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SUPPORT FOR THE IMPLEMENTATION OF THE
NATIONAL BIOSAFETY FRAMEWORK FOR LITHUANIA

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Perspectives for Safe use and Application of Modern Biotechnology in Lithuania

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1. Preface – Introduction

In this study we have tried to analyze public perception and scientific concerns regarding the use of genetic modification (GM) in agricultural and therapy products. The perception of GM in most countries is quite negative. The GM products are considered unsafe and dangerous. However, there is little scientific proof of the risks and threats of GM products. Furthermore, there is little objective and well prepared information available for non-scientifically oriented public and administrative-governmental officials. We have tried to discuss some of the most important GM-related issues and tried to orient the introductory study of the subject towards general population.

The study consists of five parts.

The first and largest part (Chapter 2), written by Prof. Leonas Grinius, addresses genetically modified organisms, their use in agriculture and impact on our food. Situation in the world regarding GMO and several debates on the safety of GMO are presented in detail.

The second part (Chapter 3) addresses biomedical research and industry in the World with some discussion on the situation in Lithuania, especially the biosafety and ethics issues related to the production and therapeutic use of recombinant products, human genetic modification, cloning, and issues related to human embryos and stem cells.

The third part (Chapter 4) addresses biotechnology from commercial point of view with the emphasis on the threats and advantages of using GMO in commercial products. Some recommendations for Lithuania are also given.

The fourth part (Chapter 5) addresses the impact of biotechnology on the environmental issues with the emphasis on pollution treatment and monitoring.

The fifth part (Chapter 6) addresses several new and emerging technologies where GM is widely used, especially nanobiotechnology, DNA, and protein array technologies.

We emphasize that these technologies have been developed worldwide and are slowly advancing into Lithuania. Our country is trying to find a way to both safely use them and avoid all risks associated with the use of GMO. We hope that this study will help to shed some light on numerous discussions and issues related to GMO and other high technologies

2. Genetically modified organisms (GMO): their effect on environment, health of humans and animals, and on production of ecologically clean food

Specific issues addressed in the chapter:

- Major trends in GMO technology;
- Analysis of safe use and application of GMO technology in agriculture and food industry;
- Effect of GMO on the health of humans and animals, and on environment;
- Production of ecologically clean food;
- Biosafety principles and guidelines for safe use of GMO technology in agriculture and food industry;
- Recommendations for safe use of GMO in Lithuania.

2.1. Trends in development and use of genetically modified organisms (GMO)

The potential of GMOs is enormous. For instance, environmental benefits such as nitrogen fixation may be built into genetically modified (GM) crops, and their yields may be increased. The use of external inputs such as pesticides, fertilizers or other energy demanding and potentially environmentally damaging substances may decrease as a result of herbicide resistance and insect resistance. Moreover, health benefits may occur, e.g. if a GMO contains extra vitamins or minerals.

In the past, selective breeding was widely used to alter genetic properties of organisms rather haphazardly. Many important improvements have been achieved using this approach, but it is a slow process. But it was genetic engineering that opened up a new area in biotechnology, as the insertion of foreign genetic material provided faster and more systematic way of directly altering the genome to produce GMOs. It is expected that genetic engineering would help agronomists make the productivity gains that are necessary to supply enough food at reasonable prices.

However, there are some risks and disadvantages related to GMO. For example, herbicide resistance may spread from genetically modified crops to weeds or wild relatives, and conventional or organic crops, or their seeds, may be contaminated. Also, non-target organisms may suffer because of loss of food resources, or the insecticides being broad-spectrum. Moreover, the yield improvements may not be as large as expected, or the use of external inputs may increase instead of decrease. Indications exist for potential health problems related to consumption of some GMOs. Therefore, a careful case-by-case approach is necessary, since each GMO behaves differently and may have unexpected effects to some extent.

Another aspect of the current GMO technology is intellectual property rights and dominance of multinational corporations over seeds and external inputs. This may lead to economic dependence and disempowerment of the farmer, especially in developing countries.

Regulations and directives both at the global level and at the European Union (EU) level regulate GMOs. Also, GMOs are regulated at the national level by the EU member states to some extent. The Convention on Biological Diversity (CBD) and its Cartagena Protocol on Biosafety are the only glo-

bal environmental agreements focusing on GMOs.

The use of GMOs involves many stakeholders such as consumers, farmers who are using GMO and the non-GMO farmers, as well as the environment industry and biotech industry. The stakes are high and the interests diverse. Therefore, the field of GMOs is a difficult and sensitive issue to regulate. The potential negative consequences must be anticipated and prevented, while benefiting from the potential positive attributes must be facilitated.

On the other hand, the growth in world population and the impact that has had on farmlands has to be taken into consideration. Thus, world population in 1900 was roughly 1 billion people. In the year 2000, world population was about 6 billion people. And world population is projected to grow to 9 or 10 billion people by the year 2050. Until the Green Revolution spread to South America and then to Asia, beginning about 40 years ago, the only way for developing world farmers to keep up with population growth was to convert forests, jungles and deserts into farmland. More productive crop varieties developed during the Green Revolution allowed farmers to grow vastly more food on only slightly more land.

It is, of course, possible to increase crop yields by simply planting and harvesting more existing crops. This can be done by planting them more densely, or by increasing the number of acres devoted to growing them. Other methods include increasing the use of fertilizers, pesticides, herbicides and irrigation, each of which have well-known risks. Though effective at boosting yields, vast monoculture regions of intensively farmed land have had significant ecological affects, especially including the loss of biodiversity. Unless a viable alternative is devised the destruction of important ecosystems will increase as the need for more food production increases. One way, or another, increasing food production most often means the ability to produce better yields under the same conditions or, more generally, the ability to better resist weeds, insects and diseases.

In the developed Western countries, advances such as hybridization, agricultural chemicals, and farm machinery have boosted food production to the point where it appears that the amount of food produced has reached the limit of the ability of existing crop plants to convert sunlight to energy. As these Western countries produce all the food they need – and are likely to need in the foreseeable future (climate change permitting) – their current problems are not the same as those in the undeveloped countries, where poverty requires immediate implementation of low-cost solutions.

Local populations in the developing world will have to rely on low cost solutions that do not require unrealistic practices, such as local farmers buying expensive chemicals or equipment. The engineered seeds should have the added benefit of pest resistance and tolerance to extreme environmental conditions, such as drought that are needed to sustain village farms. To fulfill its promises, GMO technology should provide the seeds to farmers that are better adapted to their cultivation requirements. Although there is ample reason to believe that GMOs may in the long term have substantial benefits for food production, there are many hurdles still to be overcome, both scientific and political.

This puts the proponents of GMOs in the dangerous position of over-selling the technology, and thus looking foolish when on occasions it fails to live up to its promise, or fails to do so quickly enough. The opponents of GMOs are equally in danger of denying access to a potentially useful technology for many people who might benefit from it.

For instance, Peter Mandelson, EU Trade Commissioner, has warned that unless the EU closes the gap between its own GM approval system and those of countries exporting feed, hungry cows and struggling farmers will be the result. There is an economic risk in Europe, if we fall behind the global economy in approving safe biotechnology, he stated in a speech at the European Biotechnology Info Day in Brussels.

The commissioner cited a recent report from the European Commission that suggests that Europe might experience increasing problems in sourcing and importing animal feed approved under EU rules, thereby putting heavy strain on the EU livestock sector. He added that …isolation from international trade in agricultural biotech products that have passed credible safety standards may not be a viable option for the EU.

Mandelson argued that, with the population of the world projected to reach nine billion by 2050, food demand will double, while the fight against climate change will also require agriculture to produce more energy crops and raw materials for industry. It is simply not responsible or defensible to calmly refuse to assess the role of GM food in meeting those demands, the commissioner stressed.

This "asynchronous authorization" already has caused trouble for food and feed producers, such as in cases of GM maize approved in the USA, but not in the EU. However, a new soy bean variety, Roundup Ready 2 from Monsanto, is likely to have an impact unseen before. The main EU-importing countries USA, Argentina and Brazil are likely to have adopted the new variety by 2009/10, whereas the process will take several years in the EU and potentially will lead to a shortfall of soy imports.

The slow approval procedures for GM plants in the EU likely will affect the European meat industry, according to an internal report of the European Commissions' DG AGRI cited by Agrar Europe. While an average of only 15 months is needed for the approval of a new GM plant in the USA, 2.5 to 10 years are required in the EU.

Due to the importance of soy as feed in the farming of pigs and poultry, the report predicted extreme changes in the EU meat sector. In the worst-case scenario, the EU would be faced with an import deficit of 32 million tones, of which only approximately 20 percent could be substituted by increased local production.

In 2010, the production of pork may fall by more than one third, poultry by almost 50 percent, and only beef production is expected to remain unaffected. Pork imports are estimated to increase more than 50-fold, imports of beef almost 3-fold and of poultry by 150 percent. Exports would fall drastically: no poultry and beef would remain for exports, and pork exports may be lowered by 85 percent.

2.2. Public perception of genetically modified organisms (GMO) and genetically modified (GM) food

The issues of public perception can be broken down into two areas: intrinsic and extrinsic. Intrinsic concerns are those that have to do with moral concerns about the very process of GMO: that it is unnatural or against religious views for one or more reasons. If intrinsic objections are held, then the extrinsic ones are irrelevant, in the same way that if capital punishment is objected on moral grounds, then there is no need to argue about the methods by which it should be carried out.

Intrinsic objections include the following:

- Developing GMOs is unnatural;
- Scientists are trying to play God;

- We are arrogating to ourselves historically unprecedented levels of power;
- We are disrespecting life by patenting it;
- We are "commodifying" life and illegitimately abrogating species boundaries and exhibiting arrogance, hubris, and disaffection.

Also, some people refuse food of animal origin. Therefore, it is against their believes to consume transgenic plants containing animal genes. Such objections are difficult, if not impossible to refute, because they rest on strongly-held beliefs, rather than on facts. It is fair to say that 'intrinsic' spiritual arguments are the ones which cannot be refuted by any scientific committee.

Extrinsic objections to GMOs rest more on facts and reasoning. They have to do with consequences arising from the application of the GMO technology. Such objections include claims that GMOs may have disastrous effects on animals, ecosystems, and humans. Potential harms to ecosystems include possible environmental catastrophe, inevitable narrowing of biodiversity, and irreversible loss or degradation of air, soils, and waters. Possible harms to humans include risks to the food security of future generations, decreased food security for women and children on subsistence farms in developing countries, perpetuation of social inequities in modern agriculture, a growing gap between well capitalized economies in the Northern hemisphere and less capitalized peasant economies in the South, and the promotion of reductionistic and exploitative science.

In this context one may consider the fable of Prometheus giving fire to mortals. When he brought fire, did mankind extinguish it? On the contrary, humans attempted to learn how to use it to the best of their ability.

Obviously, people draw should draw their conclusions based on considerations having to do with moral facts, such as individual human rights, the duty to do no harm to innocents, the duty to take into consideration the beauty, integrity, and balance of nature, the duty to help liberate the oppressed and to maximize the ratio of good over evil in the world.

However, the GMO proponents contain that main consideration for what is impermissible should not be drawn on a categorical basis: such as prohibiting GM crop plants, or GM microorganisms for environmental remediation. Each individual application should be evaluated on the basis of the potential dangers it is likely to pose and the dangers it is likely to avert.

At the beginning, producers of GMO were focused first on introducing production traits that most directly benefit farmers, millers, and manufacturers. The need to communicate with the general public was lost in the process. As result, consumers in some countries became skeptical towards GMO.

Numbers of societies, especially within European Union, have an entrenched fear of GMO technology because of deep mistrust in regulatory agencies, as well as in corporate ethics. For instance, mad cow disease and other food-related scandals have made many Europeans fearful of their food, and this prompted them to think that regulatory agencies could have prevented such a disease from happening. On the contrary, prevention of it in the US has coincided with a majority of consumers worrying little about genetically engineered foods.

Trade protectionism has motivated many European food producers to help fuel fear of GMO products made by their competitors overseas. The failure GM foods in the European market is more of a failing in education system that has left many people so scientifically illiterate that they are easily manipulated by misinformation.

More, people in developed countries take food for granted, and benefits of GMO technology are not always clear to them. For instance, people very clear understand how medicine saves them from dying, but it is less clear for them how the use of GMO in agriculture keeps them from dying.

As GMO debate continues, GMO proponents argue that it is ethically justifiable to develop GMO that will, without any adverse environmental or social consequences, help to feed hungry children. On the other hand, they indicate that it is ethically unjustifiable to develop GMO that will do no good, but may kill hungry children. According to this line of argument, it is ethically justifiable to develop GMO that will allow more efficient use of arable land, provide nutrients and vitamins to malnourished people, and reduce the use of synthetic chemicals in agriculture. GMO proponents indicate that it is ethically unjustifiable to develop GMO that could produce super-weeds without a consideration of how to prevent this from occurring, or mitigate against it. Below we discuss few specific examples of public perception of GMO technology.

2.2.1. We do not need your Frankenfood

Opponents of genetically modified food often refer to it as Frankenfood, after Mary Shelley's character in her novel Frankenstein. The term was coined in 1992 by Paul Lewis, an English professor at Boston College who used the word in a letter he wrote to the New York Times in response to the decision of the US Food and Drug Administration to allow companies to market genetically modified food. The term Frankenfood has become a battle cry of the European side in the US-EU agricultural trade war.

Some argue that there is more than enough food in the world and that the problem is food distribution, not production, so people should not be offered food that may carry some degree of risk. Arguments are made that genetic modifications might have unforeseen consequences both in the initially modified organisms and in their environments.

A number of scientists, however, like Henry I. Miller of Stanford's Hoover Institution and Gregory Conko of the Competitive Enterprise Institute make the case that foods modified by recombinant DNA splicing present no new or special dangers, but in fact may improve the lives of countless millions worldwide. Specific safety problems in the use of GMO and GM food are discussed in the chapter 4 below.

Perception of GMO in three countries is discussed below. Lithuania has been chosen as a country where this study was written. Also, public perception in the US is described because that country is a world's leader in biotechnology. The UK is a biotechnology leader in Europe and, therefore, UK's situation is described below. More, the British are well know for applying their common sense in different areas of their endeavors, biotechnology included. Also, their scientific community does not shy away from involvement in public debates and their expert opinions are extremely valuable while tackling such a complicated issue as GMO.

2.2.2. Perception of GMO in Lithuania

The use of Genetically Modified Organisms (GMOs) is embedded in the Lithuanian program for Biotechnology development (http://www3.lrs.lt/pls/inter3/dokpaieska.showdoc_l?p_id=224096). According to that document, biotechnology is defined as integration of life science and technical science in order to use genetically modified cells and organisms for purposes of production and

services. So, it is well recognized in Lithuania that biotechnology does require using genetically modified organisms (GMOs) for technological purposes.

Lithuania formalized its regulatory and decision making process for biotechnology in 2003 after five years of development. The Ministries of Health, Economy and Agriculture all have a voice in Lithuania's biotechnology policies and regulations with the Ministry of Environment having the final word on any such decisions.

Two committees provide input into the government's decision-making process. The participants on both committees are nominated and accepted in an inter-Ministry process. The Advisory Committee makes recommendations directly to the Ministry of Environment. It is a policy-level committee comprised of 20 voting members and up to 10 additional nonvoting observers. These members include representatives from Government Ministries, such as the Ministry of Health, Environment and the State Veterinary Service, and nongovernmental organizations such as Greenpeace, and universities.

Members usually have a scientific background or hold an influential position in their organization. The Scientific Committee is comprised of technical experts currently working in laboratories or teaching institutions. They meet as requested by the Advisory Committee to provide scientific opinions on a variety of issues.

After reaching a consensus opinion, the Advisory Committee presents its recommendation to the Ministry of Environment, and the Ministry uses that information at its discretion and develops a recommendation to be circulated to the other Ministries for feedback. Once their opinions are solicited and considered, the Ministry of Environment makes the final decision. A wide range of policies are developed using this process including co-existence regulations, field trial approvals, and opinions on upcoming EU Committee votes concerning biotechnology.

The Lithuanian government in October 24, 2006 has approved a plan for development of high technologies in the country which specifically indicates biotechnology among them.

The measures for biotechnology development in Lithuania include the following:

- Design enzymes with programmable properties;
- Produce technologies for detection of pathogens;
- Develop transgenic plants resistant to pathogens;
- Propose methods for identification of malignant cells using their surface markers;
- Develop new generation of individualized anti-cancer treatments;
- Expand studies of adult stem cells and their use in therapy;
- Produce media ingredients required to cultivate stem cells;
- Create bank of stem cells and prepare guidelines for the use of stem cells.

Thus, development of transgenic plants, which are genetically modified organisms, is an integral part of the biotechnology development in Lithuania, which has been approved by the Government.

Research in this area of agro-biotechnology is carried on in the following institutions: Lithuanian Institute of Agriculture (in Dotnuva), Lithuanian Institute of Gardening (in Babtai), Lithuanian Institute of Forestry (in Girionys), Lithuanian University of Agriculture (in Kaunas), Institute of Biotechnology (in Vilnius) and Vilnius University. All together, these institutions employ

about 100 people involved in agro-biotechnology. Some of those groups have already developed contacts with internationally acclaimed leaders in production of transgenic crops.

As result of those contacts, BASF applied in October 2006 for a permit to field test GM rapeseed in Lithuania. This request was discussed by the Biotechnology Advisory and Scientific Committees and approval of the trails was recommended. Also, EU Commissioner, Mrs. M. Fischer Boel, supported experiments with genetically modified plants during her visit top Lithuania in April, 2007. She pointed out that genetically modified plants which have been found safe for humans and harmless to environment can be cultivated in the EU. To prevent gene transfer, GMO plants have to be cultivated separately from other plants. Mrs. M. Fischer Boel indicated that she recommended to the EU member states to introduce legislative measure to ensure that gene transfer between GMO plants and other plants is prevented.

Initially, both the Ministry of Environment and the Ministry of Health were in favor of approving the petition. But representatives of "green" organizations lobbied aggressively against the field trial. In the end, the Ministry of Environment, which has the final say on these decisions in Lithuania, rejected the request. Officially, the Ministry of Environment noted that it took into account public opinion, opinions of relevant institutions and a possibility of negative effects on the environment in making its decision.

Monsanto has also requested approval for GM (Roundup Ready) corn field trials in Lithuania. The Ministry of the Environment issued its negative decision in April, 2007 without soliciting input from the National Biotechnology Advisory or Scientific committees. A significant factor for such a decision was opposition of a parliamentary committee on the environment to plantings of GMO crops in Lithuania.

Banning of the MON863 seemed to be justified by a paper by French scientists who published a new re-interpretation of Monsanto's field trial data raising doubts about the MON863 safety. After assessing this publication, the European Food Safety Authority (EFSA) has reaffirmed its safety assessment of the genetically modified maize MON863 in July, 2007. Therefore, the GMO Panel of EU saw no reason to revise its previous opinion, that the MON863 maize would not have an adverse effect in the context of its proposed use.

USA authorities monitoring unfair trading practices around the globe have indicated their concern that the Lithuanian government's decision to ban field trials of the transgenic crops lacked a basis in sound science. For details, see recent report on the Lithuanian biotechnology prepared by the Global Agriculture Information Network (GAIN) of USDA Foreign Agricultural Service in April 2007. This report LH7002, "Biotechnology in Lithuania" can be found on net at: http://www.fas.usda.gov/gainfiles/200704/146280747.pdf.

Americans have noticed the negative decision on rapeseed ran counter to the opinion of scientific experts. The USDA indicates that they will continue to monitor this situation and work with FAS/Brussels to ensure Lithuania remains in compliance with its EU and, ultimately, wider trade obligations concerning transgenic crops.

A recent survey of Lithuanians, commissioned by the Ministry of Environment, was conducted in early 2007 at the Fonitel call center. It consisted of 1,000 Lithuanians over the age of 18 throughout the country. The survey found that 60 percent of them disapprove of planting of genetically modified plants in Lithuania. Some individuals

acknowledged the benefits, with about 30 percent saying that the technology could help producing more food and alleviating starvation, and about 20 percent agreeing that GMOs were important for scientific advancement.

In general, there is a lack of knowledge about biotechnology's use in food production among the general population, as in this survey almost 70 percent of Lithuanians say that they do not consume any food derived from GMOs. In fact, foods produced from transgenic plants are plentiful in Lithuania. Those products are the following:

COOKING OILS: Brolio, Lanku, Sodziaus, Kolumbo, Teviskes, Augalinis aliejus, Dolores, Maxima, Optima linija, Perla, Karolina, Zemaicio, Aukselis, Saulute, Omili, Huilor, Oilio, Vitela, Luccija, Jasmine, Caroli, Zitos soju aliejus.

SWEETS: chocolate Dinastija, Safari, chocolate waffles Smakdown, candies Vkus lesciny-siurpriz, chocolate creme Cikonella, nut spread Finetti.

MARGARINE: Optima linija, Aukselis, Aima, Listte, Extra, Osrini.

MAYOINNAIZE: Sodziaus, Provanso.

The "green" parties in the Baltics, particularly in Estonia and Lithuania, are regaining popularity and are actively anti-biotechnology. The parties lost almost all of their seats in parliament in the Baltics in the early 1990's as the focus in those countries became rebuilding their economies. However, focus on global climate change and in part concerns about GMO's have helped their resurgence. For example, the Estonian Greens won almost 7 percent of the votes in the early 2007 parliamentary election. The Lithuanian green activists are becoming increasingly vocal as evidenced by their effort to lobby the government and public against permitting any field trials of transgenic crops.

