente se tiende a utilizar el término AMG (Animal Modificado Genéticamente) para referirse a los animales transgénicos.

Algunos argumentos en contra del uso de los animales transgénicos en investigación tienen que ver con una cuestión previa a su aplicación, como es que, en la creación de un animal transgénico, no se respeta la integridad genética de los animales ya que se produce la mezcla de material genético entre diferentes especies e incluso entre diferentes reinos, por ejemplo, entre animales y plantas. Algunas personas consideran que esta mezcla de material genético entre especies, o la creación de quimeras, que en ocasiones es parte de la estrategia técnica para la obtención de un animal transgénico, altera el concepto de *"especie"* y es una intervención antinatural que podría interferir en la concepción de lo que hace que un animal sea tal. Se argumenta que la modificación genética directa es meramente una extensión de las técnicas de cruzamiento tradicionales. Las modificaciones genéticas de animales proporcionan argumentos para acusaciones como tratar a los animales como cosas o mercancías.

Palabras clave: Animales Transgénicos, Biodiversidad, Organismos Genéticamente Modificados, Bioética, Biotecnología, DNA Recombinante

Introduction

German Fritz Jahr proposed in 1927 the first construction of the word bioethics as an equation *"Bio=Ethik"*, in addition to a *Bioethical Imperative*, which also should consider other living beings in their related natural frames, together with the contributions given by wisdom. There has to be included artistic perspectives and/or the lived religion - constructed and experienced by historical, or anonymous prominent figures - in order to have guidelines of conduct that were able to protect life (Bios) as a whole, not only of human beings. All of this with the reverential respect towards life, within natural functioning, as it was proposed by the Noble-Peace-Prize-Winner A. Schweitzer (ROA-CASTELLANOS; BAUER, 2009).

More recently, POTTER (1970) methodologically proposes in his article *Bioethics, Science of Survival* to include ancient knowledge from the fields of philosophy and science, as well as renowned knowledge, as for example empirical knowledge, exact sciences and books of the Holy Scriptures in order to establish guidelines of conduct that have allowed different human groups to survive. For POTTER, wisdom was a goal that would guarantee survival. But nowadays it can be perceived that the appreciation for each interlocutor has been more present in the real founders of bioethics, than in their legatees. This happens including with the so-called solver of moral medical problems, HELLEGERS (1971) that proposed the same neologism based on respect, even for the Georgetown school, though its proposal now has reduced for several years bioethics to its medical and legal problems. (CICCONE, 2006).

The term *transgenesis* developed in parallel designs the process of transferring genes in an organism. The transgenesis is used nowadays to create new plants and animals. Life as a meaning for some reason is not longer dignified or respected as an end by itself. There are different transgenic methods to modify cell reportoires or whole hosts, such as the use of pistols of genes or the use of bacteria or viruses as vectors to transfer the segments of genetic information (LACADENA, 1996).

Anyhow, the word *transgenic* refers to a plant or an animal into whose cells receive a fragment of exogenous DNA or DNA that cannot normally be found in the organism in question has been introduced. A transgenic mouse, for example, is one that has been injected with foreign DNA, through a fertilized-modified ovum that is implanted into an adoptive mother. The formed animal has not only its own DNA, but also the fragment of exogenous DNA that was reinjected in the stage of fertilization of the ovum.

In this fashion, it is possible to study what effect this gene has on the whole organism instead of only observing a single cell in a cell culture. This is important because many diseases do not affect one single type of cells, but the interactions between many different types of cells, even the so-called stromal cells. This type of technology allows exemplifying human diseases in other species, where it is possible to study the biology and possible therapies for the disease.

Transgenic animals

In the last decades, especially due to the huge advances in the knowledge of the molecular bases of diseases, there has appeared the need to have genetically defined models, that is, models in which the genetic mutations that predispose, or take part in the development of the disease, could be controlled. This fact, together with the significant advance in technologies for genetic manipulation, has led to the development of genetically modified animal models, which are then called *"transgenic animals"*[1]. This term refers to an animal whose genome has been deliberately modified by means transferring exogenous DNA into all its cells, including the germinal ones. In 1981, GORDON and RUDDLE coined the term transgenic as an animal variant, result of the introduction of a gene, or genes, into its genome.PALMITER and BRINSTER described in 1986 the introduction of genes into cells of the germinal line[2].

The simplest form to generate a transgenic animal is the one that involves the isolation of the gene that shall be introduced *(transgene)*, its cloning and manipulation, so that it can be expressed by target organism, as well as its insertion into the organism. To reach that all the cells of the organism express this new gene, it needs to be incorporated in an embryo, in a zygote stage or very early phases. Once the scientist is sure that the embryo incorporated the transgene, the new set of cells is implanted into a receptive animal, which acts as mother (in a procedure where is possible to use *in vitro fertilization techniques*).

If, on the other hand, one is not interested in an animal whose entire genome contains the transgene, but only certain type of its cells, a procedure similar to the previously described is being carried out, but instead of injecting the transgene into a zygote, it is injected, for example, into an already existing blastocyte. The result of this procedure is an organism with normal cells, as well as cells that contain the transgene.

1. More recently, there has been seen a tendency to use the term GMA (Genetically Modified Animals) to refer to transgenic animals.
2. The first transgenic laboratory mouse appeared in 1974 and was called *Brinster´s Mouse*; nowadays there exist approximately 1.000 strains of knockout mice. A *knockout* is a mutant animal which lacks the specific expression of a gene, eliminated by genetic mutation. The so-called knockin has a new gene included.

An example of the use of this technology is the production of transgenic sheep or goats. These are created by injecting the gene that codifies the desired protein into a fertilized ovum, which is implanted into a mother sheep or goat. Then, the presence of the desired gene in the offspring and those goats that present it, are induced to produce milk with some special characteristics when producing proteins.

The creation of transgenic animals presents new opportunities, but also creates new challenges. Among the first ones there is a possibility of studying the function of certain proteins, including some causes of human diseases. One of the major problems is the randomized insertion of the desired genes and the epistasis phenomena.

Animal transgenesis includes the addition *(Knock in)*, substitution, elimination *(Knock out)* or inactivation of one, or multiple genes.Their applications include the area of basic investigation (creation of animal models for the analysis of animal and human pathologies, discovery of new therapies, etc.), the food supply (improvement of productive characters in livestock, resistance to diseases, etc.), the industry (synthesis of new textile compounds, therapeutic proteins, etc.) and the medicine (possibility of xenotransplants (PETROCELLI et al. 2003)[3], models for gene therapy, etc.) amongst others that can be summarized as follows:

- Genetic bases of diseases and therapy designs.
- Models for the investigation of infections and gene therapy.
- Models or bioreactors as basis for the testing of drugs and medicines.
- Biotechnological designs in agricultural industries.
- Animal models for the analysis of the effects of the modulation, activation or suppression of the gene expression.

The study of genetic syndromes, chronic metabolic diseases, generation of new medicines to treat diverse diseases and the transplant of organs, are possibilities in transgenesis that should be deepened with respect to biological and ethical principles. The most common animals for this type of transplant are pigs, which, because of the similarities with the humans are the animals that show proportional size and if managed lowest rejection rates.

In case of medicines, there are several advances from transgenic animals: insulin, growth hormone and anticoagulant medicines are some of them.

Through the insertion of certain genes into mice, diseases can be studied thoroughly, in order to know how they work.To test different treatments to find the best one, it is necessary to create transgenic mice that develop different types of cancer. Nowadays these experiments have been mainly carried out in mice, though it can be expected to be done further with bigger animals, whose similarities with the humans are remarkable.

Besides, transgenic animals improve quantitatively and qualitatively certain elements as those in milk in the case of the cow-derived products, for enhancing human growth and the protection against diseases.

In the case of milk it is to be said that transgenic cows are able to generate lactose-free milk as well as enriched milk that can provide a major nutrition to babies and elders[4]. There also exist transgenic hens that synthesize human proteins in the egg white of their eggs.

GMO - Derived food

The food products subjected to genetic engineering, also known as transgenic food, refers to products that were produced on the basis of a genetically modified organism by means of genetic engineering. In other words, it refers to food obtained from an organism to which genes derived from another organism have been added to the receptor in order to obtain desired characteristics. At present, there can be observed a major presence of food products coming from transgenic plants such as corn, barley or soybean.

The improvement of the species that would be used as food for mankind or domestic animals has been a common cause in the history of Humanity. Between 12.000 and 4.000 b. C. there already existed the procedure of improvement by an artificial selection of plants. After the discovery of the sexual reproduction in vegetables, the first intergeneric crossing was realized in 1876. In 1909 the first merger of protoplasts was carried out and in 1927 there were obtained plant mutants of major productivity by means of X-ray irradiation of seeds. In 1983 the first transgenic plant was produced. In those days, some biotechnologists manage to isolate a gene and to introduce it into a genome of the bacterium *Escherichia coli*.

Three years later, in 1986, Monsanto, a multinational biotechnological company created the first genetically modified plant. It was tobacco plant, whose genome received a gene of resistance to the antibiotic Kanamicyn. Finally,

3. Petrocelli, A; Rodríguez, D., Spadafora, C., Tamino, G.; Zannini, P. (2003).- Cap. 1; In Ravarotto, L; Pegoraro, R. (ed). Transgenesi, Clonazione, Xenotrapianto. Ed. Piccini. Padova

4. In Canada, some researchers did go further and used the genes of a spider in a goat, so that silk could be extracted from the milk of the latter.

in 1994 the commercialization of the first genetically modified food, tomatoes *FlavrSavr*, created by Calgene, a biotechnological Company, was approved. An antisense gene was opposed to the normal gene of the poligalacturonase, an enzyme that leads to the ripeness of the tomato, so that it would remain live longer.

Nonetheless, few years later, in 1996, this product had to be withdrawn from the market of fresh products because of unforeseeable consequences such as a soft peel, a strange flavor and some other changes in their composition. Even so, these tomatoes are used for the production of elaborated tomatoes in industry.