Lithuanian greens are emboldened by their successful protest action, which resulted in banning of all experiments with transgenic plants in Lithuania by the Environment Ministry. Currently, they aggressively promote idea of genocide of Lithuanians by genetically modified foods supplied by transnational corporations (www.zalieji.lt). More, the green activists dispute authority of Lithuanian scientists in GMO issues by blaming them to be too eager to accept donations from multinational corporations in exchange for ability to conduct their scientific research.

As scientific proof of deleterious effect of so called "GM foods", Lithuanian greens quote publications of A. Pusztai from 1999 and I. Ermakova from 2005. While doing that, the Greens completely ignore controversy surrounding A. Pusztai work. Also is ignored the fact that I. Ermakova has not yet published her experiments in any peer-reviewed scientific journal. The only source of her sensational claims about debilitating effect of transgenic soy on experimental rats is her private website. It is also very worrisome, that NIH in the US did not find any scientific merit to reproduce in I. Ermakova's work despite lobbying by her supporters.

Farm groups in Lithuania are not as influential as they are in neighboring Poland, but the most influential farm group in Lithuania is a green-farm coalition, and they are vocally anti-GMO. They express their desire to keep Lithuania "GMO-free."

Despite those controversies, the Lithuanian population trusts scientists' opinion on the GMO technology more than any other group such as farmers, the government and environmental organizations. But the vast majority of Lithuanian scientists, even though they recognize the potential benefits of the biotechnology, have no interest group or organization vocally promoting use of the technology.

In 2007, the Ministry of Environment has started an aggressive public awareness and educational outreach program on biotechnology. The goal is to provide scientifically based and balanced view on the GMO technology. The targeted groups include teachers, farmers, politicians and legislators, as well as consumer groups. In mid-February 2007, the first of these programs took place near Vilnius, the capital of Lithuania. The program was designed to explain the science and its current uses. It was directed to government officials, consumer groups and the interested general public. A variety of scientists led these seminars, which were followed by question and answer sessions and open discussions. Several post-seminar discussions were positive toward the technology, although representatives from environmental organizations expressed their opposition to the GMO technology in any form. In April 2007, the Ministry held an educational seminar specifically designed for the members of parliament. Also, a risk assessment seminar on biotechnology was offered to interested scientists in mid-April. Several more outreach sessions are planned throughout the summer and fall of 2007.

2.2.3. Perception of GMO in the US

National telephone surveys (Hallman et al. 2003, 2004) have shown just how uninformed the American public is. While 48% of those surveyed thought GM foods were in their supermarkets, less than one-third believed they had ever eaten GM foods. Considering the prevalence of GM crops in American processed foods, this belief is a gross underestimation.

When asked, 94% of respondents wished to see `labeling of GM ingredients. Of those who initially disapproved of GM crops, 31% were more likely to buy GM food if it was grown in a more environmentally friendly way, and 26% were more likely to buy if it contained less fat than ordinary food. When asked, the public was also uneasy about the health consequences of growing GM crops. Over one-third (37%) of respondents did not believe GM food was safe to consume, while another 18% were unsure.

Furthermore, when asked what topics they would like to see covered in a hypothetical television show featuring genetically modified foods, respondents were more interested in learning about possible health and environmental effects than in issues related to cost. Therefore, the survey found the US public being uninformed about agricultural biotechnology. But, when asked, people were concerned about the technology's health and environmental implications, and about its potential risks and benefits.

Research by the Pew Initiative on Food and Biotechnology has also shown that in 2005 Americans' knowledge of genetically modified organisms and foods continues to remain low, and their opinions reflect that they are particularly uncomfortable with animal cloning. The Pew survey also showed that despite continuing concerns about GM foods, American consumers do not support banning new uses of the technology, but rather seek an active role from regulators to ensure that new products are safe. To better understand the US public's lack of knowledge of GMO issues, Kramer and Thompson examined the 2004 coverage of agricultural biotechnology in four national newspapers, the Los Angeles Times, the New York Times, the Wall Street Journal, and the Washington Post. They found coverage did not reflect those aspects of agricultural biotechnology that most interest the public. Articles were primarily framed in terms of public accountability. However, the angles covered in these articles primarily addressed social and eco-

nomic issues, not health and environmental concerns. Furthermore, social, economic, and legal/regulatory controversies were the most commonly reported controversies rather than health or the environment.

Kramer and Thompson concluded that articles on agricultural biotechnology did not address the concerns of the general public and may not be viewed as relevant by them. The authors noted that emphasis of reporting in newspapers was not likely to change unless a major environmental or public health event related to agricultural biotechnology occurs.

Interestingly, about 550 Amish farmers in Pennsylvania, known of their resistance to technological innovations, have adopted GM crops, because they allow for less intensive farming (less pesticides, etc.), are more productive (under these specific conditions), and do not conflict with the Amish lifestyle (see http://www.whybiotech.com/index.asp?id=3947 and http://www.squidoo.com/amishfarm/).

Amish farmers grow some of the best food in the world, and extremely conscientious. Their free range animal husbandry, organic foods, and careful management and processing of their products are second to none. At the same time, the challenges for these and other small farmers are increasing year-by -year, driving some of them out of the business altogether.

Therefore, it hardly may come as a surprise to learn that some Amish farmers, who have shunned innovations like the telephone and electricity, have embraced biotechnology. In fact, a growing number of Amish in Pennsylvania have been using genetically enhanced seeds because they see them as another tool to help them continue their traditional agrarian lifestyle. For instance, Amish farmers in Pennsylvania say they can earn twice as much with biotech tobacco.

I myself like biotechnology, Amish farmer Daniel Dienner told the Associated Press. I feel it's what the farmers will be using in the future. Dienner is one of about 550 Amish farmers in Pennsylvania who have been growing a genetically enhanced, nicotine-free tobacco plant since 2001. Other Amish farmers have been growing a biotech potato, which is resistant to pests and viruses, on a test basis.

The biotech tobacco has been commercialized by Vector Tobacco and is used in Quest cigarettes, which are designed to help smokers quit the habit. Dienner says Vector Tobacco has been paying about $1.50 per pound for the nicotine-free tobacco – nearly doubles the 80-cent-per-pound rate for traditional tobacco.

The increased income – genetically enhanced tobacco can earn up to $3,500 per acre compared with $300 to $400 per acre with corn – has allowed more farmers to continue farming. Without tobacco, I wouldn't be at it anymore, one Amish farmer told the Associated Press. We have a three-year contract. I wish it would be 10 years.

Amish scholars say genetically enhanced crops are not inconsistent with the simple life that is central to Amish beliefs because it helps them continue their ties to agriculture, allowing families to work together.

2.2.4. Perception of GMO in UK

GMO perception by general public in the UK was reviewed in depth by D. Burke (2004), who served as a Chairman of the UK Advisory Committee on Novel Foods and Processes from 1989 to 1997. Here we address some of topics covered in his review.

As noted by Burke (2004), the conclusive influences on the GM debate in the UK were those of the media and the non-governmental organizations (NGOs). British newspa-

pers run campaigns in the fierce competition for circulation, readers like scare stories although they may not believe them and scientists do not understand the workings of the media; the stage was set for trouble. In fact, the media had a field day when Árpád Pusztai claimed on television on 10 August 1998, and later in a press conference in the UK House of Commons on 12 February 1999, that feeding rats with genetically modified potatoes caused them damage. Despite its eventual publication in a peer-reviewed journal (Ewen and Pusztai, 1999), the Royal Society (1999) stated, after a careful investigation by a peer group found no convincing evidence of adverse effects from GM potatoes. Although Pusztai's claim was not supported by evidence (Chen et al, 2003), the headlines of many newspapers from that period stagger the imagination. Reactions in the press to Pusztai's press conference, collected by Burke (2004), are presented below:

Are we at risk from mutant make-up? Express on Sunday, 21/02/99;

Scientists warn of GM crops link to meningitis. Daily Mail, 26/04/99;

Scientists raise the fear of GM foods triggering new allergies. The Express, 30/04/99;

Lifting the lid on the horror of GM foods. The Express, 12/05/99;

The GM pollen that can mean a cloud of death for butterflies. Daily Mail, 20/05/99;

Mutant porkies on the menu. News of the World, 23/05/99;

GM risk in daily food of millions. Guardian, 24/05/99;

GM food 'threatens the planet'. Observer, 20/06/99;

Meat may be tainted by Frankenstein food. Daily Mail, 06/07/99;

M&S sells genetically modified Frankenpants. Independent on Sunday, 18/07/99.

Just before the results of the Farm Scale Evaluation were released on 16 October 2003, a large number of anti-GM headlines appeared in several British newspapers, clearly aiming to influence public opinion. Burke (2004) provides a sample of the following headlines:

Is GM the new thalidomide? Daily Mail, 08/10/03;

How GM crop trials were rigged. Independent on Sunday, 12/10/03;

Flaw in crop trials destroys the case for GM. Independent on Sunday, 12/10/03;

Stop the rush to GM crops (leader). Independent on Sunday, 12/10/03;

Curb on GM crop trials after insect pollution. Daily Telegraph, 14/10/03;

Polluted for generations. Daily Mail, 14/10/03.

Even after the report was published, many newspapers stated the results as the end of GM in the UK. In fact, the trials did not assess the effects of genetically modifying crops but rather the effect of different types of weed control. Results of these studies, which have little to do with genetic modification, are discussed in detail in the section 5.1.1 of this chapter.

In addition to the hostile attitude of many newspapers, the NGOs involved in the GM debate in the UK have proved themselves to be very skilled at presenting their position to the media. They are highly organized, have clear points of view and are well funded. They know how to 'spin', or change the way journalists approach a story. Their mission is not to debate facts and findings but to influence public opinion, and any debate with them is unlike a standard scientific debate. NGOs are not looking to find a mutually agreed solution, but rather to promote a single uncompromising message. As soon as one objection is dealt with, they move on to the next, never admitting that they might be wrong.

As noted by Burke (2004), scientists, in contrast, know that science at the cutting

edge is not always able to provide clear conclusions. In that sense, scientific findings are always provisional, but faced with the crisp, clear and often outrageous claims of NGOs, they are unimpressive in the public debate.

In the debate following Pusztai's claim, the scientific community was continually losing out, while the pressure groups released one news story after another, winning new headlines about every three days. Scientists were always on the defensive, and often too busy to respond quickly to news stories.

Finally, the media reaction infuriated the UK scientific community as never before. In 2003, UK scientists together with more than 150 scientists across the world, including Nobel laureate of DNA structure fame James Watson, signed a letter delivered to British Prime Minister Tony Blair drawing attention to the positive impact that biotechnology is contributing to conventional agricultural practices in many parts of the world.

Amongst the signers of the letter included Peter Raven of the Missouri Botanical Gardens; Ingo Potrykus, developer of 'Golden Rice'; Gurdev Khush - the legendary rice breeder and winner of the World Food Prize; Florence Wambugu, author of 'Modifying Africa: How Biotechnology can Benefit the Poor and Hungry'; Charles Arntzen, the developer of edible vaccines in crops; and Roger Beachy of the Danforth Center for Plant Science in St. Louis.

Professor James Ochanda of the University of Nairobi co-sponsored the letter campaign because he believes that in Europe, biotechnology is based on ideology as opposed to rational choice. For Africans, biotech crops are an important means of fighting hunger and malnutrition. While Europe is debating about biotechnology, this is a technology that the developing world needs in order to address some of our most pressing societal problems.

The UK and the EU need to move forward with biotech crops, just as has happened elsewhere in the world, says Prof. Kameshwar Rao of the Foundation for Biotechnology Awareness and Education in India, who also sponsored the drive. Biotech crops are helping to address critical needs for increased agricultural productivity and food security. They are not the problem; they are an essential component of the solution.

The scientists cited firsthand global experience that GM crops are providing farmers with cost-effective means of controlling pests while using fewer pesticides and reducing the impact of agriculture in the face of increasing environmental pressures. According to the letter's authors, it is distressing to us to see the impacts that anti-science efforts in the UK have had on the development of excellent basic research into new technologies, as well as those engaged in it.

Leading international scientists overwhelmingly support integrating biotech crops into existing agricultural systems, said Dr. C.S. Prakash of the United States-based Tuskegee University and signer of the letter to Blair. In reality, there is overwhelming scientific evidence that this technology is a safe and useful approach to improving agricultural production and environmental sustainability, and contributes significantly to better health.

The letter to the Prime Minister also outlined the scientists' concerns that the government's science-based reviews of new technologies, including crops enhanced through agricultural biotechnology, were adversely impacted by politics. The scientists urged that government decisions should be science-based policies that foster the development of demonstrated safe technologies with significant environmental and economic benefits in the UK.

The letter was delivered to Prime Minis-

ter Tony Blair on 30 October 2003, and he replied on 7 November: I believe that the technology has great potential in the UK [and the Government] will take decisions on the basis of scientific evidence ... and will not react to scare mongering, but will continue to build a firm evidence base. valuable while tackling such a complicated issue as GMO.

2.2.5. Anti-GMO sentiment in the European Parliament

Some regional members of European Parliament are expressing anti-GMO sentiment. They want the European Commission to delegate them authority of banning GMO in their regions. To articulate their position, they have prepared a report, or opinion as it is known, which claims the present system for monitoring risk to human health and the environment from GM crops is inadequate and accuses the European Food Safety Authority, which advises the Commission, of failing to heed concerns expressed by experts in Member States.

The opinion's author, Pietro Marrazzo, President of Lazio region in Italy, argues that the Commission's approach focuses only on economic issues and do not address the potential health and environmental risks associated with GM plants. He says a more rounded strategy, combining a mix of objectives, is required.

He stresses that co-existence farming cannot be properly managed without first introducing effective monitoring procedures to assess risk for health and the environment. He points out that the existing system allows assessments to be carried out by the companies wishing to market the GM product and also voices concerns about the inadequacy of legislation on seed purity, which he views as essential for effective implementation of co-existence rules.

The report presses for a redefinition of the rules requiring producers to label crops and products which have a GM content of 0.9% or above in any one ingredient. He says this threshold should be lowered for conventional farming, where recurrent contamination can quickly result in high pollution levels in the environment and in the food production chain.

In the case of organic farming, he suggests the threshold should be as close to zero as possible so that the presence of GMOs is reduced to a technically unavoidable level.

Reinforcing these arguments, Marrazzo highlights studies (but do not indicate whether they have been published) carried out in Lazio, which allegedly have shown that GMOs may remain in the soil long-term, especially in certain climatic conditions, and can seep from the soil into water.

He also calls for a simplification of the procedure for applying the 'safeguard clause', which allows Member States or regions to ban the cultivation of GM products on their territories when danger threatens and scientific knowledge is insufficient for a full safety assessment to be made. Marrazzo, a member of the Party of European Socialists, says that the current procedure needs to be strengthened to prevent deliberate GMO release or its contained use, while awaiting the withdrawal or amendment of authorization.

Until such concerns are met, the regionalists insist to keep the existing bans on the use of particular GMO products. Raccording to them, regions should also be able to declare themselves as GM-free.

Marrazzo underlines that the regional level is the most appropriate for implementing and evaluating the effects and risks related to coexistence of GM plants and non-modified plants, and that the Commission should, therefore, take more account of re-

gional views in its future proposals and encourage more funding for research.

2.3. Understanding the GMO and GM Food

As indicated by Burke (2004), scientists and the public work under different value systems. Scientists and technologists see novel applications from new discoveries as logical and reasonable, and characterize all opposition as unreasonable: If only they understood what we are doing, the public would agree with us. This is often untrue. Indeed, the public's reaction to risk is often rather different to that of scientists, and can occur as outrage (the way the public regards Monsanto), dread (as many would regard a nuclear power station explosion) and stigma (the way the public regards food irradiation).

Having acknowledged that still is very important to understand scientific foundation of GMO technology. Without this, productive discussion of GMO issues becomes impossible.

2.3.1. Genetic engineering as a tool to produce GMO

Usually genetically modified organism is defined as an organism whose genetic material has been altered using techniques generally known as recombinant DNA technology. Recombinant DNA technology is the ability to combine DNA molecules from different sources into the one molecule in a test tube. Such a recombinant DNA molecule is introduced into an organism to alter its abilities, e.g., the phenotype of the organism.

Technically speaking, both genetic engineering and conventional technologies lead to genetic modification of an organism. Nevertheless, the GMO term generally does not cover organisms whose genetic makeup has been altered by conventional cross breeding or by mutagenesis breeding, as these methods predate the discovery of the recombinant DNA techniques. Strictly speaking, current term "GMO" encompasses organisms that are "transgenic" (see below).

The origins of genetic engineering represent a series of sequential scientific advances from the discovery of DNA to the production of the first recombinant bacteria (E. coli) expressing a frog gene (Cohen et al., 1973). Such a genetically modified bacteria represented first transgenic organism in which a bacteria ((E. coli) received a gene from different species (frog). This led to concerns in the scientific community about the possible risks of gene shuffling between species and construction of transgenic organisms.

At the Asilomar Conference in Pacific Grove, California, scientists agreed that government should oversee the recombinant DNA research until the technology is deemed safe (Berg et al., 1975). Nevertheless, Herbert Boyer soon founded the first company, Genentech, to use recombinant DNA technology. In 1978, the company announced that it had produced a strain of E. coli that could produce the human insulin protein.

Today construction of transgenic organisms is a common practice when genetic material is shuffled between genomes of different species to cause both new and useful traits. Often these novel traits would not be possible by conventional breeding without using genetic engineering to overcome genetic incompatibilities between species.

Combining genes of diverged species in the same genome by transgenic gene shuffling is the subject of controversy in its own right. Some see the science itself as intolerable meddling with natural order, despite many known examples of natural genetic

crossings occurring throughout history, like, for example, horizontal gene transfer.

Some activists would like to see GMOs banned, while others push simply for required labeling of genetically modified food. Other controversies include the definition of patent and property pertaining to products of genetic engineering and the possibility of unforeseen global side effects as a result of modified organisms proliferating. The basic ethical issues involved in production and use of GMOs are beyond the scope of current review.

2.3.2. Production of GM food

A genetically modified (GM) food is a food product derived in whole or part from a genetically modified organism such as a crop plant, or an animal, or a microbe, such as yeast. Genetically modified foods have been available since the 1990s. The principal ingredients of these GM foods are derived from soybean, maize, canola and cottonseed oil.

Some governments, such as those in the European Union and Japan, have emphasized risks over benefits from GM foods and require mandatory labeling and traceability, while others, such as the United States, have regulatory agencies that have no such requirements. This has led to the United States claiming that bans on the sale of GM products violate free trade agreements and has resulted in trade wars over the requirements for GM food products. Many scientific institutions, even in the European Union and Japan, however, do not judge the risk of unintended changes in composition of GM foods to exceed the risk currently exhibited by conventional crops.

2.3.3. Development of genetically modified crops

The first commercially grown genetically modified (GM) food crop was a tomato called the FlavrSavr created by Calgene in California. The company submitted this crop to the U.S. Food and Drug Administration (FDA) for assessment in 1992. The agency determined that the FlavrSavr was in fact a tomato, which did not constitute a health hazard. Therefore, it did not required special labeling.

Calgene released the GM tomato into the market in 1994 setting a price that was at two to five times higher of the price of standard tomatoes. Even though the FlavrSavr faced production problems and the competition from a conventionally bred Long-Shelf-Life (LSL) variety, Monsanto bought Calgene in 1995.

A variant of the FlavrSavr was used by Zeneca to produce tomato paste, which was sold in Europe during the summer of 1996. It's labeling and pricing were designed as a marketing experiment, which proved that, at the time, European consumers would accept genetically engineered foods. This attitude would be drastically changed after outbreaks of mad cow disease weakened consumer trust in government regulators, and protesters rallied against the introduction of Monsanto's Roundup-Ready soybeans.

The next generation of GM crops included insect protected cotton, introduced into the United States and Australia in 1996, and herbicide tolerant soybeans. Other successful GM crops include insect protected maize and herbicide tolerant maize cotton and rapeseed varieties.

Unlike the European Union, which agriculture is heavily subsidized by the government, these crops have been widely adopted both in the United States and in the countries like Australia that do not depend heavily on subsidized farming. GM crops have also been extensively planted in several developing countries (Argentina, Brazil, South Africa, India, and China) where agriculture is a major part of the total economy.

Currently, GM crops are grown commercially, the principal ones being herbicide- and insecticide-resistant soybeans, corn, cotton, and canola. Other crops grown commercially, or being field-tested, are the following: a sweet potato, which is resistant to a US strain of a virus that affects one out of the more than 89 different varieties of sweet potato grown in Africa; golden rice with increased iron and vitamins; maize with enhanced levels of the essential nutrient lysine to provide better quality protein for animal feeds, and a variety of plants able to better tolerate non-biological stresses. Those stresses are commonly encountered in a normal growing season, such as water and nitrogen limitation, soils of high-salinity or acidicity, or hot weather (Oh et al., 2005, Kasuga et al., 2004, Pellegrineschi et al., 2004, Zhang et al., 2001). Such traits can provide more reliable crop performance over an extended period of cultivation.

Transgenic rice has been developed by a Californian company, Ventria Bioscience (http://www.ventria.com/news), to improve oral rehydration therapy for diarrhea. In sub-Saharan Africa and parts of Latin America and Asia, diarrhea is the number-two infectious killer of children under the age of five, accounting for some two million deaths a year. Recent trials in the Instituto Especializado de Salud del Niño (Children's Hospital) and the Instituto de Investigación Nutricional (Nutrition Research Institute) in Lima, Peru have demonstrated that specialized milk proteins lactoferrin and lysozyme, brand name Lactiva/Lysomin ORS), made in transgenic rice plants improve the effectiveness of oral rehydration solution used to treat diarrhea.

In a randomized and blinded clinical study, 140 children were evaluated. They were admitted to the hospital suffering from acute diarrhea. Results showed the following beneficial results for children who consumed oral rehydration solution with Lactiva/Lysomin ORS:

- Duration of diarrhea was 30% shorter. Specifically, children consuming Lactiva/Lysomin ORS were sick for 3.67 days on average, as compared to 5.21 days for children receiving ORS without Lactiva and Lysomin;
- 85.1 percent of children who consumed Lactiva/Lysomin ORS recovered, while only 69.2 percent of the control group recovered;
- The percentage of children who relapsed after 48 hours without diarrhea was lower in the Lactiva/Lysomin ORS group than in the control group without Lactiva/Lysomin ORS (8.5 percent compared to 18.7 percent).

2.3.4. GM crops around the globe

The majority of commercially available GM crops have well documented agronomic advantage, like herbicide tolerance or insect resistance. These traits offer major benefits to the farmer and the environment. Importantly, economic benefits of GM crops in developing countries are more significant compared to industrialized countries because agriculture in these countries is a larger part of the economy, and employs a larger fraction of the labor force, and often agriculture suffers from losses of crops to insects which are remedied in insect protected GM crops. However, in industrialized countries, the consumer benefits from GM traits are mainly indirect, and channeled through their benefits to the environment, including promotion of efficient use of available arable land and water.

GM crops have shown to contribute to significantly reduced greenhouse gas emissions from agricultural practices. This reduc-

tion results from decreased fuel use, about 1.8 billion liters in the past nine years, and additional soil carbon sequestration because of reduced plough or improved conservation tillage associated with biotech crops. In 2004, this reduction was equivalent to eliminating more than 10 billion kg of carbon dioxide from the atmosphere.

A recent study, GM crops: the global socio-economic and environmental impact – the first nine years 1996–2004, by Brookes and Barfoot from PG Economics Ltd, UK reported (see http://www.pgeconomics.co.uk/GM_global_study.htm) that GM crops contributed to significantly reduced greenhouse gas emissions from agricultural practices. This reduction resulted from decreased fuel use, about 1.8 billion liters in the past nine years, and additional soil carbon sequestration because of reduced plough or improved conservation tillage associated with biotech crops. In 2004, this reduction was equivalent to eliminating more than 10 billion kg of carbon dioxide from the atmosphere, or removing 5 million cars – one-fifth of the cars registered in the United Kingdom – from the road for one year.

The authors indicated that the largest environmental gains from changes in pesticide spraying have been from GM soybeans and cotton, which have reduced the associated environmental footprint by 19 percent and 17 percent, respectively. According to the authors, GM crops have reduced the volume of pesticide spraying globally by 6 percent since 1996, equivalent to a decrease of 172.5 million kg. That's equivalent to eliminating 1,514 rail cars of pesticide's active ingredient. The global pesticide usage savings in 2004 were equivalent to about one third of total pesticide active ingredient used on European arable crops.

According to the study, substantial net economic benefits at the farm level have been realized in addition to environmental gains from biotech crops. The industrialized nations of the United States and Canada, as well as the developing nations of China, South Africa and Argentina, experienced the greatest reductions in the environmental impact of crop production.

Since 1996, global farm income has increased by a cumulative total of $27 billion derived from a combination of enhanced productivity and efficiency gains. This increase in farm income is equivalent to adding 3 percent to 4 percent to the value of global production of the four main biotech crops. Herbicide-tolerant soybeans have generated the greatest gains at more than $17 billion in increased income, while biotech cotton farmers improved their income by $6.5 billion in the past nine years.

Growers in the United States and Argentina have reaped the greatest rewards, each gaining approximately $10 billion in the past nine years, while farmers in China have experienced a $4 billion income increase from planting biotech cotton.

In addition to the significant measurable benefits, valuable indirect benefits that are more difficult to quantify can be credited to biotech crop adoption. These include increased management flexibility, facilitating reduced tillage practices, reduced production risk and improved crop quality.

Graham Brookes, director of PG Economics, and one of the authors who conducted the study stated: As the world is increasingly focused on the need to reduce greenhouse gas emissions, it is clear biotech crops are already making an important positive contribution to achieving this goal. The EU is currently missing out on these environmental and economic benefits. As a European citizen, I find it difficult to see why we are denying ourselves a clear opportunity to improve our environment and to improve

the incomes and efficiency of our agricultural sector.

In the US, 89% of the planted area of soybeans, 83% of cotton, and 61% maize was genetically modified varieties by 2006. Genetically modified soybeans carried herbicide tolerant traits only, but maize and cotton carried both herbicide tolerance and insect protection traits. The latter trait was due to expression of the Bacillus thuringiensus insecticidal protein, e.g., Bt protein. In the period 2002-2006, there were significant increases in the area planted both to the Bt-protected cotton and maize, and to the herbicide tolerant maize. The Grocery Manufacturers of America estimate that 75% of all processed foods in the U.S. contain a GM ingredient.

Although most GM crops are grown in North America, in recent years there has been rapid growth in the area sown in other countries. Notably, in 2005 Iran grew its first crop of biotech rice, the first biotech planting of this important food crop globally. The Czech Republic planted Bt maize for the first time, bringing the total number of EU countries growing biotech crops to five with Spain, Germany and the Czech Republic being joined by France and Portugal, which resumed planting biotech maize after four and five year gaps, respectively. This could signal an important trend in the EU.

As indicated by the International Service for the Acquisition of Agri-biotech Applications (ISAAA, 2005), two-thirds, or 14 of the 21, countries growing biotech crops achieved "mega-country" status by planting 125,000 acres or more in 2005, including the United States, Argentina, Brazil, Canada, China, Paraguay, India, South Africa, Uruguay, Australia, Mexico, Romania, the Philippines and Spain.

Brazil experienced the most significant growth, increasing its biotech soybean area by 88 percent to reach a provisional 23 million acres in 2005. India displayed the largest proportional growth, nearly three-fold, by planting 3.2 million acres of Bt cotton in 2005 compared to 1.24 million acres in 2004.

When biotech crops were first commercialized, critics suggested the technology would never be valuable in the developing world. Now, resource-poor farmers in developing countries account for 90 percent of the 8.5 million growers who benefit from biotechnology, while developing nations represent more than one-third of 2005 global biotech area.

Dr. Clive James, chairman and founder of ISAAA indicated the following: Biotech crops have increased the income of 7.7 million resource-poor farmers in China, India, and South Africa, the Philippines and seven other developing countries, helping alleviate them from abject poverty. The broader commercialization of biotech rice, the most important food crop of the world's 1.3 billion poor and the 850 million hungry and malnourished, can further this effort. Biotech rice could make a substantial contribution to the formidable U.N. Millennium development goal of reducing poverty, hunger and malnutrition by 50 percent by 2015.

In 2005, the GM crops were grown by 8.5 million farmers in 21 countries, 90% of whom were resource-poor farmers from developing countries. Specifically, 60% of global soybean area, 28% cotton, 18% canola, and 14% global maize were sown to genetically modified varieties.

According to ISAAA (2005), the area used to cultivate GM crops grew constantly, as depicted in the table below.

Year	Area used for GM crops worldwide (square km)
2002	587,000
2003	676,000
2004	809,000

Four countries represented 99% of total GM surface in 2001: United States (68%), Argentina (22%), Canada (6%) and China (3%). It is estimated that 70% of products on U.S. grocery shelves include GM-derived ingredients. In particular, pesticide resistant Bt corn is widely grown in the US, as are soybeans genetically designed to tolerate glyphosate herbicides.

2.4. Future developments

Future envisaged applications of GMOs are diverse and include drugs in food, bananas that produce human vaccines against infectious diseases such as Hepatitis B, metabolically engineered fish that mature more quickly, fruit and nut trees that yield years earlier, and plants that produce new plastics with unique properties. While their practicability or efficacy in commercial production has yet to be fully tested, the next decade may see exponential increases in development of GMOs and their products as researchers gain increasing access to genomic resources that are applicable to organisms beyond the scope of individual projects. Safety testing of these products will also at the same time be necessary to ensure that the perceived benefits will indeed outweigh the perceived and hidden costs of development.

According to the ISAAA, the future looks promising for continued increases in adoption levels of GM crops in the next decade. As Dr. Clive summarized his outlook for GM crops: I am cautiously optimistic the stellar growth experienced during the first decade of commercialization will not only continue, but will be surpassed in the second decade. The number of countries and farmers growing biotech crops is expected to grow, particularly in developing countries, while second-generation input and output traits are expected to become available.

Other indicators of continued growth include China's expected near-term adoption of biotech rice, more nutritional biotech food and feed, products and the anticipated introduction of novel crop products used as renewable resources for more sustainable and affordable production of biofuels. ISAAA projects the global value of the biotech crop market to increase from $5.25 billion in 2005 to $5.5 billion in 2006.

2.5. Safety of GMO and GM food

2.5.1. Testing GMO safety

In the USA, regulation of a genetically modified food is determined by the objective characteristics of the food and the intended use of the food, irrespective of the way it was developed. This situation is recognized in the concept of Substantial Equivalence that was developed in 1993 as a criterion for identifying whether a novel food is at least as safe as the equivalent existing food. The FDA takes a safety assessment approach in their regulation of novel foods (including those made by recombinant DNA methods. This policy is outlined in an FDA statement (FDA, Statement of Policy: Foods Derived from New Plant Varieties, (GMO Policy), Federal Register, Vol. 57, No. 104 (1992), p. 22991).

The FDA policy states that a formal premarket review is to be taken when the objective characteristics of any substance added to the food raises issues of safety (Foods Derived from New Plant Varieties. Federal Register 57 104, 22984, May 29 1992, FDA, U.S., Department of Agriculture). Therefore, prior to marketing a new GM food product, manufacturers are required to submit documentation to the FDA to demonstrate its safety and then await approval before selling

it to consumers (United States Food Safety System, FDA, U.S. Department of Agriculture).

Critics of GM food believe this regulatory model fails to sufficiently protect consumers and claim that the FDA is subject to pressure and influence by biotech industry. One concern these critics voice is that novel crop may have unintended changes in composition that have been unintentionally created during the insertion of new genetic material. On the other hand, plant scientists, backed by results of modern comprehensive profiling of crop composition, point out that crops modified using GM techniques are less likely to have unintended changes than are conventionally bred crops (http://www.isb.vt.edu/news/2006/news06.jan.htm#jan0603, FDA, Statement of Policy: Foods Derived from New Plant Varieties, (GMO Policy), Federal Register, Vol. 57, No. 104 (1992), p. 22991).

Fates of DNA and novel proteins from GM crops were compared to their non-GM counterparts after ingestion by animals. A comprehensive fingerprinting of protein profiles, a proteomics approach (Kärenlampi and Lehesranta, 2006) and comprehensive fingerprinting of metabolites (a metabolomics approach) was performed (Catchpole et al., 2005).

These studies found that there were no significant differences in feed or nutritional value of genetically modified crops as compared to nutritional performance of the corresponding conventional crop, and that no residues of recombinant DNA or novel proteins were found in any organ or tissue sample obtained from animals fed modified materials.

Comprehensive chemical fingerprinting of GM potatoes in comparison with conventional potato varieties has shown that, apart from the intended changes in food composition, the GM potatoes appeared to be substantially equivalent to traditional cultivars (Catchpole et al., 2005). Other detailed comparisons of detailed protein profiles of both GM and conventional potatoes (reviewed by Aumaitre, 2004) detected a great deal of variation in protein profiles of different conventionally potato varieties, but found considerably fewer differences in protein profile due to insertion of a new trait by genetic engineering.

2.5.2. Effects of GMO on the health of humans and animals

In August 1998 widespread concern, especially in Europe, was sparked by remarks by nutrition researcher, Dr Árpád Pusztai, regarding some of his research into the safety of GM foods.

Pusztai claimed his experiments showed that rats fed on potatoes genetically engineered to express a lectin from snowdrop had suffered serious damage to their immune systems and shown stunted growth. The lectin expressed by the genetically modified potatoes is toxic to insects and nematodes and is allegedly toxic to mammals. He was criticized by leading British politicians, the majority of scientific peers with expertise in the area and by the GM companies because the announcement of his results in a television interview preceded the scientific publication of his results. When his studies were finally published in The Lancet (Ewen and Pusztai, 1999), no evidence of stunted growth or damage to immune system was substantiated.

The Lancet paper's actual summary was the following: Diets containing genetically modified (GM) potatoes expressing the lectin Galanthus nivalis agglutinin (GNA) had variable effects on different parts of the rat gastrointestinal tract. Some effects, such as

the proliferation of the gastric mucosa, were mainly due to the expression of the GNA transgene. However, other parts of the construct or the genetic transformation (or both) could also have contributed to the overall biological effects of the GNA-GM potatoes, particularly on the small intestine and caecum.

The paper's publication was accompanied by a Lancet's editorial explanation genetically modified foods: "absurd" concern or welcome dialogue? in the same issue. Also, publication was followed by an independent critique Adequacy of methods for testing the safety of genetically modified foods, which had a contrary evaluation of the published data (Kuiper et al., 1999). This was followed by a lively follow up debate in several later issues of the journal.

The British Royal Society sent Pusztai's data to six independent reviewers whose expertise included statistics, clinical trials, physiology, nutrition, quantitative genetics, growth and development, and immunology (37). The reviewers regarded the data as not adequate to support the conclusions because of the following reasons:

- Poor experimental design, possibly exacerbated by lack of 'blind' measurements resulting in unintentionally biased results;
- Uncertainty about the differences in chemical composition between strains of non-GM and GM potatoes;
- Possible dietary differences due to non-systematic dietary enrichment to meet Home Office and other requirements;
- The small sample numbers used in experiments testing several diets (all of which were non-standard diets for the animals used) and which resulted in multiple comparisons;
- Application of inappropriate statistical techniques in the analysis of results;
- Lack of consistency of findings within and between experiments.

Nonetheless, controversy about Pusztai's assertions still lingers, caused by strongly held opposing views on his conclusions and data. Public perception of these issues was discussed above in the section 2.3 of this chapter. Basically, experts are concerned with the public's emphasis on matters well removed from the actual laboratory observations, as well as ignorance in the public debate of hundreds of studies that support the safety of GM foods and feeds (see Burke, 2004).

On the other hand, Pusztai has sent his research protocols to 24 independent scientists in different countries, including experts in physiology, medicine, toxic pathology, nutrition, microbiology and biochemistry. This group of experts provided no additional data but, as in any referee's report, they gave their summary assessment and concluded that the Pusztai's data would be acceptable for scientific papers (see their comments at http://plab.ku.dk/tcbh/Pusztaimemorandum.htm).

Another controversy recently arose around Monsanto's data on a 13-week rat feeding study on a strain of GM corn. In 2004, the Scientific Panel on Genetically Modified Organisms of EFSA has given careful consideration to the arguments set out in the report. Following its investigation of the report, and of the retrospective evaluation of renal tissues and data derived from the 13-week rat feeding study performed by independent peer reviewers, the GMO Panel has concluded that there is no evidence presented in the report that changes the conclusions already reached by the GMO Panel earlier this year in its Opinions on the safety of the insect-protected genetically modified maize MON 863 (EFSA 2004a, b). These opinions state that the results of the rodent toxicity study with MON 863 maize did not indicate concerns about its safety for human

and animal consumption. (http://www.efsa.eu.int).

Thus, the EU regulatory authorities that examined the Monsanto data concluded that the observed small numerical decrease in rat kidney weights was not biologically meaningful, and the weights were well within the normal range of kidney weights for control animals. There were no corresponding microscopic findings in the relevant organ systems, and all blood chemistry and organ weight values fell within the normal range of historical control values for rats.

2.5.3. Presumed toxicity of GMO: Showa Denko debacle

Third biggest Japanese manufacturer Showa Denko K.K. also contributed to the GMO controversy. Often issues related to the Showa Denko cause are completely misquoted in popular media. Some journalists even claim that Showa Denko produced GMO corn that killed people. Although such a claim is complete nonsense, a food supplement produced by Showa Denko harmed people. Therefore, it is necessary to address this issue in this study.

In 1989, the Showa Denko K.K. began marketing a genetically engineered supplement of the amino acid L-tryptophan in the U.S.

In producing it, a gene to increase tryptophan yield was spliced into the DNA of bacteria, from which the substance was then extracted. Within a few months of entering the market, the bioengineered supplement caused an epidemic of an unusual malady (called EMS) that resulted in the death of 37 people and the permanent disability of at least 1,500 others (FDA's Regulation of the Dietary Supplement L-Tryptophan. Human Resources and Intergovernmental Subcommittee of the Committee on Government Operations, U.S. House of Representatives, Washington, D.C., 1991).

To settle this case, Showa Denko paid out a total of over $2 billion in compensation to more than 2,000 victims.

For many preceding years, other manufacturers had marketed L-tryptophan supplements produced from bacteria without use of gene-splicing. Epidemiological evidence from the Center for Disease Control does not link any tryptophan from these other manufacturers with outbreaks of EMS. (Kilbourne, E. Journal of Rheumatology Supplement, vol. 46, Oct. 1996) Further, Showa Denko's genetically engineered tryptophan was found to contain at least one unusual toxic contaminant never before seen in any conventionally produced tryptophan.

Although there was no conclusive proof that EMS resulted from the genetic engineering, the link has not been ruled out; and many experts think it likely that whatever toxins caused the disease were unexpected side effects of the bioengineering procedure. It is well recognized that this procedure can alter cellular activity and generate novel toxins (see also T.J. Simat, et. al. Synthesis, Formation and Occurrence of Contaminants in Biotechnologically Manufactured L-Tryptophan, Proceedings of the 9th International Meeting on Tryptophan Research, Hamburg, Germany, 10-14th Oct., 1998). The main reason a definitive answer has not been reached is that the relevant evidence in Showa Denko's laboratory was destroyed before it could be examined.

FDA scientists confirm that the genetic engineering by Showa Denko scientists might have caused the EMS. On September 27, 1991, Dr. James Maryanski, Coordinator of FDA's Biotechnology Working Group, discussed the matter with other government officials. According to his record of the meeting (FDA Administrative Record at 22,923):

we do not yet know the cause of EMS nor can we rule out the engineering of the organism. In subsequent years, Dr. Maryanski continued to acknowledge that bioengineering cannot be ruled out (FDA Public Meeting on Bioengineered Foods, Washington, D.C. November 30, 1999)

Despite Showa Denko Debacles the decision-makers issued a policy statement In 1992 asserting there is overwhelming consensus among scientists that GE foods do not entail different risks than conventional ones. Accordingly, the policy presumes every GE food is as safe as its conventional counterpart unless demonstrated otherwise. (The only exception is for foods from one of the few species involved in the most common food allergies.) The FDA does not require any testing, and testing is done on a purely voluntary basis by the manufacturer, with all critical decisions left to its discretion. Thus, U.S. law declares that new foods such as these cannot be deemed safe unless there is a reasonable certainty they will not be harmful. Further, determination of safety must be based on solid evidence from standard testing (21 CFR 170.3(b) & (h)).

2.5.4. Are GMO allergenic?

As more genetically modified plants become present on the market, the more people will be consuming proteins new to the human diet. The possibility that isolated cases of allergic reactions to a new protein could arise is not out of the question. Automatically assuming that genetically modified foods cause allergic reactions, however, is not justified.

In 1993, the Pioneer Hi-Bred International genetically engineered a soybean variety with an added gene from the Brazil nut despite the fact that Brazil nuts were already known to produce food allergies in certain people. The company's intention was to increase the levels of the natural essential amino acid methionine, a protein building block commonly added to poultry feed to improve effective protein quality. Unfortunately, the methionine-rich protein chosen by the Pioneer Hi-Bred turned out to be the major source of Brazil nut allergy (Nordlee et al., 1996).

Investigations of the GM soybeans developed by the Pioneer Hi-Bred International revealed that these soybeans produced immunological reactions with the people who were suffering from Brazil nut allergy. As a result of this mishap, the company discontinued further development of the GM soybean and had all material related to the modified soybeans destroyed.

While this study indicates the possible risks of GM foods, and indeed any new food source, some point out it establishes the commitment the developmental community has toward consumer safety, as well as the competence of current safeguards because of food allergy problems occurring with many conventional foods. For instance, Kiwi fruit, as a relatively new food in many communities, has become widely eaten by people despite provoking allergies in certain individuals.

Another allergy issue was described in studies on transgenic amylase inhibitor in peas (Prescott et al. 2005). The authors indicated that transgenic expression of a plant protein (R-amylase inhibitor-1 from the common bean (Phaseolus vulgaris L. cv. Tendergreen)) in a non-native host (transgenic pea (Pisum sativum L.)) led to the synthesis of a structurally modified form of this inhibitor. Employing models of inflammation, they demonstrated in mice that consumption of the modified amylase inhibitor, but not the native form of it, predisposed to antigen-specific CD4+ Th2-type inflammation. Furthermore, consumption of the

modified inhibitor concurrently with other heterogeneous proteins promoted immunological cross priming, which then elicited specific immuno-reactivity of these proteins. The authors concluded that transgenic expression of non-native proteins in plants may lead to the synthesis of structural variants possessing altered immunogenicity.