Worldwide, the damages produced by weeds destroy almost 10 % of the crops. To avoid this, the farmers use herbicides with the resulting economic expense and contamination of water and soil. Generating plants that are resistant to these crops would improve this situation. To achieve it, vectors that transport genes of resistance to herbicides are transferred. An example of the aforementioned, is the resistance to the herbicide gliphosate in the GMO soybean and corn. This substance is effective in low concentrations, but as chemical agent remains toxic for humans and the scavenging microorganisms of the soil.

The action of the glyphosate is on the *enzyme enolpyruvyl-shikimate-3-phosphate synthase (EPSP synthase),* important for biosynthesis of amino acids, and therefore, by inhibiting this enzyme, the plant dies. The use of those organisms turns out to be a great problem since it would mean the development of *"superundergrowths"*, due to the massive application of this herbicide, which with after some time generates resistance in undergrowths (weeds), in addition to possible crossings with similar non-transgenic plants. Many Latin-American populations are in a situation of fight, due to the loss of the native corn and varieties cultivated in remote times, that through policies are replaced by non replicative-GMO seeds, known as Terminator seeds. This type of technology try to prevent genetic mixture with wild types due to the high rate of crossing of this species since the way of dispersion of the pollen is the wind. However, this trait has been perversively used for economic purposes by attacking native-fitted varieties seen as competences for lab seeds. It is important to note this is not a scientific problem itself, but a problem of ethics in government agents and companies that allow such an unfair and anti-biodiversity use in times of environmental challange.

Nowadays, GMO corn and soybeans that are resistant to glyphosate compose the majority of raw material in markets of the USA and other countries (resistance observed for the first time in 1996). Since it introduction to the market, there has been a spectacular increase in the development of crops of the transgenic soybean. Sadly, they are harvest on regions of tropical forests where worldwide flora and fauna have their refugee. Consequences, besides that damage, are losses of soil and erosion, due to the cultivation and lack of a post-harvest-coverage. Similar things happened with the production of corn, cotton and rape (canoil) that also have had a high development rate at an almost equal level, but lower than the soybean. Of all these crops, the USA produces two-thirds of the worldwide production of GMO plant crops.

Nourishing along increasing qualities of crops

During the last 50-100 years, the genetic improvement of the plant cultures has resulted in an important improvement of the productivity and increase in the nourishing capacities, but in the last years there has been decreases and even stagnation in the productive levels, which might be due to a lack of policies for soil protection. An example of cultures where biotechnology has helped to correct a nutritional deficiency is the one of golden rice, which shows increased levels of beta-carotene, a predecessor of the vitamin A.The lack of this vitamin is a fact in many parts of Asia and Africa, where each year numerous children become permanently blind due to this deficiency. There has been detected a lack of nutrients in Latin America and the Caribbean. There are other studies that have been designed to increase the levels of fatty acids, of antioxidants and of other vitamins and minerals in the plant cultures (CAPÓ and DRANE, 2013).

Transgenic plants and edible vaccines

Vaccines need a manufacturing process under controlled condition. Nevertheless, in underdeveloped countries, there are problems regarding the production, transport or storage of the vaccines since the majority of them need refrigeration and all of them need to be under sterile conditions. It is because of this that there are inexpensive, synthesized vaccines that are being developed by synthesizing edible plants. GMO biotechnology may play a role for solving this situation. This way, the gene that codifies the antigenic subunit of the hepatitis B vaccine has been transferred to a tobacco plant and the same gene has been expressed in its leaves. In the same way, this technique is used to beat the cholera, as well as the use of other vegetables or fruit-plants as the potato or the banana that are being considered *edible plants*.

Nevertheless, considerations for the use of this tool it is understandable since what reaches our intestine is only the gene and not the complete virus or bacteria, there is no possibility that the person contracts the disease, but benefit is enough in order that our immune system reacts by protecting us against a possible infection.

Transgenic tobacco plants for the decontamination of soils

In this case, the transgenic plants are used for the biorremediation of soils. This study was carried out in a zone of a military training camp and firearm manufacturing site during the Second World War. The soil was poluted by residual TNT. To eliminate this problem, genetically modified tobacco plants have been planted. Those plants are capable of generating a major number of decomposing bacteria for this explosive without harmful elements.

Ethical aspects and processes

Scientists have admitted that science is not capable of predicting the whole set of risks and the impact obtained by the environmental release of genetically modified organisms. Therefore, transcendence of GMO release remains unknown and it is uncertain which effect they have on biodiversity, human and animal health, environment, producing systems and even on food safety.