As result, the Australian Commonwealth Scientific and Industrial Research Organization, the national government body for scientific research in Australia, has scrapped its $5 million project to develop a genetically modified pea because of published allergic reactions in trials on mice.

Respected plant scientist Maarten J Chrispeels from University of California in San Diego has made interesting comments about this example that illustrate how foods offer many different types of risks (http://www.agbioworld.org/newsletter):

First of all, amylase inhibitor is a food protein, but also a "toxic" protein because it inhibits our digestive amylases. This is one of the reasons you have to cook your beans! The other toxic bean protein is phytohemagglutinin, and it is much more toxic. This particular amylase inhibitor is found in the common bean (other species have other amylase inhibitors). Even though it is a food protein, it is unlikely ever to be used for genetic engineering of human foods because it inhibits our amylases. What the results show is that the protein, when synthesized in pea cotyledons has a different immunogenicity than when it is isolated from bean cotyledons (the native form). This is somewhat surprising but may be related to the presence of slightly different carbohydrate chains. Is there some difference in the folding or in the C-terminal processing at the two C-termini? The results fully support the notion that approval of every GMO should be based on an evaluation of the crop and of the transgene.

2.5.5. GM food may have positive side effects like reduction of mycotoxin level

Mycotoxins are chemicals made by molds that are detrimental to human health. Many different mycotoxins are produced by various fungi such as Aspergillus or Fusarium species that grow on plants. Some of these chemicals cause liver damage, or cancer. In the case of the chemical called fumonisin, which is mycotoxin produced by certain Fusarium fungal species that are natural colonizers of maize plants, the fungal toxin is known to cause (i) severe human birth defects when pregnant women ingest food such as tortillas made from moldy maize, and (ii) cancer in adults when either men or women drink maize based alcoholic beverages fermented from mouldy maize. These food safety problems are serious health issues in regions where maize is a staple food in Central America, South Africa and China.

World-wide trade losses from mycotoxin presence in maize are hundreds of millions $US annually, with the United States, China, and Argentina suffering the greatest losses. The reduction of mycotoxins provided by Bt corn has been estimated to provide the United States alone a total benefit of $23 million annually (Wu et al., 2004). Fungal growth on maize is promoted by moisture, climatic factors, and most notably, insect predation. Several reports demonstrate that insect protected GM maize can have lower mycotoxin levels due to reduced insect damage to the crop.

2.5.6. Position of independent European science journalists regarding safety of GM food

European perspective on GMO of independent science journalists is represented on the GMO Compass website (www. gmo-

compass.org), which is financially supported by the European Union within the European Commission's Sixth Framework Program.

These European authors indicate that knowledge on allergens has increased significantly. Databases now exist that contain extensive information on myriad allergens. Although there is no such thing as absolute certainty, many criteria are now known that characterize known allergens. As result, tests for allergenicity have been becoming more and more accurate and reliable. That allows checking new proteins from GMOs to see if they possess any of these criteria.

When new genetically modified plants are being approved, their allergenic potential is reviewed. Since GMOs tend to differ from conventional foods by only one or a few proteins, these "allergy checks" can be done quite straightforwardly. Obviously, if a GM plant is found to contain a potential allergen, its chances of receiving approval in the EU are slim to none. Only GM plants containing new genes that have a very low probability of causing allergies receive a positive assessment from scientific reviewing committees.

Some genetically modified plants contain no novel proteins. Sometimes, an existing gene is simply switched off by means of incorporating a reversed copy of the gene, canceling out the existing version. An example of this is the Favr Savr tomato, in which an enzyme involved in ripening was repressed.

The GMO Compass indicates that developing new cultivars by conventional plant breeding and new processing methods can also change the properties of proteins found in food and increase its allergenic potential. But allergenicity of a new food, such as a new exotic fruit, is difficult to predict because the number and characteristics of the new proteins remain completely unknown. Therefore, no one can know for sure if new allergens are lurking in novel products when an exotic fruit or food is to the market. As was the case with kiwis, the first cases of allergic reactions come up only years after a new food's introduction to the market.

The European GMO Compass concludes that although it isn't easy to predict the allergenic potential of new foods, rejecting GMOs because of allergies is unjustified.

2.5.7. Conclusions about safety of GM food

Although no major health hazards have come to light since GM food was introduced 12 years ago, and close to 150 studies are published to attest their safety, consumer rights groups such as the Organic Consumers Association (http://www.organicconsumers.org/) and Greenpeace (http://www.greenpeace.org/) emphasize the long term health risks which GM could pose, or that the risks of GM have not yet been adequately investigated.

In this regard, it seems relevant to discuss a review of publications regarding safety of GM foods conducted by Preston (2004). He searched the PubMed database using the search terms (genetically and modified and food) coupled with crop species with known genetic modifications, including maize, soybean, canola, cotton, potatoes, tomatoes and peas. Searches also included the word transgenic instead of genetically and modified. A large number of hits were obtained by this search strategy, with most having little or nothing to do with GM food tests.

He collected papers that had the following: (1) an abstract in PubMed, (2) were a research publication, not a review or commentary, (3) reported a feeding study involving food or food products from GM crops (not purified proteins from other sources such as bacteria or other GM products) in the abstract, (4) test subjects were mammals, birds

or fish, and (5) reported at least one measure of comparison with non-GM food.

He found 42 publications abstracted in PubMed that passed these tests. Of the 42 publications, most examined the effects of feeding GM crop products to livestock including cattle, pigs and poultry. A smaller number examined effects on rats and mice with two on fish. As reported in the abstracts of the publications, 36 studies found no significant effect of GM crop products on the parameters measured or concluded GM and non-GM products were equivalent.

Four studies reported a positive effect of the GM feed. However, two of these were GM plants engineered for improved food quality. Negative effects were reported in two studies published in 1998 and 1999 by A. Pusztai. Those publications have been discussed above.

Almost two thirds the publications extracted by Preston (2004) from the database have been published since 2002. Many of these examined the potential effects of GM crop on livestock performance and were clearly aimed at determining whether the reports of dangers of GM crops to livestock in the press were true. According to Preston (2004), studies published since 2002 all have reported no negative impact of feeding GM feed to the test species.

2.6. Environmental and ecological impacts of GMO

2.6.1. Case study in UK: effect of GM oilseed rap on insect populations

Some fear that certain types of genetically modified crops will further reduce biodiversity in the cropland, and eventually even lead to the extinction of certain species. For example, herbicide-tolerant crops may be treated with the relevant herbicide to the extent that no wild plants ('weeds') survive. This would reduce population of insects as well as other wildlife, like birds, which feed on weed seeds and on insects.

The UK Farm Scale Evaluations (FSEs) were established because of concerns that the introduction of genetically modified herbicide-tolerant (GMHT) crops could have negative impacts upon farmland biodiversity (Firbank et al. 2003a, b). It was feared that control of weeds in GMHT crops tolerant to broad-spectrum herbicides might be so efficient that it could help to clean up previously weedy fields (Watkinson et al. 2000), exacerbating long-term declines in weeds and the wildlife depending on them (Hails 2000). By contrast, others suggested that GMHT crops might ameliorate intensification by delaying and reducing herbicide use (Firbank and Forcella, 2000; Carpenter et al., 2002), or allowing weeds and associated wildlife to remain in fields for longer (Strandberg and Pedersen, 2002; Dewar et al., 2003).

It has already been demonstrated that the herbicide regimes associated with spring-sown GMHT varieties of beet, maize and spring oilseed rape had direct effects on weeds (Heard et al. 2003), as well as indirect effects on invertebrate abundance and diversity (Brooks et al. 2003; Haughton et al. 2003; Hawes et al. 2003; Roy et al. 2003).

As a follow up, an important paper complementing the earlier studies has been published (Bohan et al., 2005). In that study, authors investigated whether there is no difference between the herbicide management of glufosinate-ammonium-tolerant winter oilseed rape (Brassica napus L. ssp. Oleifera) and that of comparable conventional varieties, in terms of their effect on the abundance and diversity of weeds and invertebrates. The authors estimated the magnitude of any observed differences in weed and invertebrate abundance or diversity, and related those to herbicide management.

Publication of this study resulted in a highly passionate debate about these studies and often misquotation of the results. Therefore, it seems necessary to discuss them in detail.

Typically, winter oilseed rape (WOSR) is a break crop in cereal rotations and is grown one year in every three or four. WOSR is sown from late August to early September. Over-wintering may be difficult in dry years if establishment has been poor, and the crop is frequently grazed by pigeons (Isaacson et al. 2002). WOSR plants form a rosette until March or April, when stem extension begins. Vigorous, dense crops resist broad-leaved weed competition, but slow or sparse crops (late drilled or exposed to draught) may be vulnerable.

As WOSR is a broad-leaved crop ('dicot'), selective herbicides can be used to control grass (monocot) weeds and cereal volunteers, while the herbicides most commonly used to control dicots work best when applied pre-emergence. The GMHT SeedLink variety (Bayer CropScience, Cambridge, UK) used by the authors in their experiment was modified to be tolerant to the herbicide glufosinate-ammonium, the same herbicide that was used in the spring oilseed rape and maize in the UK FSEs. This herbicide has foliar activity against most dicots at a wide range of growth stages, but is less effective on monocot weeds (Petersen 2000), particularly as they become larger.

For total weeds, the authors observed few treatment differences between GMHT and conventional cropping, but large and opposite treatment effects were observed for dicots and monocots. In the GMHT treatment, there were fewer dicots and more monocots than in conventional crops. At harvest, dicot biomass and seed rain in the GMHT treatment were one-third of that in the conventional, while monocot biomass was threefold greater. Also, monocot seed rain was almost fivefold greater in the GMHT treatment than in the conventional. Thus, compared with conventional winter-sown rapeseed, GMO herbicide-resistant plants kept the same number of weeds overall, having more grass weeds, but fewer broad-leaved weeds.

These differential effects persisted into the following two years of the rotation. Flowers of broad-leaved weeds provide food for insects, while their seeds are an important food source for other wildlife. Bees and butterflies that forage and select for dicot weeds were less abundant in GMHT WOSR management in July. Year totals for Collembola were greater under GMHT management. There were few other treatment effects on invertebrates, despite the marked effects of herbicide management on the weeds.

Researchers indicated that fewer butterflies and bees were found in the fields planted with the GM version of winter oilseed rape because of the way herbicide was sprayed, but not because the crop was modified genetically.

Green groups, however, were aghast. These results are yet another major blow to the biotech industry. Growing GM winter oilseed rape would have a negative impact on farmland wildlife, Friends of the Earth campaigner Clare Oxborrow said.

2.6.2. Is Bt corn toxic to insect?

There were claims that certain strains of GM maize (Bt corn) are toxic to plant eating insects. It has been alleged those strains cross-pollinated with other varieties of wild and domestic maize and passed on these genes with a putative impact on maize biodiversity (58).

Subsequent to the publication of these results, several scientists (59) pointed out that these conclusions of the authors, which were

based on results from the Polymerase Chain Reaction method, were lacking appropriate controls for sample contamination and experimental artifacts. After this criticism, the Nature, scientific journal where this data was originally published, concluded the evidence available is not sufficient to justify the publication of the original paper (60).

2.6.3. Honeybees vanish, leaving keepers in peril. Are GMO to blame?

As described in a recent article (A. Barrionuevo, February, 2007) published by the New York Times Company, in 24 states throughout the US, beekeepers have gone through similar shocks as their bees have been disappearing inexplicably at an alarming rate, threatening not only their livelihoods but also the production of numerous crops, including California almonds, one of the nation's most profitable.

In fact, bees are flying off in search of pollen and nectar and simply never returning to their colonies. And nobody knows why. Researchers say the bees are presumably dying in the fields, perhaps becoming exhausted or simply disoriented and eventually falling victim to the cold.

Beekeepers have fought regional bee crises before, but this is the first national affliction. As researchers scramble to find answers to the syndrome they have decided to call colony collapse disorder, growers are becoming openly nervous about the capability of the commercial bee industry to meet the growing demand for bees to pollinate dozens of crops, from almonds to avocados to kiwis.

Some 15 worried beekeepers convened in Florida in February, 2007 to brainstorm with researchers how to cope with the extensive bee losses. Investigators are exploring a range of theories, including viruses, a fungus and poor bee nutrition. One of beekeepers, Mr. Bradshaw, thinks that the quality of forage might make a difference. Last week he used a forklift to remove some of his bee colonies from a spot across a riverbed from orange groves. Only three of the 64 colonies there have died or disappeared.

None of beekeepers considers GMO as a possible culprit. But some of commentators on the Internet (see for example, www.care2.com/news/member/947049031/312650) were quick to declare: Bees also won't pollinate GMO crops. Bye-bye bio-diversity in our food chain.

A literature review on bees by genetically modified plants conducted by Louise A. Malone in 2002 indicates that bees may collect pollen, nectar, resins and honeydew from genetically modified plants and incorporate these into bee products such as honey, pollen and propolis. Of those, GM plants have a real potential to produce pollen containing both transgene DNA and novel proteins, while production of nectar, resins and sap containing both transgene DNA and novel proteins is not at all certain.

Thus, pollen, which commonly occurs in honey at concentrations ranging from 20,000 to 100,000 grains per 10 g (and rarely to a maximum of 5 million grains per 10 g), is thought to represent the most likely source of GM material in bee products. As, according to L. A. Malone, average pollen grain weighs 0.03 g, these values are equivalent to honey containing 0.0006% to 0.03% w:w pollen, with a maximum value of 1.5% w:w in rare occasions.

GM food labeling legislation allows for a food to contain up to a certain percentage of GM material where its presence is unintentional. At present this percentage is 1% w:w in New Zealand, Australia, the European Union and Saudi Arabian South Korea and Japan allow higher concentrations of GM

material in honey (3% w:w and 5% w:w, respectively), while in Canada and the United States there are presently no requirement to label foods containing GM material.

L. A. Malone indicates that traces of transgene DNA were detected by PCR in shop-bought honey from regions where field trials of herbicide-resistant GM oilseed rape were grown (Friends of the Earth study). Also, a novel protein responsible for kanamycin resistance was detected by sensitive ELISA technology in a sample of honey taken from a hive near flowering herbicide-resistant GM oilseed rape in the United Kingdom (MAFF study). Real concentrations (w:w) of GM material in those honey samples remain to be determined.

Although honey containing GM material cannot be certified as organic, L. A. Malone points out that organic beekeeping rules do not always specifically mention GM crops. Perhaps this problem can be addressed by keeping hives at a certain distance from GM crops. As indicated by L. A. Malone, hives must be kept at least 3 km from conventionally-grown crops.

In most cases, novel proteins and GM plants have little, if any, direct effect on bees. Thus, L. A. Malone quotes experimental evidence that bees (both larvae and adults) did not change patterns of their survival, while being fed either Bt toxins (both lepidopteran-active and coleopteran-active) or Bt corn. L. A. Malone also indicates that cysteine protease inhibitor-expressing oilseed rape and chitinase-expressing oilseed rape caused no effect on bees.

On the other hand, L. A. Malone points out that bee survival is reduced by a few days if bees are fed some serine protease inhibitors at high concentrations, while low concentrations of the same inhibitors have no effect on bee survival.

2.6.4. Can GMO cause environmental threats such as appearance of super weeds?

Clearly, the world in which we live would be a vastly different place without the selective breeding for desirable crop traits that has occurred across several millennia, and more recently, the sophisticated ability to move single genes among species. If we were to revert back to growing 'natural', pre-selected fruits and vegetables, the world could support only a fraction of the people currently on the planet owing to greatly decreased crop yields. Yet, there are ecological risks with such tinkering of genomes.

The transfer of herbicide resistance from crop to weed is a possibility, and one that presumably increases with the likelihood of cross-pollination. However, agronomists know that many weeds and some crop plants develop resistance to herbicides through natural selection and evolution, over long-term exposure to certain herbicides. It is currently unclear whether the transfer of herbicide resistance is greater for genetically engineered resistance than the type of resistance that arises as a result of natural selection.

No matter what methods of weed control we use, the weeds that survive become super weeds for that method. An example is silver leafed nightshade in cotton fields. Before herbicides, persistence of silver leafed nightshade was a different type of problem: that is, there were no resistance issues, but farmers had to hoe the weeds twice to keep them under control. Soon after farmers started using herbicides, resistant strains began to arise. The same is true for every herbicide or management practice. Resistance to herbicides is an on going problem and it will require the continued development of new herbicides regardless of what technology we use. GE is just another tool. However, many

of the newer herbicides developed over the past several years have much less impact on the environment than the ones they replaced. On balance, that should be viewed as a positive step.

The past few years have witnessed a dramatic growth in the genetic modification of commercial crops, with some crops in the USA now being composed of a majority of transgenic material. Crops that have undergone selection for advantageous traits and/or have been genetically modified have conferred a vast benefit on agriculture, by increasing yields and reducing chemical inputs to control weeds and insect herbivores. As 30–40% of agricultural productivity is reduced worldwide by insect herbivory (Oerke et al., 1994), plants selected or created to resist such herbivory are highly beneficial to farmers.

The foregoing concerns have been amplified in recent years, sometimes unnecessarily, by a few notable mistakes and confusions. For example, the report by Quist and Chapela (2001) that transgenic constructs had been found in a native maize landrace in Oaxaca, Mexico, where transgenic maize had not been previously grown, was used by Greenpeace and Friends of the Earth as evidence that GM crops are not safe (Hodgson, 2002).

Following criticisms of the techniques used for the detection of the transgene (e.g. Metz and Futterer, 2002), this paper was ultimately retracted. By this time, however, public concern over the possibility of transgene escape had already been heightened. A recent analysis of over 150 000 maize kernels from the same region failed to find evidence of the presence of the transgene (Ortiz-Garcia et al., 2005). A second example concerns the presence of the transgene from Starlink corn (only approved for release as animal feed) in taco shells and a number of other related products destined for human consumption (Dorey, 2000; Fox, 2001). While this sort of contamination is clearly a cause for concern, it remains unclear whether it resulted from hybridization between GM and non-GM crops in the field, or whether batches of non-GM seed were contaminated by GM seed before planting or after harvest.

On the other hand, there is little doubt that transgenes will eventually move into weeds, although it is less clear what the ecological consequence would be. Thus, despite the financial benefits of growing such plants, there are concerns that the same genes (alleles) which confer a growth advantage to the crop plant could cause ecological problems by escaping and becoming introduced into plant species in the wild (Ellstrand et al., 1999; Haygood et al., 2003; Pilson & Prendeville, 2004; Lu & Snow, 2005; Chapman & Burke, 2006).

This is not an empty concern, as Reichman et al. (2006) recently provided the first evidence of the escape of transgenes into native and naturalized plant populations in the USA. Glyophosphate-resistant creeping bent-grass was identified up to 3.8 km from the control area. Movement of crop genes into wild relatives could potentially result in the evolution of a weedier or more invasive plant species. It is already known that 22 out of the 25 most important crop species hybridize with wild relatives, so it seems probable that such a hybridization event could occur in most systems (Ellstrand, 2003).

Such gene flow depends on two processes. First, in order for a gene to move to a wild relative, there must be a hybridization event between the crop and the wild species. Thus, factors such as pollinator behavior and density, and timing of flowering, will directly influence the rate of such gene flow. Wind-pollinated plants will potentially undergo even less-constrained hybridization owing

to their independence from pollinators. Second, despite the initial assumption that the rate of gene flow is the primary determinant of successful hybridization, there is growing recognition that the fate of hybrid offspring under natural selection has an even greater influence on gene escape. Theoretical work has demonstrated that even with low rates of allele migration, the success of hybridization depends mainly on the selective advantage provided by the allele (Slatkin, 1976; Morjan & Rieseberg, 2004; Chapman & Burke, 2006).

Assuming that the mating event was successful, is the offspring more or less 'fit' compared with its parents? How does it fare in the natural environment, where pathogens, herbivores and competitors conspire to make life difficult? Surprisingly, we know very little about these key questions. Owing to the fact that a phenotype is the product of an interaction between the genotype and its environment, it is perhaps only a matter of time until a certain combination of genes is in the appropriate habitat, thus allowing the hybrid to establish outside the crop setting.

Research on the spread of crop genes to wild relatives is the study of rare events coupled with difficult-to-predict outcomes. It is an inherently complicated field (see recent reviews by Chapman and Burke, 2006, Wolfe and Blair, 2007).

Reports by other researchers also indicate the need for cautionary approach regarding ecological impact of gene flow. To study the transgene movement from crops to their weedy wild relatives, Stewart and colleagues (Halfill et al., 2005, Moon et al., 2007) extensively studied hybridization of crop Brassica napus to weedy Brassica rapa. In their experiments, weedy accessions of B. rapa were transformed with Bacillus thuringiensis (Bt) cry1Ac- and green fluorescence protein (gfp)-coding transgenes using Agrobacterium. Then, ecological performance was assessed of the wild biotype relative versus introgressed hybrids in which the transgenic parent was the crop. Regenerated transgenic B. rapa events were characterized by progeny analysis, Bt protein enzyme-linked immunosorbent assay (ELISA), Southern blot analysis, and GFP expression assay.

GFP expression level and Bt protein concentration were found to be significantly different between independent transgenic B. rapa events. Similar reproductive productivity was observed in comparison between transgenic B. rapa events and B. rapa × B. napus introgressed hybrids in greenhouse and field experiments. In the greenhouse, Bt transgenic plants experienced significantly less herbivory damage from the diamondback moth (Plutella xylostella). No differences were found in the field experiment under ambient, low, herbivore pressure. Thus, the hybrids were the least competitive with wheat compared with parental Brassica competitors, although differences between transgenic and nontransgenic hybrids varied with location. Also, hybridization, with or without transgene introgression, resulted in less productive and competitive populations.

Brassica rapa grows as a wild and weedy species throughout the world and is the most likely recipient of transgenes from GM oilseed rape. For transgene introgression to occur, the critical step that must be realized, is the formation of an F1 hybrid. Concerns exist that hybrid populations could be more vigorous and competitive compared to the parental species.

The authors examined the effect of simulated herbivory and interspecific competition on the vegetative and reproductive performance of non-transgenic F1 hybrids and their parental lines. Several vegetative and reproductive performance measures

were used to determine the effect of simulated herbivory and competition on the Brassica lines, including leaf length and biomass for herbivory and seedling height and biomass for competition. For defoliation experiments, B. rapa showed little response in terms of leaf length but B. napus and the F1 hybrid responded negatively. Brassica rapa showed elevated biomass responses, but B. napus and the hybrid demonstrated negative responses to defoliation.

Defoliation at the cotyledon stage had a slight effect upon final biomass with the F1 hybrid performing significantly worse than B. napus, although seed counts were not significantly different. For the series of competition experiments, hybrids seemed to be more similar to B. rapa in terms of early seedling growth and reproductive measures. The underperformance of hybrid plants when challenged by herbivory and competition, could potentially decrease survivorship and explain the rarity of hybrids in field surveys. However, should transgene introgression occur, the dynamics of hybrids could change radically thus increasing the risk of gene flow from a transgenic oilseed rape crop to the wild recipient.

Recently, Campbell & Snow (2007 published a study based on the use of late-generation hybrids, rather than the direct products of the primary hybridization event. They have provided an insightful assessment of the impact of crop-to-wild plant hybridization by investigating the performance of advanced-hybrid genotypes under realistic field conditions. Specifically, they used a well-studied system, consisting of a weedy radish species (Raphanus raphanistrum) and third-generation hybrids between R. raphanistrum and R. sativus.

It appears in nature that the crop–wild hybrids have replaced the original populations of R. raphanistrum throughout California (Hedge et al., 2006). Campbell & Snow initiated this experiment in Michigan by planting three F1 hybrid populations and three wild populations of R. raphanistrum in 2002. The populations experienced simulated agricultural management and natural environmental conditions through time.

Once the F3 generation was produced, the parent species and hybrids were grown in a semi-natural agricultural garden, under varying plant densities, to examine the effect of competition on life history traits and adult fecundity. Plants were grown (1) alone, (2) with intrabiotype competition (i.e. R. raphanistrum vs R. raphanistrum) or (3) with interbiotype competition (i.e. R. raphanistrum vs F3 hybrids).

Using an elegant path analytic approach, the key finding of this large experiment was that whereas wild plants, when grown alone, generally outperformed the hybrids, overall fitness measures of hybrids were enhanced under competitive conditions. Thus, plant–plant competition may actually serve to increase the evolutionary impact of hybridization by promoting the movement (introgression) of crop alleles into wild populations. As noted by Campbell & Snow, 'the persistence of crop genes within weed populations also depends on the competitive ability of advanced-generation hybrids when growing near its wild relatives, as well as other weed species'.

Thus, body of experimental evidence demonstrates the potential for crop wild hybrids to be successful in realistic environments, and remind us that gene flow between such organisms may prove to one day be more common than previously anticipated. Although a transgene that affords some level of protection against certain biotic or abiotic stress might provide a selective advantage in the wild, it is important to keep in mind that the strength and direction of selection in

such cases may well be context dependent. For example, a transgene that affords protection against a certain pest species is likely to provide a benefit in the presence of the pest, increasing the fitness of the individuals that carry such a trait.

In the absence of the pest, however, any such advantage would disappear. When this is combined with the fact that resistance often comes at a cost (e.g. Coley et al., 1985; Bazzaz et al., 1987), those individuals that carry the transgene might actually find themselves at a relative disadvantage when reared in a pest-free environment.

This phenomenon – known as a 'cost of resistance' – highlights the importance of carefully considering the various effects that a transgene might reasonably have when investigating its likely impact on a wild plant population.

2.6.5. How to prevent transgene escape?

In view of the prevalence of crop × wild hybridization, it seems likely that transgenes will be transmitted, at least occasionally, to wild populations (e.g. Colwell et al., 1985; Goodman & Newell, 1985; Raybould & Gray, 1994; Ellstrand et al., 1999; Stewart et al., 2003; Pilson & Prendeville, 2004). Given the potential for many such transgenes to increase the fitness of wild plants, attention has turned to the development of gene containment strategies to provide a suitable barrier to transgene escape into wild species. Additional details can be found in a number of recent reviews (e.g. Gressel, 1999; Daniell, 2002; Stewart et al., 2003).

2.6.5.1. Keeping the transgene in the crop

Several approaches have been proposed to prevent transgenes from 'escaping' into wild populations and/or non-GM crops. Some of these strategies, such as the production of apomictic or cleistogamous crops (Daniell, 2002), are still in their infancy. Others, such as those detailed below, are somewhat better developed, but all have their shortcomings.

Indeed, Baucom & Mauricio (2004) found that glyphosate tolerance in the agricultural weed Ipomea purpurea (morning glory) carries a strong fitness cost in the absence of the herbicide, and concluded that crop rotation (along with parallel rotation of the herbicides applied to the fields) could have delayed or even prevented the evolution of tolerance. Similarly, the presence of refugia may allow the maintenance of susceptible source populations (Rausher, 2001). These sorts of considerations are of paramount importance in light of the scale at which GM crops are now being grown. For example, Bt cotton is currently being planted on such a large scale in India (Jayaraman, 2005) that resistance of the target pest, cotton bollworm (Helicovera armigera), is predicted to evolve within a few years (Kranthi & Kranthi, 2004).

In the case of a polyploid crop (e.g. cotton, oilseed rape, or wheat (Triticum spp.)), it has been suggested that targeting the transgene to a specific sub-genome will prevent, or at least substantially reduce, gene flow into a wild relative that does not share this genome. While this strategy has the potential to reduce the flow of transgenes into wild relatives, it is only suitable for crops that differ in their genomic composition from local wild populations. It therefore remains unclear whether or not this strategy will be generally effective.

Another logical strategy would be to target the transgene to the chloroplast or mitochondrial genomes. Indeed, in species with strict maternal inheritance, this sort of strategy would prevent transgene escape via pollen flow. In fact, this strategy has been successfully implemented in both tobacco (Nicotiana tabacum) (Daniell et al., 1998)

and tomato (Lycopersicon esculentum) (Ruf et al., 2001).

Unfortunately, although maternal inheritance is widely assumed to be the rule in most angiosperms, rare paternal leakage has been detected in a number of cases (reviewed in Smith, 1989) including, ironically, tobacco (Avni & Edelman, 1991). In fact, one would have to survey > 3000 progeny in order to be 95% certain that the rate of paternal leakage is no higher than 0.10% (Milligan, 1992). Those very low levels of leakage may be sufficient for the escape and spread of a moderately advantageous transgene (Haygood et al., 2004). Another drawback of this approach is that it would do nothing to stop transgene escape via seed. Thus, if any seeds were to escape or be left behind following the harvest, the transgene could easily be incorporated into a wild population via chloroplast (or mitochondrial) capture.

An alternative method of preventing transgene escape via pollen flow would be to insert the gene into a male-sterile line (Mariani et al., 1990). In the case of seed crops, this approach would require the planting of nontransgenic pollen donors to ensure seed set. As was the case for organellar transgene containment, however, this strategy would do nothing to prevent gene escape via seed – even in the case of nonseed crops where no pollen donors are grown, seed can be produced on male-sterile crops when they are pollinated by compatible wild species.

There are also a variety of molecular 'tricks' that can be used to prevent transgene escape by inducing seed sterility, as summarized by Chapman and Blair (2006). For example, the seed-specific gene activation system described by Odell et al. (1994) could be used to induce seed suicide. To achieve such a goal, an external cue (in this case, treatment with tetracycline) can be used to induce a site-specific recombinase (Cre) which excises 'spacer' sequence flanked by lox sites. Removal of the spacer brings together a seed-specific promoter with a target gene that is turned on during seed development. Specifically, if a lethal gene such as a ribosome-inhibitor protein (RIP) was incorporated into this system, induction would result in the production of in viable seeds.

As indicated by Chapman and Blair (2006), one major disadvantage of this type of approaches is that they rely on an external cue to induce the system. Thus, unless all relevant cells are induced, some fraction of pollen grains and/or seeds might still be able to serve as vehicles for transgene escape.

To combat this possibility, Kuvshinov et al. (2001, 2004) suggested the use of a 'recoverable block of function' (RBF) system to induce seed sterility. The blocking construct prevents some vital biological process in the seed, rendering it in viable. More, this blocking system is 'on' until the trigger turns it 'off'. Specifically, the transgene is flanked by a blocking sequence and a recovering sequence. Therefore, blocking construct can be repressed by the activation of the recovering construct by a chemical or heat treatment, which would not be encountered under natural conditions (Kuvshinov et al., 2001). It is important to note, that the blocking sequence can be inserted into an artificial intron within the transgene, thereby preventing the two from being separated by recombination. Hence, incomplete induction of RBF is not a concern in the context of transgene escape because system is always 'on' until the trigger turns it 'off'. It remains to be seen how well this advantage of RBF over the inducible seed-suicide mechanism plays off in real life situations.

2.6.5.2. Transgenic mitigation

Each of the above strategies for transgene containment has certain disadvantages

and, to a varying degree, may not completely eliminate the possibility of gene flow. Because even a low level of gene flow can be sufficient to allow the spread of a moderately advantageous allele (e.g. Burke & Rieseberg, 2003; Haygood et al., 2004), a strategy that reduces the rate of gene escape to a low but nonzero level may not be enough to prevent the establishment and spread of transgenes. A promising alternative to the above approaches would be to couple a potentially advantageous transgene with a gene that is neutral or beneficial in an agricultural setting, but selectively disadvantageous in the wild. This basic approach has been dubbed 'transgenic mitigation' (TM; Gressel, 1999), and a simple example is shown in Fig. 6. In this case, the transgene is directly linked to a gene conferring dwarfing (Fig. 6a), which is not detrimental in an agricultural setting (Fig. 6b). However, if this construct were to be passed to a weedy population, the recipient individual(s) would be less able to compete with 'normal' plants (Fig. 6c), thereby limiting the spread of the transgene.

The success of TM relies on: (1) the mitigation gene being tightly linked to the transgene, such that the chance of recombination between the two is extremely low, and (2) the fitness disadvantage of the mitigation gene being at least as great as the advantage provided by the transgene. An additional concern is that the mitigation gene might be silenced, via either mutation or methylation. However, the insertion of the transgene between two copies of a mitigation gene in a so-called 'tandem construct' greatly reduces the likelihood of the transgene recombining away from the TM construct, and the presence of two mitigation genes makes the inactivation of both copies exceedingly unlikely (Gressel, 1999). Proposed mitigation genes include those conferring agricultural traits such as dwarfing, a loss of shattering, and a lack of seed dormancy, as these sorts of traits are likely to be deleterious in the wild (Gressel, 1999).

Recent work in A. thaliana has resulted in the identification of a gene (GAI) that responds to gibberellic acid; mutation of this gene (gai) renders the plant dwarfed (Peng et al., 1997). The GAI gene is homologous to the mutant genes conferring dwarfing in 'green revolution' wheat (Peng et al., 1999) and the mutant version has become a candidate for testing the efficacy of TM (Al-Ahmad et al., 2004). In this case, a herbicide-resistance gene coupled with gai was transformed into tobacco, and the competitive abilities of the backcross progeny (semi dwarf, herbicide-resistant) were evaluated in competition with wild-type tobacco under glasshouse conditions. At high density, no dwarf individuals survived to flower, whereas at lower density only those dwarf plants on the periphery managed to flower, indicating a very poor ability to compete with wild-type plants (Al-Ahmad et al., 2004).

Because this work was performed in a glasshouse, however, it remains unclear whether or not these results will transfer to the field. Thus, while TM appears to hold great promise as a strategy for reducing the risks associated with transgene escape, the general applicability of this approach awaits further verification.

2.6.6. Future directions

As discussed above, it has become increasingly clear that hybridization between crop plants and their wild relatives is the rule, as opposed to being an exception. Moreover, population genetic theory has shown us that the likelihood of establishment and rate of spread of an allele are governed primarily by the strength of selection, as opposed to the migration rate. Thus, even if crop × wild

hybridization is a rare occurrence, a moderately advantageous transgene would be expected to spread quickly following its escape. Chapman and Blair (2006) point out that increased individual fitness do not necessarily translate into increased invasiveness; fitness remains the best predictor of allelic spread. Thus, the fitness effects of a gene in the wild are a far more important consideration than the overall rate of gene flow (see also Hails & Morley, 2005).

With this in mind, it seems that efforts to assess the risks associated with transgene escape should be primarily directed at quantifying the costs and benefits associated with a given transgene, as well as investigating the possibility that it might provide recipient individuals with unintended (i.e. pleiotropic) benefits. As discussed by Chapman and Blair (2006), such work should be based on direct estimates of fitness, as indirect estimates (such as disease incidence in the case of white mold resistance in sunflower; Burke & Rieseberg, 2003) may not be reliable. Adding to the difficulty of this sort of work is the fact that fitness costs and benefits are likely to vary across environments, taxa, genes, and even insertion events (e.g. Jackson et al., 2004). Indeed, research to date show that the effects of transgenes can be highly variable, indicating a clear need to replicate studies across space and time, and to consider the risks and benefits of GM on a case-by-case basis.

Given that it is virtually impossible to contain genes under field conditions, the idea of countering the advantage provided by a transgene via linkage to one or more selectively deleterious mitigation genes holds great promise. While this strategy has already been tested and shown to be effective in a glasshouse trial (Al-Ahmad et al., 2004), however, it still has not been proved effective in the field. It may well be that the best strategy going forward will be to employ a combination of these strategies – for example the use of a transgenic mitigation construct in conjunction with organelles transformation.

The most obvious benefit of such "Terminator" technology is to ensure that the rights of plant breeders are protected. Meanwhile, farmers will always have the right to grow their own seed. Growing someone else's seed is another thing entirely. The fundamental right rests with the producers of GM seed to be compensated for their inventions. In most cases, farmers are purchasing the enhancement just as much as they purchase fertilizer or other inputs that help them grow more or better crops.

When farmers buy GM seeds, it is common practice for them to make a promise to only use that seed one time, just as purchasers of computer software make an implied promise not to make duplications of their software. Saving seed from the harvest is a violation of the farmers' promise. Terminator technology would only enforce the obligations on potential cheaters, while sparing everyone the cost and aggravation of going to court.

2.7. GMO policy around the world

Many opponents of current genetic engineering believe the increasing use of genetically modified (GM) crops has caused a power shift in agriculture towards Biotechnology companies, which are gaining excessive control over the production chain of crops and food, and over the farmers that use their products, as well.

Many proponents of current genetic engineering techniques believe it will lower pesticide usage and has brought higher yields and profitability to many farmers, including those in developing nations (15). A

few genetic engineering licenses allow farmers to save seeds for next year's planting in less economically developed countries.

Countries with large populations to feed, like India and China, often view risks and benefits of GMO differently then the European Union where more than enough food is produced without using GMO. Some countries, like USA and Canada, produce enough food by traditional technologies, but due to traditions of innovations and entrepreneurial spirit of their populations they are more receptive to GMP.

In August 2002, Zambia cut off the flow of Genetically Modified Food (mostly maize) from UN's World Food Program. Although there were claims that this left a famine-stricken population without food aid, the U.N. program succeeded in replacing the rejected grain with other sources, including some foods purchased locally with European cash donations. In rejecting the maize, Zambians cited the "Precautionary Principle" and also the desire to protect future possibilities of grain exports to Europe.

In December 2005, the Zambian government changed its mind in the face of further famine and allowed the importation of GM maize (16). However, the Zambian Minister for Agriculture Mundia Sikatana has insisted that the ban on genetically modified maize remains, saying We do not want GM foods and our hope is that all of us can continue to produce non-GM foods (17, 18). Similarly, Hugo Chávez in 2004 announced a total ban on genetically modified seeds in Venezuela (19).

The European Union (EU) has since 2003 used a cautious approach, where GMO products are assessed on a case-by-case basis before being approved for market access or cultivation. The executive body of EU, European Commission (EC) is charged with assessing the safety of GM products. It has recently approved the potato created by BASF since a six-year moratorium ended in 2004.

However, within the member states opinions differ widely: Some member states are very positive to GMOs, while others have banned specific GMO products. For instance, Austria banned two authorized GM maize varieties in 1999 and 2000. The Hungarian government announced a ban on importing and planting of genetic modified maize seeds in January 2005, but agreed to be authorized by the EU. Although the European Food Safety Authority ruled in March 2006 that there was no health risk from T25 created by Bayer of Germany, or from MON810 produced by Monsanta (USA), Greece also bans GM plants.

The EU's cautious approach and the member state bans, resulting in a de facto moratorium on GMOs before 2003, have led the United States, Canada and Argentina to start a trade dispute within the World Trade Organization (WTO), challenging EU legislation. This resulted in the so-called EC - Biotech case.

This case reached its conclusion in 2006, when a WTO dispute settlement panel ruled against the EU's handling of biotechnology applications in two ways. It said the EU violated the procedural obligations for implementing decisions under the Agreement on Sanitary and Phytosanitary Measures when it maintained a de facto moratorium for four years ending in August 2003, and violated the substantive obligations of the agreement with scientifically unjustified bans on GMOs already approved by EU authorities. However, Austria pointed to the United Nation's Biosafety Protocol, which allows countries to ban GM crops if there is a lack of scientific certainty over their safety. Austria argued that the WTO in its ruling disregarded this protocol because the complainants – the US, Canada and Argentina – had not ratified it.

In December 2006, environment ministers of the EU threw out a European Commission proposal to force Austria to lift its bans on the GM maize. As a result, Austria retained its right to ban the growing of genetically modified corn. The European Commission will now have to carefully consider the legal and scientific bases that would underpin any further proposals in face of almost certain opposition by the EU ministers.

Ms Helen Holder of the Friends of the Earth Europe said in the wake of the voting by the EU environment ministers: … vote was a complete rejection of the WTO's ruling on GM foods. This is a major defeat for the biotech industry and their friends in the European Commission. Every country must have the democratic right to protect its citizens and environment.

2.8. Guidelines for the use of GMO and GM food

In March 2000, Barun Mitra of the Liberty Institute, a progressive free-market think-tank in India, sent questions concerning agricultural biotechnology to the AgBioView experts with the hope that these experts would be able to address them. He received a great number of responses, which he compiled and edited with the help of Andrew Apel and Gregory Conko (see www.agbioworld.org/biotech-info/articles/agbio-articles/critical.html). Selected expert opinions are presented below.

2.8.1. GMO can ensure environmental sustainability and increase food production

There are two ways in which GMO can help promote environmental sustainability. One way is to increase total food production, thereby making it unnecessary to put marginal or environmentally sensitive areas under plow. The other way is to employ crop production methods that place fewer burdens on the environment.

First, consider productivity. Growing more food on a given area of land means that for any level of output (whether it's enough to feed six billion people today, or nine billion people in 50 years) more land is available for other purposes. That's important, because adding new cropland has historically meant plowing under virgin wilderness area. Greater productivity can be achieved with a combination of processes, including more traditional methods, as described in the answer to Question 1 above. But GE technology is an important tool that allows agronomists to alter plants more quickly and more precisely than do older techniques.

Next, consider the ability to use less agricultural chemicals, including pesticides, herbicides, and synthetic nitrogen fertilizers. Rainwater tends to make these chemicals run-off farms into rivers, streams, and sensitive lands, sometimes upsetting the ecological balance of those systems. Agronomists know, however, that some crop plants, such as certain legumes, have the ability to "fix" nitrogen, absorbing it from the air. If we can splice the ability to fix nitrogen into other crop plants, we could reduce the need for synthetic chemical fertilizers and make a giant step in sustainability.

Similarly, if we can increase disease resistance in crop plants, that added trait would allow farmers to reduce the use of fungicides and improve no-till methods. Genetically engineered plants that are drought resistant, or enable the use of less toxic herbicides could also help achieve these goals. Glyphosate tolerance has shown itself to be a sound technology in this respect, as glyphosate is far less toxic than many other herbi-

cides and becomes effectively inactive within a few days after spraying.

Seed banks have been established around the world at CIMMYT, CGIAR, and other research centers to maintain diverse germplasms that may provide useful traits for cultivated species. These seed banks are also a source for biotechnologists to identify useful genes that they can move between related species to improve crops. For example the Mlo gene was recently cloned from barley and it provides resistance to powdery mildew. Powdery mildew is a problem worldwide in cereals and other crops. GE allows us to take that gene and introduce it into other cereals. Then we can give that seed to farmers and the resistance should restore productivity in fields that are typically devastated by the disease.

An important part of the question is whether this technology will "ensure" sustainability. There is no reason to believe that GE or any one technique will by itself ensure environmental sustainability. There are numerous factors that lead to environmental degradation. For instance, the emissions and other waste created by an affluent, formerly starving nation could have a considerable impact. However, it is highly likely that the use of GE technology can help promote sustainability quite significantly.