Science can originate, under parameters of rationality, technology that is intended to be essentially productive. Such a pretension, on the other hand, legitimates the use of knowledge (PFEIFFER, 2001). Technology sets out the following issues within its area of duties subjected to the principilist frame of bioethics, as follows:

- Enhancement tool for human regulation of natural processes only through a moderate form and only if there is a need to do so - non- maleficence principle-.
- In a form that is reasonably useful for life, far away from conflicts of interest that can be detected due to private business - beneficence principle-.
- In conformity with desired rational and logical will of all the affected stakeholders - rational principle of autonomy - and in an impartial way for all living dependant creatures - principle of justice-

Any person who is employed at a laboratory of molecular biology or genetic biology will affirm that there is no ethical problem in the production of genetically modified bacteria or yeasts: they do not suppose any ethically relevant challenge beyond questions of biosafety. When these technologies came up, a moratorium regarding the use of these technologies took place. Back then, a series of experiments on DNA recombination was voluntarily postponed by the scientific community. This process ended with some ethical recommendations by Asilomar Conference regarding the issue in question.

The ethical challenges that arise due to genetically modified animals (GMA) are polyhedral since for the research and production of compounds for medical use, the use of the genetically modified animals is mainly being accepted, as long as few regulations for the manipulation and treatment of those are respected. But when these genetically modified organisms (GMO) are designed to produce tastier meat, the reticence grows and when GMOs of pets are created for capricious purposes, the ethical doubts increase.

For many researchers, the ethical problems of genetically irreversible modifications to invertebrate animals are nonexistent: the response is quite similar and the doubts are principally reduced to the problems of biosafety/biosecurity. The doubts appear when there are plans of modification in vertebrates and in the cases of animals close to the human phylogeny.

Concerns regarding the use of transgenics

The majority of the genetically modified products contain an introduced gene that codifies a protein that confers the desired character to the products (resistance to herbicide, to insects, etc.). Are there any environmental consequences for our health because of this?

In general, if the proteins are neither toxic nor allergic they do not have any negative physiological effect. In case of consuming the EPSP[5] gene of resistance to herbicide together with the plant, it will degenerate rapidly.

In Europe, unlike in the USA, it is obligatory to label transgenic food. As for the risks, a constant debate exists due to a great disagreement about whether there is or not any type of risk. Until now, there is not a solidly proven theory regarding that since there is no scientific proof to demonstrate that transgenic crops by themselves do possess direct risk. Everything lies under suspicion since veiled interests are mutual accusations on used data.

Intellectual Property

A frequently used argument against transgenic food is related to the management of the intellectual property rights and/or patents that force the farmers through market restrictions to pay royalties to the institutional improvement agent. In addition, some allude to the use of molecular

3. Petrocelli, A; Rodríguez, D., Spadafora, C., Tamino, G.; Zannini, P. (2003).- Cap. 1; In Ravarotto, L; Pegoraro, R. (ed). Transgenesi, Clonazione, Xenotrapianto. Ed. Piccini. Padova
4. In Canada, some researchers did go further and used the genes of a spider in a goat, so that silk could be extracted from the milk of the latter.
5. Enzyme enolpyruvyl-shikimate-3-phosphate synthase (EPSP synthase).

strategies that prevent the reutilization of the native tomato, that is, the employment of a part of the crop for cultivation in consecutive years. A known example of the latter aspect is the mentioned technology called Terminator, included in those restrictions of use (GURT)[6] , developed by the Department of Agriculture of the USA and the *Delta and Pine Company* during the decade of the 1990s. This technology has not been incorporated yet to commercial crops and its sale is not yet authorized. The patent restriction operates for example by inhibiting the germination of seeds.

In this point, it is necessary to emphasize the use of the hybrid vigor, one of the most frequent strategies in plant improvement used in the non-traditional varieties. This procedure is based on the crossing of two lineages that act like parental lines, giving place to an offspring with a mixed genotype that possesses advantages as for quality, fitness, and agricultural performance.

As for the possibility of patenting transgenic plants, these they cannot be a patent eligible subject in strict sense, but can be subject of rights of the breeder, managed by the *International Union for the Protection of new Varieties of Plants* (UPOV)[7]. From this perspective, transgenic plants are protected at a level that is equivalent to the one of the varieties generated by conventional procedures. This fact necessarily demands the possibility of using varieties that are protected for agriculture survival and scientific research. In 2003, the UPOV declared about the technologies of restriction of use such as the previously mentioned Terminator the following: in agreement to the existence of a legal frame of protection of the new varieties, is indicated that the application of these technologies is not necessary.

On the initial issue, since the creation of transgenic animals is one of the current applications of the technology of the recombinant DNA, the analysis of its safety and of the ethical implications of its use is a part of the social debate and penetrates the barriers of scientific analysis.At this moment, the use of transgenic animals, or genetically modified animals represents one of the most powerful and complete tools of research of the biological sciences. This is therefore the leading cause because of which the human being should treat animals humanly: for the respect that it owes to itself as another being. Humans cannot degrade its dignity with a conduct that does not bear in mind the animal suffering, and/or put in peril its own survival. Obviously, this conduct that respects the human dignity implies that the human being adequately understands the value of the living creatures that allow his/her own life and that of the nature. Furthermore, mankind should understand the need to pass on to future generations a world in good conditions, without excessive degradation produced by its own selfish desire, but, surprisingly, the key point because of which the human being should do all of this is the maintenance of its own dignity.

As happens with other applications of this technology, our societies are debating two visions that are *a priori* opposites. On the one hand, there had never before existed a major ethical sensibility regarding the respect towards the use of other creatures. On the other hand, the applications of this technology reach fields that are of an enormous social and economic interest and their use can be converted into considerable benefits for the humanity in times future survival is questioned.

This double way is responsible for the presence of different sensibilities as for the use of the animals (transgenic or not). In this way, some defend the abolition of the use of animals on the basis of the rights of these organisms, whereas others defend that society is legitimized to use the animals, regardless of whether animals are considered to have rights or not. Survival acts as a bottom line in both cases. This debate, which is not exclusive for transgenic animals, serves nevertheless as frame for some arguments against its use, especially regarding its application to experimentation and lab animals.

There are arguments against the use of the transgenic animals in research related to an issue prior to its application. For example, during the creation of transgenic animals the genetic integrity of the animals is not respected since takes place a recombination of genetic material of different species and even different kingdoms (between animals and plants for example). Some consider that this recombination of genetic material between species, or the creation of chimeras, which in occasions is a part of the method, alters the concept of *"species"* and is an unnatural intervention that might interfere in the conception of what makes that animal. By thinking this way, questions arise, such as: What is the pig that possesses human genes for avoid transplant rejection[8]?

6. Genetic Use Restriction Technologies.
7. The International Union for the Protection of new Varieties of Plants (UPOV) is an intergovernmental organization with headquarters in Geneva (Switzerland); it was created by the International Convention for the Protection of New Varieties of Plants. The Agreement was adopted in Paris in 1961. Its mission is to provide and to promote an effective system for the protection of new varieties of plants, with respect to the development of new plant varieties for the benefit of the society. Brazil, Spain, Bolivia or Chile are in this union that counts with a total of 66 members in December of 2008.
8. Evidently, in order to be able to observe this difference between human beings and other animals and to affirm the superiority of the human being, it is necessary to depart from a series of observations that show the similarities and differences between the human being and other animals; in this respect, a reasonable description offers the chapter 2 of Fox MA. The Case for Animal Experimentation. An Evolutionary and Ethical Perspective. Berkeley and Los Angeles: University of California Press, 1986; 262.

In response to these moral issues, it has been argued that in genetic engineering there is no recombination of genomes, but that there only are transferred one or two genes, a small fraction of the genome of the majority of the receiving species. That is why it is not possible to speak about "humanization" of the pig, when human genes are transferred to obtain a transgenic pig. "Humanized strains", however, is a word used in laboratories for those cases and individuals. In addition, it should not be ignored that many genes are preserved between different species, for what the presence of certain sequences does not seem to be determinant at the moment of defining the essence of a species.

As for the transgression of the barrier of species, and probable vulnerabilities towards infection agents, the discussion is complex from a scientific point of view since the barrier of species is sometimes neither clear nor immutable.

One of the central criteria has always been the one of cost and benefit. That is one of the the basis for utilitarian ethics analysis. Not only in the economic sense, but in term of knowledge, this means, what kind of knowledge has been obtained at the cost of the sufferings inflicted to the laboratory animals. With this, the ethical balance should be set out - this unstable equilibrium between benefit and sacrifice - and there should be a constant search for alternative methods.

The well known three *R´s*, (RUSSELL AND BURCH, 1959) correspond to the initial letters of three basic principles that identify alternative methods[9]:

- *Remplacement* of the procedures that use animals by others that do not require them.
- *Reduction* of the number of animals used.
- *Refinement* of the methods used.

We as scientists consider that there should add a fourth *"R"*, which would be, at a personal level, the one of the scientist's *Responsibility*[10]. According to Jonas *"The human being is the only being known for having a sense of responsibility. Only human beings can choose consciously and deliberately between different alternatives of actions and this choice has its consequences."* (SIQUEIRA, 2001).

In addition, from another perspective, doing research with animals does generate high economic costs[11]; in many cases, this cost is provided by public funds. For that reason, the insistence on futile experiments will find an additional criticism if it is done without enough racionality.

Thus, *"It is perverse that the principal aim of certain activists of animal rights is science, precisely the area in which there is major moral justification for the death of animals."* (BALLESTEROS et al., 2004). Professional knowledge and suitability, therefore, is indispensable to think in the most appropiate and convenient use of these alternatives for society.

Apart from the individual valuation with regard to the use of animals for food supply or for other uses related to research or to industrial production, and independently of the moral consideration that one has on the manipulation of the animal genomes, it is important, from a global point of view, to indicate that the use of genetically modified animals, which is under a constant control[12], is generating important scientific and sanitary benefits that in the future can produce significant applications of industrial interest.

It is necessary to indicate as well that, from a technical point of view, the current procedures of genetic modifications in animals as well as biotechnological procedures are supervised[13].

At the moment, the use of transgenic animals, or GMO represents one of the most powerful and complete research tools for the biological and medical sciences.