2.8.2. Establishing the sound scientific basis for GMO to be safe

Safety is a relative concept. Agriculture and animal husbandry have inherent dangers, as do the consumption of their products. Any sound evaluation of the safety of genetic engineering must also consider the "safety" of current methods of producing food. As mentioned above, nothing is risk free.

Nonetheless, every GE crop plant that is now on the market has been extensively studied in toxicity and environmental impact tests. In most countries, the results of those tests are available through the government. Second, many GM crops have been placed into the field over the past 20 years and experience has shown they are not a problem. Thus, the experience of over 200 million consumers in North America over the past four years, and the planting of tens of millions of acres of genetically engineered crops over that time, gives us additional evidence that the products of genetic engineering we have today are safe.

The GMO critics argue that GM foods are widely used for less then decade, and, therefore, it is premature to declare GM foods to be safe. Again, each government tackles this issue based on the economical and political situation of a given state, as well as traditions and specific demographic situation of a given society. Nations across the globe differ greatly in their approaches to the GMO risk benefit analysis.

Are more risks associated with GMO because they are produced unnaturally? In an important sense, everything about modern agriculture is "unnatural." If we were to have to grow only wild tomatoes, maize or soybeans, we would all starve. The entire recorded history of the human race has been fueled by "unnatural," that is, man-made advances in agriculture by intervening in the DNA of plants and animals.

In conventional breeding within species, it is said that "vertical transfer" of genes takes place. However, biotechnology allows "horizontal transfer" of genes across species. Isn't such horizontal transfer unnatural, and therefore possibly unsafe, as well as unethical?

The question makes a false assumption. Horizontal transfer of genes across species has been occurring naturally for millen-

nia. Therefore, it is natural. For example, one of the techniques scientists use to create transgenic plants is to splice new genes into a naturally occurring soil bacterium called Agrobacterium tumefaciens. This is especially useful, because A. tumefaciens is known to readily insert genes into the DNA of live plants, a naturally occurring case of horizontal gene transfer.

It would be better to ask why anyone would think it is "unethical" to improve foodstuffs. It's much more unethical to leave millions of innocent people hungry.

2.8.3. It is unrealistic to demand zero risks from GMO

We do not demand zero health or environmental risks from anything else–including medical treatment, providing water and power to cities, building cheap housing for poor people. In all these cases, risks are minimized and policed to an acceptable safety standard. But these things can never truly be made "risk free".

The question we should ask is whether there is evidence of risk or harm beyond what we are already experiencing when we grow traditionally bred crops and eat the foods made from those traditionally bred crops. There is no hard evidence that food or environmental safety is any less than what we are used to with non-engineered crops or foods.

Conventional breeding mixes tens of thousands of genes from two (or more) organisms together, and involves sorting through many progeny for the desired characteristics. The functions of many if not all the genes being introduced to each other are not known. Consider the genes that are being introduced to each other in two hypothetical cases:

Conventional breeding mixes about 40,000 genes from one plant with 40,000 genes from another plant. Genetic engineering mixes just 1-10 genes with known functions with the 40,000 genes of the recipient plant. Of course, zero risk cannot be promised by any technology. Even with these older methods of breeding, there have been some unwanted traits. For example, wheat is allergenic to many people. Nevertheless, during the last 10,000-plus years of agriculture human population has enjoyed substantial biological success.

Similarly, considering the risks associated with pesticide use we should assess gains by having a GM plant that requires less pesticide. Next, we should evaluate if the risk of GM plant is known or unknown, probable or implausible. The analysis here should focus on the risks associated with the practices GM will help curtail.

We need not forget about the possible benefit of GM foods. For example, if we increase nutrient content in rice resulting in less disease or blindness, what risk are we willing to take to solve such a problem? Too often when dealing with GE issues we forget to look at the dangers we are reducing with the new inputs as well as forget to look at the tremendous societal advantages that can come from GM seeds. Only when these critical factors are examined alongside any possible risk of GMO, can we determine our risk tolerance level.

2.8.4. Herbicide-tolerant and pesticidal GE crops can reduce the use of agrochemicals

Most current complaints about pesticides and genetic engineering concern the introduction of genes allowing the plants to produce biological insecticides such as Bacillus thuringiensis toxin.

Introduction of this toxin in plants di-

rectly reduces the need for applied synthetic chemical pesticides. Herbicide tolerance enables the use of fewer types of herbicides (reducing usually to one) and reduces the number of applications needed. For example, Bt crops have saved about 1,000,000 liters of insecticide applications in the US during the past 4 years.

Fewer, higher doses of the resisted herbicide are possible without damaging the crop. The end result is that close to the same amount of the resisted herbicide is used, but many other herbicides are eliminated–an overall reduction.

2.8.5. Biotechnology companies have to be totally liable for any harm GMO to environment and public health

In the US and most other countries, standard product safety laws already cover this issue. Furthermore, there is the opportunity for harm to be redressed by lawsuits. In other words, biotech companies are clearly liable for harm to the environment and public health as well.

Such responsibilities are the same as they have previously been: Inventors are liable for the safe operation of their products; growers are responsible for following guidelines to safeguard the environment; processors are responsible for safe, hygienic handling of materials; and consumers are responsible for knowing their own health concerns (e.g. allergies to foods like wheat or dairy) and consuming prudently.

2.9. Recommendations for safe use of GMO in Lithuania:

2.9.1. Define national interests in GMO, assess risks and benefits;

2.9.2. Formulate national policy regarding GMO in Lithuania;

2.9.3. Lithuania should have its own policy regarding GMO within EU;

2.9.4. Lithuania should introduce transgenic plants resistant to climate warming and transgenic plants designed for production of biofuel;

2.9.5. GMO proposals should be evaluated based on rigorous scientific analysis, avoiding influence by politics and emotions;

2.9.6. Develop effective system to control GMO effects on human health and environment in Lithuania;

2.9.7. Lithuania should not shy away form GMO innovations, it should be a GMO leader in the Baltic region;

2.9.8. Provide unbiased information to Lithuanian public about GMO;

2.9.9. Identify and address consumer's needs in Lithuania;

2.9.10. Lithuanian government and scientific community should have open lines of communication with nongovernmental organization and ecological activists.

2. 10. Literature

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3. Biomedical Research and Industry: Biosafety and Ethics Issues

3.1. Introduction

The term biotechnology came into use in the early 1970s following the invention of genetic modification techniques. Thus in the minds of many people biotechnology has been equated with genetic modification, but actually it encompasses a wide range of activities, including medicine and environment [1]. However, here we primarily focus on genetic modification, especially its ethical and safety issues.

Does genetic modification take humankind's ability to alter nature a step too far, thus transgressing boundaries that should not be crossed? Certainly some believe this is to be the case and have intrinsic objections to the whole idea of moving genes. There are several main reasons for holding this view. For example, some conservative religious views embody the idea that species are fixed entities, their genes are part of their essential nature, and that genetic modifications distort that essential nature. Others think that moving a gene from one organism to the other will destroy the complex web of life and disturb the balance of nature. There are also those who consider such activities to be off limits to humans.

However, much more expressed are not the intrinsic objections to genetic modification, but the concerns of great risk that introduction of a foreign gene into an organism will have unforeseen effects. There are at least the following possible risks associated with GMO:

1. Possibility of escape of newly designed microorganisms (or other species) that pose dangers to humans or environment. For example, a new crop may become more vigorous and out compete other species in the wild;
2. The risk of specific, intentional, misuse of genetic modification. For example, microorganisms or viruses created to kill selected part of population based on specific biological difference.

In 1975 a conference was held in USA to discuss safety and risk issues related to newly forming trend of research – genetic modification. A number of precautionary steps were introduced, especially with regard to containment of engineered species. The scientists who attended the conference agreed to use many safety measures in their research. However, the opponents of GMO technology still believe that the conference was only a device to make it appear that the scientists are genuinely concerned about public risks while actually allowing them to continue their work with little restriction.

Over the past 30 years, a number of safety guidelines were relaxed since it has been shown that in most situations the actual risks are much smaller than originally envisaged. Still, in countries like UK, GM technology is operated in a strictly structured set of procedures for regulating this work. For example, any organization that carries out GM activity must form a committee with non-scientific representation from the society to oversee risk assessment, containment details of any new line of research. Some of the regulations are much stricter than many people suppose. For example, in the UK, those working with GM plants must ensure that they do not escape into the environment.

Opponents of GM technology often re-

quire providing proof that the technology is risk-free. However, all human activities involve risk, even lying in bed. It is impossible to prove the negative; therefore, it is also impossible to prove that anything is risk-free. History shows that human activities always carried some risk. For example, airplane invention was very risky, but it provides us a relatively safe means of transportation. The world economy is not imagined without airplanes or automobiles these days despite some inherent risk in using them.

The main question is how much risk is too much and how much is tolerable. One great risk is of course biowarfare. All human technological inventions were used in wars. There are a number of reasons to believe that GM technology will also be used in this way. It was suggested that GM will be used to manufacture subtle biological weapons, for example, organisms that target at particular population groups. Entire biological warfare was banned in 1925 (updated in 1972) by the Geneva protocol. Therefore, there is no need to specifically ban GM weapons. Nevertheless, there have been always concerns that biological weapons are being produced in secret by a number of countries, including USA, USSR, and UK. Allegations are without proof, but the risk remains.

We will now discuss several aspects in medicine where genetic modification technology has already helped or promises to bring cure to various diseases in the future.

3.2. Biopharmaceuticals

The first and best known example is the development of recombinant human insulin. Isolation and cloning of the gene encoding human insulin was reported in 1977. The gene was then expressed in a microorganism, produced by the microorganism, and purified in large quantities. Three phases of clinical trials followed and in 1982 the first GM product was used in human therapy.

Insulin is essential in the treatment of some cases of diabetes. Before the availability of recombinant human insulin, the insulin isolated from pig was used. However, the supplies of pig insulin were scarce. Second, many people were allergic to pig insulin that contains several amino acid differences from human insulin. Therefore, a number of advantages are clearly visible from using GM technology:

1. Reduction of cost;
2. Produced in relation to demand;
3. Quality control of GM-made insulin is much easier than of pig-made insulin;
4. Allergy issues were almost absent with GM insulin.

However, it is notable, that a small part of population is allergic to human insulin and not allergic to pig insulin. The small amount of overall insulin production is therefore being continued by non-GM methods.

Following insulin success, a number of other recombinant biopharmaceuticals were made by GM methods, including human growth hormone, drugs for cancer treatment, many vaccines, and other products. Some of these products were made before GM technology was available. However, most of the biopharmaceuticals could not be made by any other means than GM technology. Furthermore, the GM technology is regarded as much safer with less possibility of side effects than isolation and purification of biopharmaceuticals from organisms in nature.

Some of these aspects are well illustrated by human growth hormone. It is used for children who do not produce enough of the hormone. The previous source of the hormone was pituitary glands of dead people. However, after several years of this use, it became apparent that many of these hormone prepa-

rations were contaminated with the agent that causes fatal neuro-degenerative condition, Creutzfeld-Jacob disease. The use of pituitary-derived human growth hormone was immediately banned. Even after more than 15 years since cessation of treatment, new cases arise, because the disease has very long incubation period. When the GM-made hormone arrived, the treatment was resumed.

In some instances, however, the hormone was used for a different purpose than treatment of obvious height deficiency. Parents, whose children did not have hormone-based growth deficiency, but appeared to be somewhat short, started to ask for the hormone to be administered. Furthermore, the hormone use was abused by sportsmen who desired to get a competitive edge by enhancing their muscle.

It is obvious that GM technology, as with the case of human growth hormone, has been abused. However, it would be totally wrong to ban the technology based on its misuse and misapplication. Instead, a strict framework of regulations is needed to maximize benefit and minimize harm caused by the technology.

It is interesting that many opponents of GM technology use for crops and agriculture are not against using the technology for medicine and pharmaceutical production. As long as the technology produces relatively safe and effective medicine, it is considered beneficial for the society. Furthermore, the lack of understanding and even the lack of interest to understand the technology is often obvious making people unaware of how the drugs that are injected into their veins were produced.

3.3. Genetic Modification of Humans

Interest in human inheritance goes back very long time. However, before the availability of GM and associated technologies, study of any disease-associated gene was a frustrating business. There were only several instances where biochemical analysis of the blood could indicate a gene-associated disease (e. g. Phenylketonurea). Arrival of molecular biology techniques therefore received a warm welcome by scientists working on microorganisms, plants, animals, and humans.

In 1988 the consortium of scientists in USA persuaded Congress to fund a program to sequence entire human genome with the motivation of understanding not only heritable diseases, but also those diseases based on molecular malfunctions, such as cancer. Five percent of entire funding was set for questions relating to the ethical aspects of the project. The project ended in 2001 (99.9%, in 2003 with minor completions), several years earlier than anticipated. USA carried out about two thirds of entire project activities, despite their claim to have done it alone.

The project concluded that we, humans, have about 25,000 genes, which is fewer than the number of genetic functions (about 100,000). Therefore, many genes are multifunctional. There were also a number of other interesting findings. However, the main question remains, whether the human genome project brought significant medical benefits. This question remains somewhat open, but some medical advantages are already visible.

The genome project provided a lot of information about the involvement of genes in human disease. The process is continuous, since the biochemistry of various reactions is being worked out. So far, it is clear that genetic diagnosis and genetic screening of diseases has been greatly enhanced by the availability of human genome information. However, there is no cure for inheritable diseases. The cure could in theory be provided by gene therapy. But the method is still under investigation and creation. So far, we

are able „to cure"microorganisms by inserting genes with desired changes but not adult humans who contain a large number of cells. Targeted delivery of therapeutic genes so far appears feasible only to human embryo. However, continued research may one day create a method of genetic therapy for adult humans.

However, the correct diagnosis of genetic diseases can already alleviate suffering of the bearers of ‚incorrect'gene. There are already more than 340 genetic tests available, but most clinics can provide only several. Diagnosis can be made before the symptoms become obvious thus leaving the burden of knowing to the person. Depending on the stage of diagnosis, it can be classified as pre-implantation (before human embryo implants into the wall of the uterus of his mother), pre-natal (before birth, but after implantation), and post-natal (after birth, in a baby, child, or adult).

Early diagnosis of phenylketonurea and congenital hypothyroidism allows establishment of treatment and management programs that will eliminate or at least alleviate symptoms. In some regions, the newborns are tested for thalassemia, sickle-cell anemia, and cystic fibrosis. This is especially useful for ethnic groups where such diseases are more common. There is no cure for cystic fibrosis, but early diagnosis helps parents and later the child to manage the symptoms. Testing in adults may help them change lifestyles as necessary.

Another benefit of testing may be considered pre-natal diagnosis of Down's syndrome. Parents, whose unborn baby is diagnosed with many such diseases, may be suggested to terminate pregnancy. Some parents may be grateful for such information and follow the suggestion, while others will give birth no mater what the possible outcomes are.

When in vitro fertilization is carried out, a pre-implantation testing of genetic diseases may be carried out, especially when parents are known to possess copies of faulty genes. This may help select embryos with healthy genes.

An example of benefits brought by genetic testing may be how Ashkenazi Jews test for Tay-Sachs disease. This is a neurodegenerative disease causing, among other things, progressive loss of movement and an early death. The genetic condition is recessive: it takes two mutated genes to cause the disease. Since many of Ashkenazi Jews are carriers of one copy of the gene, they do not experience the symptoms. Testing of young people enables them to check whether the partner is also a carrier of the mutated gene. In case when both partners of a young pair are carriers, it is advised that they do not marry since there is a ¼ chance that their child would inherit the disease. The testing is hard on young people who are in love, but it has greatly diminished the rate of abortions and the rate of disease in Ashkenazi Jews.

People are usually opposed to genetic modification of humans with the exception when this is done to correct a mutation that causes disease such as listed above. It is considered that elimination of the gene that causes illness in a heritable way would bring benefit to future generations. It is already feasible to insert a new gene into a human egg immediately prior or after in vitro fertilization and then establish a pregnancy by inserting the embryo into a woman womb. However, success rates for such experiments are low. Nevertheless, as techniques improve, it may become a routine therapeutic procedure.

Here we must consider the concerns that the GM technology will be used in the future to design babies with desired qualities. So far this is impossible and only featured in

science fiction and movies. But such possibilities may be feasible in the future. However, it is already impossible to distinguish between necessary therapy and simple enhancement, e.g. for sports performance. Various aspects of cosmetic surgery fall into this category. For example, reduction of disproportionally large breasts may be of therapeutic value. Enhancement of breasts may also have therapeutic value, since it is said that it makes women to be more confident and is thus psychologically therapeutic. Another example where the boundary between therapy and enhancement is blurred is leg-lengthening.

Human cloning is also a very controversial procedure. So far it is probably not possible. However, since the cloning of sheep Dolly, the prospect of human cloning became real. Any cloned person would be an experimental material' and would be exposed to a number of unknown risks that cannot be evaluated before such experiments run their course. It is possible, though, that somebody would do it in secret and the research information would later become public, thus changing the thoughts of some people about the subject. A number of ethical questions and concerns arise here.

However, cloning is often misunderstood by the general population. It is often claimed that cloning himself could prolong his life or he would somehow be reborn. In fact, clones sometimes occur naturally. In humans this occurs when twins are born. Identical twins contain exactly same genes, but their lives are different. Twins may be very similar in some aspects but very different in other aspects. Most importantly, they are two different and independent persons. Therefore, it is impossible to create your own copy. Even if you make a cloned copy of yourself, it will only look like your twin brother.

3.4. Embryos and Stem Cells

Stem cells are cells that may be able to develop into several kinds of cells during development. Such are blood stem cells. However, the most controversial research involves embryonic stem cells. The zygote, fertilized egg, is said to be totipotent - able to develop into any kind of cell in human body. After several divisions, in human embryo there are several embryonic stem cells that can develop into most cells of human body and thus are of interest in organ production and cure of a number of diseases.

A number of ethical question arise about the use of embryonic stem cells. The most important question is to decide whether the one-celled zygote is already a human being. The following supports that zygote is already a human:

1. Each zygote has a unique human genotype that has never existed before and will never exist again (except in twins);
2. Given the right conditions in the womb (and even in the test tube with right conditions) the embryo will develop into a fetus and into a child.

Therefore, destruction of any embryo would be equal to killing a person. However, there are also opposing views that do not consider human embryo to be a person yet. The main reasons are:

1. About 80% of fertilized eggs do not implant into the womb and do not establish pregnancy;
2. It is not until several rounds of cell division have occurred that the allocation of specific cell lineages to placenta and to embryo is made;
3. Even after this, the embryo may split to form identical twins, suggesting that the early embryo is not yet a human;
4. In rare occasion two embryos may merge

into one and develop as a normal human being, as based on genetic mosaics studies;
5. Embryo cannot feel any pain since there is no nervous tissue that forms at later stages of development.

On these grounds, the use of human embryos to create stem cell lines does not mean killing a person. It is also thought that their use for stem cell production may bring major benefits to existing humans and society at large.

In UK the Warnock Report, published in 1984, urges ethical respect for the human embryos and suggested that it ought to have a special status under English law. This would mean that embryos would not be used for trivial research. However, the same act stated that the difference between human being and an embryo is so large that the treatment may be different enabling legitimate use of a means to a end that was good for other humans.

There is apparently a large ethical advantage in the use of adult stem cells over embryonic stem cells. Adult stem cells may prove themselves just as potent as embryonic stem cells if greater funding was allocated to their research.

To conclude, we should note that stem cells give great promise for treatment of diseases and organ replacement, but so far only more common techniques such as bone marrow transplant reached the stage of therapy application.

3.5. Situation in Lithuania

Unfortunately Lithuanian biomedical and biotechnological research is in its embryonic stage of development as compared to neighboring Scandinavian countries. During Soviet times all research was secret, while during the independence times it was scarcely funded and largely forgotten by the society. For example, as shown by the ScanBalt analysis (www.scanbalt.org) [2], the Medicon Valley, encompassing Copenhagen greater area (population about 3 million), has annual biotechnology funding about 530 million euro and the number of Ph.D. annual graduates about 680. There are over 320 biotech companies listed in the Medicon Valley website (http://www.mediconvalley.com/CompaniesInstitutions/ListProfiles).

On the other hand, entire Lithuania with similar population has biotechnology funding about 8 million euro and the number of annual Ph.D. graduates about 25. There are only 7 biotech companies in Lithuania with the total of 129 scientists involved in R&D [3]. Medicon Valley has grown into a well integrated research and commercialization valley, while the Vilnius Science and Technology Park is only at the planning stages.

There are two private companies in Lithuania that routinely use modern genetic engineering techniques and produce relatively significant economic output (about 20 million euro per annum), namely, UAB „Fermentas“ and UAB „Sicor Biotech“. The overall share of biotechnology in Lithuanian GDP is so insignificant that the government and the society in general do not notice this industry as the potential drivers of the future economy with significant GDP share at least as is in the Scandinavian countries.

Biomedical research and industry is in even more undeveloped stages than the biotechnology. The main reason for the delay in biomedical science growth in Lithuania is the whole medicine was fully socialistic until some private initiatives started to appear several years ago. An essential reform in the administration and funding of medicine in Lithuania is necessary.

Since biotechnology plays such an in-

significant role in Lithuanian research and economy, most people know little about the real biotech threats and exaggerate safety concerns. The government often bends under pressure from green movement and other organizations. The real threat to the society, which in my opinion is emigration and poverty, are not properly addressed. Only when the society will understand that economic development encompasses some inherent risks, the living standard in Lithuania will improve significantly. On the other hand, the economic development of all Eastern European countries occurs on a significantly larger pace than the Western European. It is believed by many that the living standards will become approximately equal in about 2050.

I believe that biotechnology will play a significantly greater GDP share and the role in the society will be much greater than today. However, it is important to significantly increase funding for research, decrease bureaucratic barriers, and implement an administrative reform according to the example of Scandinavian countries.

3.6. Conclusions

In this chapter we have overviewed several aspects of ethics and safety in biomedical research and industry. The field is very large and developing fast. The main recommendation for Lithuanian authorities is to set up a bioethics and biosafety panel that would oversee GM use in Lithuanian research and industry. Such panel should closely follow the developments in the world science and legislature and give recommendations to Lithuanian parliament and government.

Our recommendations are the following:

1. Closely follow the legislature and procedures in one or two selected Scandinavian countries (e.g. Denmark or Finland) with the goal to foresee issues arising from GM use. Adopt their legislature to Lithuanian law;
2. Make effort to inform the general public about the benefits and risks of GM in biomedicine and agricultural biotechnology. People should be able to make an informed decision based on objective information. This will help apply advanced technologies in Lithuania and reduce opposition to GM;
3. Support scientific biomedical an biotechnological research in Lithuania. Allow research proposals to be initiated from the scientific community and make sufficient funds available;
4. Try to minimize restrictions on research. However, strict regulations on sensitive objects, such as embryos, embryonic stem cells, human cloning, etc. are necessary.

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4. Biotechnology and commerce

4.1. Definition

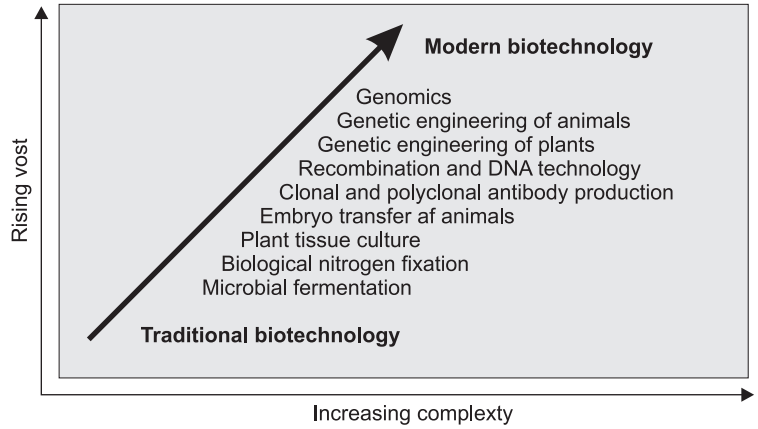
Biotechnology in its broadest sense is biology-based technology [1]. Biotechnology can also be defined as the manipulation of organisms to do practical things and to provide useful products. The UN Convention on Biologic Diversion has come up with one of many definitions of biotechnology [2]: Biotechnology means any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.

Over the years, two major biotechnology development poles became apparent. Traditional biotechnology like microbial fermentation was used as early as 10,000 years ago in fermenting beer, wine and dairy products. Another development pole represents continuously evolving techniques of modern biotechnology, such as genetic engineering. Many intermediate layers of interconnecting technological approaches exist between these poles. There is a major linking trend from traditional to modern biotechnology – increasing costs closely follow advances in technology, hierarchy represented in Figure 1.

Modern biotechnology got started with birth of gene engineering in 1972 and currently combines disciplines like genetics, molecular biology, biochemistry, embryology and cell biology, which are in turn linked to practical disciplines like chemical engineering, information technology, and robotics. Recently, the term "biotechnology" has come to be identified with modern biotechnology, specifically the use of genetic engineering techniques in medicine and agriculture. From this point we will use "biotechnology" to mean "modern biotechnology" in the text.

4.2. Basic concepts

The obvious aspect of biotechnology is the directed use of organisms for the manufacture of organic products (like beer and milk). For another example, naturally



Source: Doyle, J.J. and G.J. Persley (eds.). 1996. *Enabling the Safe Use of Biotechnology: Principles and Practices.* Washington, D.C.: The World Bank.

Figure 1. Complexity-cost gradient of biotechnology

present bacteria are utilized by the mining industry in bioleaching. Biotechnology is also used to recycle, treat waste, clean up sites contaminated by industrial activities (bioremediation), and produce now forbidden biological weapons. There are also applications of biotechnology that do not use living organisms. Examples are DNA micro arrays and radioactive tracers used in medicine. Due to diversity of approaches, biotechnology is separated into color-assigned directions listed and described in more details below.

Red biotechnology is applied to medical processes and accepted as the most important trend of biotechnologies. It finds promising applications in:

- Pharmacogenomics, the study of the relationship between pharmaceuticals and genetics;
- Drug production, aimed to produce existing drugs more easily and cheaply;
- Genetic testing, scanning a patient's DNA sample for mutated sequences;
- Gene therapy, used for treating or curing of genetic and acquired diseases.

In 2004, biopharmacology products accounted for $44.3 billions (of $550 billion net worth) and the part of in the whole drug industry is constantly increasing. Of new drugs under development, some 27% are considered to originate as biotechnology products [3].

White biotechnology, also known as grey biotechnology, is biotechnology applied to industrial processes. An example is the designing of an organism to produce a useful chemical. White biotechnology tends to consume less in resources than traditional processes used to produce industrial goods. One of most prominent aim for this industry is supply of renewable fuel, expected to double during 2006-2011 and approach €40 billion [4]. Other areas include production of pharmacological products, polymers and enzymes.

Green biotechnology is biotechnology applied to agricultural processes. An example is the designing of genetically modified plants (or organisms, GMO) to grow under specific environmental conditions or in the presence (or absence) of certain agricultural chemicals. One hope is that green biotechnology might produce more environmentally friendly solutions than traditional industrial agriculture. An example of this is the engineering of a plant to express a pesticide, thereby eliminating the need for external application of pesticides. In 1993, virus-resistant sugar beet was approved for sale and became the first GMO available on market. Nowadays, genetically modified plants are grown on about 100 million hectares, 63% of them in USA [3]. While more than 60% of all foodstuffs in USA contain certain part of GMO origin, European Union is rather skeptical regarding wide use of these products, especially for food. Whether or not green biotechnology products are ultimately more environmentally friendly is a topic of considerable debate.

The term blue biotechnology has also been used to describe the marine and aquatic applications of biotechnology, but its use is relatively rare. It is directed mainly for obtaining of pharmaceutical products from sea sources, genetic modification of sea plants and especially – fish.

4.3. Biotechnology industry in the world and Lithuania

At the moment, biotechnology is considered to be the fastest growing industry in the world. In 2005, there were more than 4,200 biotechnology companies across the globe [4]. Almost 50% of these located in the European Union (Germany is leading by number

of biotech companies); 30% in the US and the rest in Asia and Americas. The leading biotechnology firms are Amgen, Genentech and Serono. According to Burrill and Company, over $350 billion has been invested in biotech so far, and global revenues have risen from $23 billion in 2000 to more than $50 billion in 2005 [5]. The clear leader in fostering industrial biotechnology are USA, both in legitimate (in 2000, Senate approved Biomass R&D Act) and financial (over $0.5 billion allocated according this program in 2003 only) levels. Recent years face an increased activity within European Union directed towards rapid advance of this industry, too.

In 2005, revenues from biotechnology industry in Lithuania were 95 million Litas [3]. The leading companies in this field – UAB Fermentas, UAB Sicor-Biotech and UAB Biocentras are considered as possessing the most advanced technologies among the Central and Eastern Europe. It should be noted, however, that in addition to these there are quite a few more companies in the biotechnology industry in Lithuania.

Two directions appear as dominant within Lithuanian biotechnology industry: Red and White (see above for definitions). Red or medicine-linked biotechnology is represented by products or services in molecular diagnostics and solutions for molecular biology; immunodiagnostics; proteins for therapy; pharmacologic substances; bioinformatics and computational biotechnology. White, or industrial process-linked biotechnology: production of biofuel; biocatalysis; producing of various chemicals; polysaccharides; bioplastic production; optimization and management of biotechnological processes; ecologic, agricultural and forestry biotechnology. Basing on these directions, Lithuanian National Program for Biotechnology (2006) identified two priority groups of interest for developing of biotechnology industry in Lithuania up to 2025:

- Biopharmacology and molecular biology, diagnostics;
- Industrial biotechnology and agro biotechnology.

The first group of interest comprises were broad area of products, easily identifiable by use of the most advanced technologies and knowledge from the forefront of modern life- and engineering sciences. This is where the majority of global biotech enterprises are working. Lithuania also possesses several well-established companies (UAB Fermentas, UAB Sicor-Biotech and UAB Biocentras) while in the very recent years several new (UAB Biotechpharma, UAB SORPO, UAB Biota and UAB Immunolita) emerged. Pharma products, kits for molecular diagnostics and solutions for molecular biology as well as extensive efforts towards elaboration of new products characterize the areas of interest within this group. In 2005, the total revenues comprised 65 million Litas.

Industrial biotechnology is mainly crop-related. AB Malsena is well-established producer focused on flour manufacturing. Along with UAB Biopakas and UAB Tempera it focuses on production of starch and its further conversion products. Second trend: production of biofuel in both biodiesel and bioethanol forms, maintained mainly by small to medium-size enterprises.

Despite the sound achievements and rather ambitious plans (industry is anticipated to grow from 95 million Litas in 2005 to 2.5-3 billion Litas in 2013) [3] there are certain burdens to overcome. At the present state, total number of biotech companies is rather small and revenues are rather marginal, comparing to that of countries with developed biotech industry. For comparison, Finland (5 million populations) has

123 companies generating 700 million Euro per year; similarly, Israel (6 million) has 160 companies cashing 630 million Euro. At the current, small number of industry players within Lithuania is unable to create sufficient interest from universities for efficient improvement of quality of human resources and this, in turn, creates additional limitations for the expansion of biotechnology. Ties between scientific community and industry are rather irregular and inefficient, making rise of new start-up companies of low probability. Only UAB Fermentas and UAB Sicor-Biotech have own research centers pointing on insufficient competence for others to pursue efficient growth. In addition, National Platform for Biotechnology was approved only in 2006, indicating on very recent interest from legislature bodies.

4.4. Conclusions and recommendations

This chapter overviews present state of biotechnology industry in Lithuania and the world. The industry is developing extremely fast and current pressure caused by demand to further increase quality of life and diminishing energy carriers, in combination with new horizons provided by modern science provides sound basis for further growth. At the present state, Lithuania's biotech enterprises position themselves into narrow, highly specialized areas of expertise. Thus, the whole industry is at risk in future to be dropped from world-wide competition unless special measures will be taken.

Our recommendations are the following:

1. Significantly increase the support of basic research in Lithuania. Allow research proposals to be initiated not only by scientific community but also by employees from appropriate units from the industry. Create cost-effective schemes for industry to support basic research;
2. Create functional system for supporting of new start-up enterprises, provide continuous support for patent application and maintaining;
3. Further develop necessary legislature measures for industry to be effective and efficient. Closely follow success stories from countries making the biggest progress in a field and adopt this knowledge.

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5. Environmental biotechnology

5.1. Introduction

Biotechnology is the integration of natural sciences and engineering in order to achieve the application of organisms, cells, parts thereof and molecular analogues for products and services (EFB General Assembly, 1989). Environmental biotechnology is the application of these processes for the protection and restoration of the quality of our environment. Biotechnological processes to protect the environment have been used for almost a century now, even longer than the term 'biotechnology' exists. Municipal sewage treatment plants and filters to purify town gas were developed around the turn of the century. They proved very effective although at the time, little was known about the biological principles underlying their function. Since that time the knowledge base has increased enormously.

5.2. Bioremediation

Bioremediation is the use of biological agents to reclaim soils and waters polluted by substances hazardous to human health and/or the environment. Microorganisms can be used to degrade organic pollutants from contaminated soil and water into stable, non-toxic end-products. This works in situ by stimulating the biodegradative activity of competent endogenous microbial populations and ex situ by treating, under controlled conditions, soils and sediments removed from contaminated sites. Biodegradation may occur spontaneously, however, in many cases, the natural circumstances are not favorable enough for this to happen due to the lack of enough nutrients, oxygen or suitable bacteria. Introducing genetically modified bacteria into the contaminated site, bio-augmentation, has the potential to enhance bioremediation. With environmental pollution on the increase, scientists are developing genetically modified bacteria that can effectively and rapidly digest oil and that are well suited to particular environmental conditions. Others are used to remove algae from ponds and lakes, or to manufacture useful chemicals such as enzymes for plants or to provide renewable resources to make industrial chemicals from. Environmental clean up using genetically modified organisms is a promising technology. Various genetic approaches have been developed and used to optimize the enzymes, metabolic pathways and organisms relevant for biodegradation. New information on the metabolic routes and bottlenecks of degradation is still accumulating, enlarging the available toolbox. With molecular methods allowing the characterization of microbial community structure and activities, the performance of microorganisms under in situ conditions and in concert with the indigenous microflora will become predictable. Although the ability to predict design microbes or enzymes for any given remediation remains an overwhelming task, the increasing understanding of fundamental mechanistic principles generated from both genomic research or directed evolution will likely lead to the emergence of novel solutions for improved bioremediation. Some applications of bioremediation are discussed below.

5.2.1. Waste water and industrial effluents

Micro-organisms in sewage treatment plants remove the more common pollutants from waste water before it is discharged into rivers or the sea. Increasing industrial and agricultural pollution has led to a greater

need for processes that remove specific pollutants such as nitrogen and phosphorus compounds, heavy metals and chlorinated compounds. New methods include aerobic, anaerobic and physico-chemical processes in fixed-bed filters and in bioreactors in which the materials and microbes are held in suspension. The costs of waste water treatment can be reduced by the conversion of wastes into useful products. One example is the production of animal feed from the fungal biomass which remains after the production of penicillin. Most anaerobic waste water treatment systems produce useful biogas.

5.2.2. Drinking and Process Water

A very important aspect of biotechnology is its potential for the reclamation and purification of waste waters for re-use. Not only does water need to be recycled in the development of sustainable use of resources, overall quality must also be improved to satisfy consumers. In many agricultural regions of the world, animal wastes and excess fertilizers result in high levels of nitrates in drinking water. Biotechnology has provided successful methods by which these compounds can be removed from processed water before it is delivered to customers.

5..2.3. Air and waste gases

Originally, industrial waste gas treatment systems were based on cheap compost-filled filters that removed odors. Such systems still exist. However, slow processing rates and the short life of such filters drove research into better methods such as bio-scrubbers, in which the pollutants are washed out using a cell suspension and bio-trickling filters, in which the pollutant is degraded by micro-organisms immobilized on an inert matrix and provided with an aqueous nutrient film trickling through the device. The selection of micro-organisms that are more efficient at metabolizing pollutants has also led to better air and gas purifying bio-filters.

5.2.4. Soil and land treatment

Toxic heavy metals and metalloids, such as cadmium, lead, mercury, arsenic, and selenium, are constantly released into the environment. There is an urgent need to develop low-cost, effective, and sustainable methods for their removal or detoxification. Both in situ and ex situ methods are commercially exploited for the cleanup of soil and the associated groundwater. Bioremediation of land (biorestoration) is often cheaper than physical methods and its products are harmless if complete mineralization takes place. Its action can however, be time-consuming, tying up capital and land. The applicability of in situ bioremediation is and probably will remain dependent on the physical parameters of the soil, mainly its transport properties. Bioremediation using plants is called phytoremediation. Plant-based approaches are relatively inexpensive since they are performed in situ and are solar-driven. The combined use of plants and bacteria may also be possible. Certain bacteria live closely associated with the roots of plants and depend on substances excreted by the roots. Such rhizobacteria may be genetically modified to break down pollutants. Genetic engineering can potentially be used to develop plants with enhanced efficiencies for phytoextraction and phytovolatilization.

Immobilization of heavy metals into biomass or precipitation through reduction to lesser bioactive metal species, such as metal sulfide are the major mechanisms employed by nature (microorganism, animals and plants) to counteract heavy metal toxicity. These natural mechanisms can be easily exploited to optimize biosorbents that are more

efficient for heavy metal removal. Similar success in engineering enhanced biosorbents has been achieved by displaying metal-binding peptides onto the cell surface. These peptides emulate the structure of phytochelatins, metalchelating molecules that play a major role in metal detoxification in plants and fungi. Unlike nature metal-binding peptides, these designed metal-binding peptides are attractive as they offer the potential of improved affinity and selectivity for heavy metals. In addition to peptides, metalloregulatory proteins are another group of useful metal-binding moiety with striking affinity and specificity. The highly specific nature of these proteins is the result of a cleverly designed genetic circuit.

5.2.5. Solid waste

Domestic solid wastes are a major problem in our consumption society. Their elimination is both costly and warrants constant surveillance in terms of groundwater and air pollution. Yet, for a major part they are composed of readily biodegradable organics. In this respect, source separated bio-wastes can be converted to a valuable resource by composting or anaerobic digestion. In recent years, both processes have seen remarkable developments in terms of process design and control. Particularly, anaerobic digestion of solid wastes in high-rate anaerobic digesters has gained increasing public acceptance because it permits the recovery of substantial amounts of high-value biogas together with a high quality stable organic residue and this without giving rise to environmental nuisance. Moreover, anaerobic digestion of mixed solid wastes is under intensive development because in the near future it may be an important step in recycling of solid wastes and constitute an alternative to incineration.

The increasing information about the structure and function of enzymes and pathways involved in biodegradation of recalcitrant pollutants offers opportunities for improving enzymes or entire pathways by genetic engineering. Control mechanism and enzyme properties can be tailored by site directed mutagenesis, which is often guided by computer assisted modeling of the protein structures. Evolutionary approaches are extremely useful for optimization of an entire biodegradation pathway comparing to step-by-step modifications offered by rational design. At the same time, recent advances in genome shuffling between species; that allows the exchange and recombination of diverse pathways into a single species, will further accelerate the discovery of novel microbes that are useful for the remediation of even a complex mixture of pollutants.

5.3. Detection and Monitoring

5.3.1. Detection and monitoring of pollutants

A wide range of biological methods are already in use to detect pollution incidents and continuously monitor pollutants. Biological detection methods using biosensors and immunoassays have been developed. Most biosensors are a combination of biological and electronic devices - often built onto a microchip. The biological component might be simply an enzyme or antibody, or even a colony of bacteria, a membrane, neural receptor, or an entire organism. Immobilized on a substrate their properties change in response to some environmental effect in a way that is electronically or optically detectable. It is then possible to make quantitative measurements of pollutants with extreme precision or to very high sensitivities. The sensors can be designed to be very selective,

or sensitive to a broad range of compounds. Microbial biosensors are micro-organisms which produce a reaction upon contact with the substance to be sensed.

5.3.2. Detection and monitoring of microorganisms used for bioremediation

When laboratory grown micro-organisms are inoculated into a bioremediation site it often becomes necessary to monitor their presence and/or multiplication to check the progress of the process. This is especially true and even required when genetically modified micro-organisms are involved. The traditional technique to detect the presence of micro-organisms in soil is direct plating on selective media. This is greatly facilitated if the organism contains a marker which can be selected for. Newer techniques include the immunological and light-based bioreporter techniques. The spatial distribution of specific microorganisms in a sample can be determined microscopically and non-invasively. The most sensitive and specific technique is the direct isolation and amplification of DNA from soil, which is increasingly being used.

5.3.3. Detection and monitoring of ecological effects

Bioremediation is aimed at improving the quality of the environment by removing pollutants. However, the disappearance of the original pollutant is not the only criterion by which the success of a bioremediation operation is determined. Toxic metabolites may be produced from the pollutant or the biodegrading bacterium may cause diseases or produce substances that are harmful to useful micro-organisms, plants, animals or humans. To avoid unexpected effects, especially after the release of new member of the eco-system like a genetically modified organism, the monitoring of the ecological effects of a bioremediation operation may be required. The problem with monitoring ecological effects is what to monitor. Numerous ecological effects are possible but not all of them may be relevant or permanent or even the result of the bioremediation operation. The parameters to be monitored are usually determined case-by-case.

5.4. Prevention

The production of toxic or recalcitrant waste effluents by the chemical industry is leading to major problems of their disposal. New biotechnological approaches are now being used which will enable biological treatment of these wastes and will, in future, replace existing methods of effluent treatment.

5.4.1. Process improvement

Many industrial processes have been made more environmentally friendly by the use of enzymes. Enzymes are biological catalysts that are highly efficient and have numerous advantages over non-biological catalysts. They are non-toxic and biodegradable; work best at moderate temperatures and in mild conditions, and have fewer side reactions than traditional methods because they are highly specific. Production methods that employ enzymes are generally cleaner, safer and more economic in energy and resource consumption compared with other methods. The main drawback is that a specific enzyme i.e. required for a given application. New techniques and approaches to protein design and molecular modeling are enabling researchers to develop novel enzymes active at high temperatures, in non-aqueous solvents and as solids.

5.4.2. Product innovation

Biotechnology also can help to produce new products which have reduced impact on the environment. The production of new biomaterials like bioplastics avoids the use of non-renewable resources like fossil fuels. The use of genetically modified plant varieties that are resistant against insects and/or diseases may considerably diminish the use of pesticides.