The numerous possibilities of use of transgenic animals in very diverse fields of economic and scientific interest lead some researchers to think that the research with this type of animal is incompatible with the principle of reduction previously mentioned. To underline the fact that transgenic animals do not contribute to the reduction in the use of laboratory animals, here is some information: it is estimated that in the number of animals used for studies related to the creation and use of transgenics increased by 73 % between 1997 and 1998 in Canada, by 29% in Great Britain and by 20% in the USA (GRIFFIN et al., 2009). Knock out organisms are also daily used in laboratories all around the world.

9. In 1986, by means of the Directive 86/609/EEC, the European Community urges its member states to promote the legislation concerning the "three R´s", which is being done since then, however, with different diligences. Finally, the CE has created the European Centre for the Validation of Alternative Methods (ECVAM), located in Ispra, Italy.
10. The moral progress, which is the only progress to which we might aspire, relies on responsibility. Today, there is neither a person nor an institution that could take responsibility for the results of all this experimentation 20 years from now.
11. To start a research project, it is necessary to process a whole series of permissions and train all the staff involved in the project (animal carers, graduate staff and specially trained personnel).
12. Cartagena Protocol on Biosafety to the Convention on Biological Diversity. 29th of January of 2000.
13. Nagoya-Kuala Lumpur Protocol on Liability and Redress, supplement to the Cartagena Protocol. 2010.

Studies which consider the evolution of the use of animals in different countries detect a similar situation: since 1996, there has been taken place a gradual decrease of the experiments that generate severe pain in non- anesthetized animals in Great Britain, Canada and the USA. Techniques have been refined. Currently, the quota of reporting this type of procedure are at a scarce level (GRIFFIN, 2002) and society counts on Research and Ethics Comittees to make sure animal experiments are rightly perfomed. Nevertheless, though the use of mice was decreasing gradually from 1991 to 1997, from then on, a progressive increase is being detected, coinciding with the increase in the use of the transgenic animals. The relevancy of this increase detected in mice turns out to be clear if one bears in mind that the rodents (mice and rats) constitute the group most extensively used as laboratory animals: in 1999, for example, rodents counted for85 % of the total of laboratory animals used in Europe.

Therefore, the information on the use of animals seems to indicate that until the mid 1990s, the effort to reduce the number of animals used for research made by the governments was turning out to be really effective, but from this date, and coinciding with the development of the genetic modification in animals, there has taken place an important change in this reductionist trend. A different necessity created a different demanding for lab animals.

The increase in the use of animals for research that include procedures of genetic modification are linked to the fact that they constitute models to study the detailed molecular mechanism of pathologies, and on the other hand, to the fact that in order to obtain transgenic mutants, a great number of animals that are rejected for not presenting the appropriate geno or phenotype (they do not show the specific characteristics that are required), or because they are not allowed to live. In many occasions a large part of transgenic animals do not survive over a long period of time from their birth, since the physiological and anatomical defects are directly derived from the introduction of the new gene are too significant. Also, these animals by law have to be destroyed once used.

Moreover, transgenetic technologies show a low-level of efficiency in many experiments and many of the animals used for the process die early during the embryonic development or due to anatomical, physiological or behavioral defects. In some occasions the transgenetic process leads to the appearance of unexpected phenotypes, due to a limited control of the technology of insertion of genes or due to unexpected interactions of the introduced DNA with other genes of the animal used (*background* or genetic pool). As an example, depending of the methodology used, the creation of a strain of transgenic mice in the year 2000 required an average of between 365 and 900 mice (HUGHES, 2001). Certainly, technologies have slightly improved since then, but the obtaining of a transgenic animal does still cause the “loss” of a high number of animals.

Once *“founded”* a strain of a transgenic animal, the subsequent animals are created by means of conventional crossing methods, in order to obtain animals that are going to be used for future experiments.

Moreover, in relation to the second *“R”*, **refinement**, the situation gets complex. Technologies of genetic modification, as will be shown, are increasingly precise in means of the insertion of genes. This is why the unwanted effects of the process of insertion, at least in some species, are getting easier to avoid. But random insertion is a persistent deleterious effect stilla for many transgenic techniques. Those animals are prone to have additional health vulnerabilities.

The use of the genetic modification is associated with the search for phenotypical effects that are easily detectable by the researcher, effects that usually are being associated with the presence of important anatomical, histological and physiological alterations, etc. In this respect, one of the most frequent and interesting applications, from the scientific point of view, is the creation of the previously mentioned transgenic *knock-outs*, those are, animals in which a functional gene is replaced with a non-functional version by means of homologeous recombination. This type of *site-directed mutagenesis* by deletion produces the absence of a certain functional gene product. This technology has been especially developed and applied to mice with the aim of creating models of human and animal neoplasic diseases. In these situations in which the disease is *“created”*, the animals undoubtedly suffer to some degree (MEMPHAM et al., 1999).

It is questionable whether it is appropriate to apply the technology of genetic modification to specifically generate animals that experience a disease that in many cases usually would not break out in this species under natural conditions.

Certainly, there might be discussed if the suffering generated by a “created” pathology in an animal model is unnecessary or not, or if it is necessary to be relieved of a iatrogenic process, but what is clear is that for health personnel it is a “must” to cure, if possible,, unless one previously decides not to “generate” the above-mentioned animal model. Sadly, slaughter is the most frequent end for paradoxically avoiding transgenic genes spread.

As for the importance of the genetically modified animals, there are two aspects that must be considered in the third “r” of **reemplacement**. On the one hand, the possibility of creating animal models in species as the mouse, gives the opportunity to reduce the use of non-human primates in some types of clinical trials, for example, the clinical trial of the polio vaccine (GORDON, 1997), in research on neurodegenerative diseases (CHAN, 2004), on viral infections such as hepatitis B, HIV, etc. On the other hand, the development of new technologies for the gene inactivation, as the interference RNA (RNAi), enables to think about alternative methods that in the near future could replace some experiments that nowadays are carried out in mammalian *knock-outs*, such as clinical trials of gene silencing in cultures of stem cells or differentiated cells (HASUWA, 2002), or clinical trials carried out in non-mammalian animals for which the methodology of homologous recombination has not been developed up to the moment (ROIGNANT et al., 2003).

It is possible that the numerous scientific and biotechnological possibilities of the genetic modifications (studies on gene regulation, physiological research, production of proteins or specific hormones, clinical trials on toxicity of medicines, improvement of growth and of quality in agriculture, etc.) lead to an *explosion*, maybe temporarily, in its use. It is important to indicate that the justification for this explosion in the use of transgenic animals on the basis of the potential benefits that derive from their use, is a point of view that belongs to the area of the so-called "utilitarian ethics", which, nevertheless, is not shared by the whole society.

The characters introduced by means of genetic engineering in species destined to the production of edible products contribute to an increased productivity (for example by means of a major resistance to plagues) as well as the introduction of new characteristics of quality. Due to the major development of the genetic manipulation in plant species, all GMO food products correspond to derivatives of plants. A frequently used characteristic is, for example, the resistance to herbicides, as it is possible to use them in a way that they only affect the flora alien to the crop. It is to be emphasized that the employment of modified varieties that are resistant to herbicides has diminished the pollution due to the presence of these products in aquiferous and soils, though it is true that there would be no need for the use of these herbicides, which are very harmful because of their content of glyphosate (GLY) and ammonium glyphosinate (GLU) if these varieties were not planted, which are exclusively designed to resist to the above-mentioned compounds.

Insect pests are one of the most devastating elements in agriculture. For this reason, the introduction of genes that provoke the development of plants that are resistant to one or more insect orders has been a common element of many of the patented varieties. The advantages of this method leads to a minor use of insecticides in the fields sowed with these varieties, which results in a minor impact to the ecosystem that harbors the crops and to the health of the workers that manipulate the phytosanitaries.

Ultimately, the first transgenic animals are being developed. The first transgenic animal that has been approved for human consumption in the USA was a salmon called Aqua Bounty (2010)[14], which was capable of growing twice as fast and also during the winter, thanks to the growth hormone of another species of salmon and the "antifreeze" gene of another species of fish.

In several countries of the world there have appeared groups opposed[15] to the creation of transgenic organisms, principally made up of ecologists, associations that promote consumer rights, as well as some scientists and politicians. Those demand the labeling of genetically modified organisms since they worry about *food safety*, environmental impact, cultural changes and economic dependences that could arise from the use of these products. They invoke to avoid this type of food, whose production would involve damages to health, as well as environmental, economic, social damages and legal and ethical problems due to patent restrictions. Thus, the advantages and disadvantages of the process need to be taken into account. That is to say: the beneficial impact as for economy, environmental status of the near-crops ecosystem and on the health of the farmer should be taken into account, as previously described, as well as doubts with regard to the possible appearance of allergies, changes in the nutritional profile, dilution of the genetic array and the diffusion of resistances to antibiotics.

The Food and Agriculture Association (FAO) indicated the following with regard to the transgenics whose purpose is to serve as food supply: The countries in which transgenic crops have been introduced to fields have not observed notable damages to health or environment. In addition, the farmers use fewer pesticides or less toxic pesticides, reducing in this way the pollution of the water supplies and the damages to the health of the workers, allowing also the return to the fields of beneficial insects. Some of the concerns related to the flow of genes and the resistance to plagues have been approached thanks to new technologies of genetic engineering.

Nevertheless, that there have not been observed negative effects until now, does not mean that could not exist in the future. Many scientists request a careful case-by- case evaluation, before the product or process can be spread, in order to face the legitimate safety concerns.

The elimination of living autochthonous varieties due to the use of genetically improved populations, diminish the genetic range and the biodiversity for other characteristics that can be brought together with the selected characteristics. If one considers, in addition, the induced reproduction impossibility of certain populations or the lack of observation of other biological characteristics beyond the aim of the study, the damage is profound at an ecological level.

The World Health Organization indicates in this regard that the different genetically modified organisms (GMO) include different genes inserted in different ways. This means that

14. Aqua Bounty is a biotechnological company dedicated to research, the development and the commercialization of products that are intended to increase the productivity of the fish farming.

15. There was a protest by Spanish agrarian organizations against the use of transgenics in the ecological agriculture (Puerta del Sol of Madrid, on the 30th of August of 2008).

each genetically modified food (GM) and its innocuousness need to be evaluated individually and that is not possible to make generalized statements on the innocuousness of all genetically modified food. The genetically modified food that is nowadays available on the international market passed the risk assessments and it is not probable that they present risks to human health. In addition, there have not been demonstrated effects on the human health as a result of the consumption of the above-mentioned food by the general population, in the countries where they were approved. The constant use of risk assessments according to the principles of the codex and, where applicable, including the post-commercialization monitoring, need to be the base to evaluate the innocuousness of the genetically modified food.

This way, some arguments against the use of transgenic animals in research are related to an issue prior to its application, such as during the creation of a transgenic animal, the genetic integrity of the animals is not respected since there takes place a recombination of genetic material of different species and even different kingdoms (between animals and plants for example). Some consider that this recombination of genetic material between species, or the creation of chimeras, which in occasions is a part of the technical strategy for the obtaining of a transgenic animal, alters the concept of "species" and is an unnatural intervention that might interfere in the conception of what makes that an animal is such. By thinking this way, questions arise such as what makes that a pig is such in the case that it possesses human genes.

To conclude, there is argued that the direct genetic modification is merely a tool. It can be harmful or benefical; It is a mere extension of the traditional and biological technologies of crossing, so that if genetic modifications of animals provide arguments for accusations such as *"playing God"*, *"unnatural"* or *"to treat to the animals as goods"*, the same arguments would be applicable to the selective crossings that are used in a routine way (BOYD GROUP, 1999)[16].

16. Boyd Group is a forum for the exchange of points of view on questions of interest related to the use of laboratory animals.

Bibliography

1. Ballesteros J, Aparisi A (ed.). Biotecnología, dignidad y derecho: bases para un diálogo. Ediciones Eunsa 2004.

2. Castodiaris C. El ascenso de la insignificancia. Ediciones Catedra 1999.

3. Ciccone E. Bioética. Historia. Principios. Lecciones. Ediciones Palabra 2006.

4. Chan AWS. Transgenic nonhuman primates for neurodegenerative diseases. Reprod Biol Endocrinol 2004; 2:39.

5. Capó MA, Drane J. Ecoética en America Latina y El Caribe. Bioethikos 2013; 7(4):376-387.

6. Ewen SW, Pusztai A. Effect of diets containing genetically modified potatoes expressing Galanthus nivalis lectin on rat small intestine. Lancet 1999; Oct 16; 354(9187):1353-4.

7. Gordon J, Ruddle F. Integration and stable germ line transmission of genes injected into mouse pronuclei. Science 1981; 214(4526):1244.

8. Gordon JW. Transgenic technology as an alternative to animal experimentation. In: Developments in animal and veterinary sciences, 27. Animal Alternatives, Welfare and Ethics 1997; 95-112. Ed. van Zutphen LFM, Balls M. Elsevier Science B.V.

9. Griffin G, Fenwick N, Long M. The Canadian Council on Animal Care: Replacement, reduction, refinement alternatives online. Society for In Vitro Biology, Charleston SC 2009; June 6-10, p.A2007.

10. Griffin G, Gauthier C, Befus D. CCAC guidelines on: antibody production. Canadian Society of Immunology. Lake Louise AB 2002; April 6-9.

11. Hasuwa H, Kaseda K, Einarsdottir T, Okabe M. Small interfering RNA and gene silencing in transgenic mice and rats. FEBS Lett 2002; 532(1-2):227-30.

12. Hughes SS. Making dollars out of DNA. The first major patent in biotechnology and the commercialization of molecular biology 1974-1980. Isis 2001; 92:541-75.

13. Mepham TB, Crilly RE. Bioethical issues in the generation and use of transgenic farm animals. Alternatives to Laboratory Animals (ATLA) 1999; 27, 847-855.

14. Palmiter R, Brinster R. Transgenic mice. Cell 1985; 41:343-5.

15. Pfeiffer ML. El riesgo biotecnológico ¿ficción o realidad? Acta Bioethica 2001; 7(2).

16. Potter VR. Bioethics, science of survival. Perspectives in biology and medicine 1970; 14 (1):127-131.

17. Ravarotto L, Pegoraro R (ed). Transgenesi, Clonazione, Xenotrapianto. Piccin-Nuova Libraria 2013.

18. Roa-Castellanos RA, Bauer C. Presentación de la palabra bioética, del imperativo bioético y de la noción de biopsicología por Fritz Jahr en 1929. Bioethikos 2009; 3, 158-170.

19. Roignant JY, Carre C, Mugat B, Szymczak D, Lepesant JA, Antoniewski C. Absence of transitive and systemic pathways allows cell-specific and isoform-specific RNAi in Drosophila. RNA 2003; 9(3):299-308.

20. Russell WMS, Burch RL. The Principles of Humane Experimental Technique. Editorial Methuen & Co. 1959.

21. Siqueira J. El principio de Responsabilidad de Hans Jonas. Acta Bioethica 2001; 7(2).