5.5. Advances of Genetic Engineering

Recombinant DNA technology has had amazing repercussions in the last few years. Molecular biologists have mapped entire genomes, many new medicines have been developed and introduced and agriculturists are producing plants with novel types of disease resistance that could not be achieved.

5.5.1. Industrial processes

The leather processing industry has introduced enzymes to replace chemicals traditionally used for cleaning the hide. In textile production, enzymes have superseded chemicals for bleaching, including the "stone washing" of jeans. Chlorine consumption by the pulp and paper industry may soon also be reduced considerably by the use of enzymes. The grease and protein digesting enzymes in washing powders significantly reduce the quantity of detergents needed for a given washing effect. They also mean that the washing temperature can be reduced, which results in energy conservation.

5.5.2. Alternative fuels

Researchers seek ways to exploit genomic knowledge and genetic engineering methods to optimize biological organisms for efficient production of alternative fuels and for carbon sequestration. They are also undertaking large-scale genomic sequencing of environmental microbial populations to discover new organisms that might be of value for carbon sequestration or fuel synthesis. Genetic engineering might be used for more efficient conversion of glucose to fuel.

5.5.3. Pollution control

By adding the enzyme phytase to the feed of pigs and chickens the amount of phosphate which is excreted by these animals can be reduced by more than 30 %. In South Africa bacteria are used for the isolation of gold from gold-ore. This so-called bio-mining saves an enormous amount of smelting energy and generates much less waste. The biotechnological production of indigo, which uses a genetically modified bacterium containing the right enzymes, takes only three steps, proceeds in water, uses simple raw materials like sugar and salts and generates only indigo, carbon dioxide and biomass which is biodegradable.

5.6. Legislation

Because new organisms can be created by genetic engineering that may never be produced by spontaneous or selection driven evolution, concerns exist about the unpredictability of their possible interactions with the eco-system. Genetically modified organisms which are properly kept within the confines of their approved production facilities are much less a concern than genetically modified organisms which are meant to be released into the environment like disease resistant plants or soil bacteria for bioremediation. The possible ecological effects of the latter are even more difficult to evaluate due to the fact that it is well known that soil bac-

teria frequently exchange genetic material (also between species). This together with the fact that we know little about the great majority of soil inhabiting bacterial species makes it almost impossible to predict the fate of every DNA copy of a newly introduced genetic property in a soil bacterium. If the extra DNA is derived from another soil bacterium, it may on the other hand be reasonable to argue that the genetically modified bacterium might also have evolved spontaneously some day due to the frequent exchange of genetic material in the soil.

Regulation to ensure safe application of novel or modified organisms in the environment is important, not least to maintain public confidence. The European Union has two Directives on the contained use of genetically modified micro-organisms, and on the deliberate release of genetically modified organisms into the environment. These have been implemented in the national legislation of most EU Member States. They require that a detailed experimental protocol, including assessment of potential risks, is approved by competent authorities before a genetically modified organism is released into the environment. The aim of the European Commission is to maintain the EU's competitiveness globally - both in research and commercial applications- without compromising safety.

5.7. The application of environmental biotechnology in Lithuania

Environmental biotechnology is one of the safest ways of applying genetic engineering. Besides, it is used to solve pollution problems that are very important, especially considering the current growth of industry and new EU environmental legislation that are being introduced in Lithuania. Application of environmental biotechnology can result in substantial improvement of remediation, pollution detection, monitoring and treatment processes. The improvement can be achieved with already existing methods that include biotechnology. At the same time, the research in the field of environmental biotechnology should be supported.

One of the most important conditions for using genetically modified organisms for solving environmental problems is the restriction of their interactions with the ecosystem. National legislation on the contained use of genetically modified micro-organisms and on the deliberate release of genetically modified organisms into the environment has to be modified. National legislation has to be harmonized with the EU legislation. The legislation has to ensure that the release of genetically modified organisms into the environment is monitored and controlled. When these rules are introduced, there should not be any obstacles for the successful application of genetic engineering and environmental biotechnology in Lithuania.

5.8. Conclusions and recommendations

Environmental biotechnology is extending back into the last century. As the need is better appreciated to move towards less destructive patterns of economic activity maintaining improvement of social conditions, the role of biotechnology grows as a tool for remediation and environmentally sensitive industry. Already, the technology has been proven in a number of areas and future developments promise to widen its scope. Some of the new techniques now under consideration make use of genetically modified organisms designed to deal efficiently with specific tasks. As with all situations where there is to be a release of new technology into the environment, concerns

exist. There is a potential for biotechnology to contribute to the development of a more sustainable society by making a further major contribution to protection and remediation of the environment.

Genetic engineering can in principle be a very powerful tool in creating environmentally friendlier alternatives for products and processes that currently are polluting the environment or exhausting non-renewable resources. Politics, economics and society will ultimately determine which scientific possibilities will become reality. Organisms can also be supplemented with additional genetic properties for the biodegradation of specific pollutants if naturally occurring organisms are not able to do that properly or quickly enough. Bottlenecks in environmental cleanup may be circumvented. Until now this has not been done on any significant scale, since in most cases naturally occurring organisms can be found or selected for, which are able to clean up a polluted site. In the USA some genetically modified bacteria have been approved for bioremediation purposes but large scale applications have not yet been reported. In Europe only controlled field tests have been authorized.

6. Fusion of biotechnology and nanotechnology

6.1. Nanobiotechnology

Nanobiotechnology is a multidisciplinary integration of biotechnology, nanotechnology, chemical and electronic engineering and other related fields. Nanobiotechnology aims at understanding the basic principles of biological functional units as well as creating extremely small elements at nano-scale (below 100 nm), in a controlled way combining biological and technical materials and interfaces.

The recent ScanBalt Competence Region Mapping Report [1] emphasizes the scientific and technological progresses in the following areas of life sciences and biotechnology: microarrays, biosensors, protein engineering, recombinant DNA-technologies, cell cultures, monoclonal antibodies or bioprocessing technologies. These innovative technologies hold the promise to make biotechnology the dominant economic force of at least the first half of the 21st century. Furthermore, the electronic and computer science breakthroughs will allow massive amounts of genetic information to be decoded and processed at relatively low cost and within a reasonable time scale. On the other hand, by some estimates, nanotechnology promises to far exceed the impact of the Industrial Revolution and is projected to become a $ 1 trillion market by 2015 [2]. According to some other experts, by 2015 at least half of all pharmaceuticals will be based on nanotechnology [3]. Thus, the merger of life sciences, biotechnology and nanotechnology will open even broader perspectives. Among the products envisioned at the intersection of these fields are new imaging contrast agents, targeted nanoparticles capable of delivering multiple therapeutic agents simultaneously, and a new class of in vivo diagnostic agents called "reporters of efficacy", which are designed to determine if cancer drug is having its intended effect. [4]. The economical interests of investing in nanobiotechnology are obvious. According to the data from 2003, more than $3 billion could be invested worldwide in government nanotech research, including hundreds of millions of dollars in corporate R&D [5]. The same study states that 13 of the 30 companies in the world's best known stock indicator, the Dow Jones Industrial Average, mentioned nanotechnology on their website, more than $900 million in venture capital funding has gone to nanotechnology start-ups in 1999-2003. Nanobiotechnology is often considered as the most promising area of nanotechnology. This can be illustrated by the fact that about 52% of the venture capital funding has gone namely to nanobiotechnology start-ups [5].

6.2. Biotechnology and nanoscale materials science

Nowadays, nanoscopic entities and nanostructured materials are being fabricated from all kinds of regular materials, including polymers, metals, semiconductors, ceramics, composites and biological materials. However, functionally they often remain "inert". Unlike normal (inert) materials, the next generation of smart materials is designed to respond to external stimuli, adapting to their environment in order to boost performance, extend their useful lifetimes, save energy, etc. Materials will also be developed that are self-replicating, self-repairing, or self-destroying as required, thus reducing waste and increas-

ing efficiency. The three major strategies for the production of smart nanomaterials are (1) combination of regular materials from the above mentioned different classes in new, complex architectures; (2) employment of novel technologies for manufacturing and controlling structures on the nanoscopic scale; and (3) mimetics of biological systems (biomimetics) and the use of bioengineered molecules and organisms [6].

However, one of the major obstacles from the point of view of practical applications, especially in the fields of so-called biochips and other novel nanobiotechnological detection systems, is the insufficient methods for the parallel fabrication of the smart nanoassemblies on solid supports. It is generally accepted that the most promising way is the convergence of the bottom-up and top-down fabrication methods. Also, a distinguishing characteristic of nanometer scale structures is that, unlike macroscopic materials, they typically have a high percentage of their constituent atoms at a surface. In some sense, nanostructures are "all surface", i.e. their functional properties are defined mainly by their interfacial properties [7]. Thus, in nanoscale materials science of crucial importance are methods, which enable a precise control and manipulation of the surface properties, including surface charge, hydrophobicity, the amount and composition of surface reactive groups. However, it is important to stress that the unusual physicochemical properties of engineered nanomaterials also raise concerns about undesired effects on biological systems, because at the cellular level they include constituent building blocks and machineries that resemble nanomaterials in terms of their function [2]. Therefore, the safety evaluation of nanomaterials and risk assessment is of increasing importance. On the other hand, in some cases nanotoxicity is a desirable effect, e.g., it could be used to initiate programmed cell death – a new cancer chemotherapy principle.

The applications of biological materials in so-called soft lithographic fabrication methods for the engineering of surface-supported micro- and nanostructres are one of the important recent developments [8]. The invention of such methods opened new opportunities to fabricate functional assemblies that contain proteins, DNA, liposomes, viral particles and cells with high precision. Such techniques have proven to be useful even for printing arrays of single protein molecules. Printed protein entities can be employed, for example, in nanoelectronic or sensor devices and therefore companies like IBM have been active in this field.

The other group of highly interesting lithographic techniques for nanobiotechnological applications are based on scanning probe microscopy, e.g. nanografting and dip-pen nanolithography (DPN). These techniques were developed in the middle of the last decade [9]. Both nanolithographic methods achieve a lateral resolution close to 15 nm. Moreover, in the DPN process various biological molecules (DNA, proteins, and lipids) can be used as ink. Thus, direct fabrication of biological in vitro model systems as well as biomimetic materials is possible with the precision at the level of single molecules.

The principles of self-assembly of natural nanosystems, in combination with genetic engineering of proteins, have been used to fabricate first nanomechanical devices with ATP-powered biomolecular motors [10]. Such systems are believed to be essential for the creation of a new class of sensors, mechanical force transducers and actuators. Also, as shown recently, assemblies of proteins and genetically modified virus scaffolds can provide templates for catalytic synthesis of metallic nanoparticles, magnetic and sem-

iconducting nanowires, which are important building blocks of nanoelectronics [11].

So far, the main focus in nanotechnology has been on synthetic materials, such as carbon nanotubes, nanoparticles (colloidal gold, quantum dots, latex, etc.), inorganic materials (ZnO, TiO2, silica). A lot of effort has been put into biofunctionalization and biocompatibility of these nanomaterials in order to apply them as drug carriers, medical treatments, implants, tissue engineering constructs and cosmetics. Although the advances in this field are impressive, one should bear in mind that materials are available from the natural world and they have interesting properties on the nanoscale. For example, bacterial cellulose, which consists of 50-80 nm wide fibrils, can be engineered for use not only in food but also in medicine, electronics and other industries [12]. Also nanofibrils from wood cellulose have many interesting applications in materials science. They can be isolated using enzymes known as cellulases, preferably genetically modified, and they have been used for construction of nanocomposites with increased strength. In the future, cellulose nanofibriles could be also exploited as optical materials for security features, decorative coatings, automotive windows, information storage and laser optics [13].

The above mentioned examples outline the general trends in the nanobiotechnological research and industrial applications. Further on, the invention of biochips, one of the technological breakthroughs is commented in more detail owing to its exceptional importance in modern biotechnology.

6.3. DNA arrays and nanoassemblies

Microarrays of DNA probes (also know as DNA chips) were introduced 11 years ago and there has been a rapid evolution of this technology since then [14] (Fig 1). The first DNA arrays consisted of 45 complementary DNA (cDNA) probes spotted in a microarray on a glass slide. The DNA was immobilized and the resulting microarray was used for gene expression analysis. Already one year later 1000 probes were arrayed.

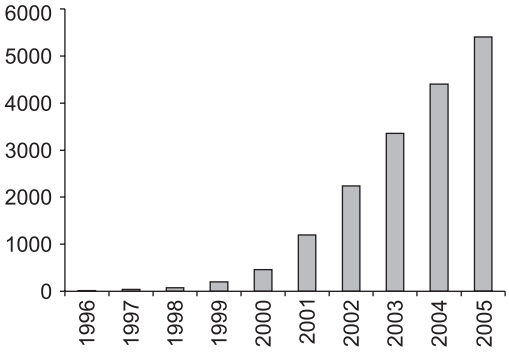

Figure 1 Number of articles with the keyword "microarray" in the ISI data base

Just like fabrication of microelectronic circuits in electronics, an alternative technology based on in situ synthesis of DNA probes on solid support directed by light allowed fabrication of microarrays of 135,000 probes. Driven by miniaturization, DNA array technology has recently allowed "global" analysis of the genome or transcriptome in one batch process. Expression of 40,000 different mRNA molecules or 100,000 different single nucleotide polymorphism (SNP) can be analyzed on a piece of glass or silicon ("DNA chip") that is between 1 and 8 cm^2 depending on technology used [14]. Moreover, this year, Affymetrix introduced the Genome-Wide Human SNP Array 6.0, which includes about 1.8 million markers that can be used to detect genetic variation in whole-genome association studies. Specifically, the newest SNP Array 6.0 contains 906 600 SNPs and about 946 000 nonpolymorphic probes [15].

Currently, short DNA strands can be precisely positioned on surfaces not only for fast

genetic screening and analysis, but also for addressable assembly of nanomaterials such as gold nanoparticles, templates for future molecular electronics [16]. Self-assembled DNA nanotemplates on solid supports, with synthetically introduced biotin molecules in the nods, have been fabricated and they also can be used in a variety of applications in nanotechnology (see below) [17].

6.4. Protein arrays and nanoassemblies

Protein arrays are an analogous, but a bit younger technology, as compared to the DNA arrays. A protein array consists of microscopic domains, which are generated on a solid support by printing, robotic dispensing and other means. The specific domains can be used to immobilize proteins or their antibodies, ligands or to attach various synthetic bioactive molecules. Every tiny domain can be used to follow a different molecular recognition or enzymatic reaction. Therefore, protein arrays are considered to become the main technological platform in the research of human proteome, which consists of about 2 mln. differently modified proteins and complicated protein networks. There is no other technology, which enables to follow simultaneously and in real time the function of different protein networks, in particular, using non-label detection techniques [18]. Currently, there are over 30 different protein array systems available on the market [19]. However, for successful applications, especially, in the field of medicine, further research is needed on the functional activity of proteins in synthetic environments and on the optimization of physicochemical properties of the solid substrates.

A general scheme of a typical protein array experiment is as follows: a large set of capture ligands (proteins or peptides) is arrayed on a solid support, after washing and blocking surface unreacted sites, the array is probed with a sample containing (among a variety of unrelated molecules) the counterparts of the molecular recognition events under study [20]. If an interaction occurs, a signal is revealed on the surface by a variety of detection techniques, including label-free detection methods such as surface plasmon resonance. By scanning the entire array a large number of binding events are detected in parallel. Protein arrays generally fall into three categories: (1) function arrays; (2) detection arrays (or analytical arrays); (3) reverse phase arrays.

In protein function arrays (which are generally aimed at discovering protein function in fundamental research) a large set of purified proteins or peptides or even an entire proteome is spotted and immobilized. The array is then used for parallel screening of a range of biochemical interactions. Protein function arrays can be used to study the effect of substrates or inhibitors on enzyme activities protein-drug or hormone-effector interactions or in epitope mapping studies [20].

In protein detection microarrays, an array of affinity reagents (antigens or antibodies) rather than the native proteins themselves, is immobilized on a support and used to determine protein abundances in a complex matrix such as serum. Analytical arrays can be used to assay antibodies (for diagnosis of allergy or autoimmunity diseases or to monitor protein expression on a large scale. In a third category of protein arrays (usually referred as reverse phase microarrays), tissues are spotted on the surface and probed with one antibody per analyte for a multiplex readout [20].

Patterning of proteins into sub-100 nm sized domains (nanoarrays) is the natural extension of the protein array technologies.

Moreover, nanobiotechnological devices can be created, which are based on the specific function of proteins on the single molecule scale. For example, one enzyme molecule, a natural biocatalyser, can perform 100-1000 chemical modifications of substrate molecule per second. It has been already demonstrated that the catalytic activity of single enzyme molecules can be monitored with so-called zero mode waveguides [21]. The small size and force-exerting capabilities of enzymes called motor proteins, in combination with genetic engineering give them unique advantages over current human-made motors [22]. Furthermore, the DNA-directed assembly of proteins can be applied to fabricate artificial multienzyme constructs with a high degree of spatial control that are otherwise not accessible by conventional chemical cross-linking [23].

Table 1 Status of microarray-based processes*, **

Transcriptional profiling	Mature, but still to be improved
Genotyping	Mature, but still to be improved
Splice-variant analysis	In progress
Identification of unknown exons	Early stages
DNA-structure analysis	Pilot phase
ChIP-on-chip	In progress
Protein binding	Under development
Protein–RNA interaction	Idea
Chip-based CGH	In progress
Epigenetic studies	Under development
DNA mapping	Mature
Resequencing	In progress
Large-scale sequencing	Under development
Gene/genome synthesis	Early stages
RNA/RNAi synthesis	Pilot phase
Protein–DNA interaction	Under development
On-chip translation	Under development
Universal microarray	Under development

*From most to least developed: mature, in progress, under development, early stages, pilot phase, idea. CGH, comparative genomic hybridization; ChIP-on-chip, on-chip chromatin immunoprecipitation.
** Reproduced from Ref. 24.

6.5. Nanobiotechnology in Lithuania

Biochips and advanced nanobiotechnological analytic platforms remain to be relatively unknown in Lithuania, although they found some first applications in the Lithuanian biotech industry. For example, a commercial DNA microarray fabrication and analysis system has been obtained by the company Fermentas. However, no systematic research has being carried out in the field of biochips specifically and in nanobiotechnology in general. Nevertheless, Lithuanian scientists are traditionally strong in closely related areas such as bioelectrocatalysis and biosensors (Professors Juozas Kulys, Vladas Laurinavičius, Institute of Biochemistry). They have been publishing in this field since 1974 and these publications are frequently cited by the colleagues abroad. Some medical applications of the developed biosensors have been demonstrated too, for example biomedical analytic systems were commercialized as early as in 1986. Another important field with world-class research, relevant to nanobiotechnology, is DNA modifications. The group of Prof. Saulius Klimašauskas (Institute of Biotechnology) has reported on new, original molecular tools based on methyltransferase mutants, studies that recently have attracted a lot of interest in the communities of chemists and biochemists. These tools are believed to be useful for different nanobiotechnological applications in the future.

The pioneer of scanning probe microscopy (SPM) methods and nanomanipulation in Lithuania is Prof. Valentinas Snitka (Kaunas University of Technology). His group is using SPM techniques for imaging of tissues and cells. Also, Prof. Arūnas Ramanavičius (Vilnius University) has employed atomic force microscopy to monitor antibody-antigen complex formation. The group of Prof. Ričardas Rotomskis is interested in applications of na-

noparticles in medicie. These two groups have recently upgraded their laboratories accordingly via EU Structural Funds programs.

The first systematic research on protein nanoarrays was carried out by the scientists from the Institute of Physics. Dr. Ramūnas Valiokas leads an interdisciplinary team of scientists, who are specialized in nanobiotechnology. His laboratory is one of few in Europe with established dip-pen nanolithography process for nanopatterning of biomaterials. Their work has been recently presented at the Nanotech Northern Europe 2007 Congress, which was held in Helsinki in March 2007.

A couple of Lithuanian research groups have participated in EC-funded nanobiotechnology initiatives. For example Prof. Aivaras Kareiva's group (Vilnius University) has been a partner in CellProm, the largest FP6 integrated project related to nanobiotech. Also, Prof. Valdemaras Razumas (Institute of Biochemistry) took part in a STREP project focusing on bio-molecular mechanisms at biological interfaces on the molecular scale.

The interdisciplinary collaboration at the intersection of nanotechnology and biotechnology will certainly benefit from the support, which comes from the State Science and Studies Foundation. A few nanobiotech-related projects have been supported through the National science priority program (nanotechnologies is among the officially recognized science priorities in Lithuania) and other programs initiated by the Foundation.

6.6. Conclusions and recommendations

As mentioned above, the current commercial path for nanotechnology ventures mirrors the early evolution of the biotechnology industry, allowing similar strategies toward both technology commercialization and investment [5]. The rapid expansion of nanobiotechnology creates exciting opportunities for multidisciplinary and interdisciplinary collaboration also in Lithuania. It would enable not only to tap the potential of life sciences, but also that of chemistry, biophysics, laser technologies, material science and other related fields. Although there have been some good examples of such collaboration, Lithuanian science is generally segregated and divided into the "classical" fields. This often can be regarded as a serious obstacle for making scientific breakthroughs on the international scale. Also, the Lithuanian biotech industry so far has shown no (or very little) interest in nanotechnological methods and applications. This can be illustrated by the fact, that the National program for the Expansion of Industrial Biotechnology (approved in October 2006 by the Prime Minister of Lithuania) contains even no reference to nanotechnology.

The success of Lithuania in the vibrant field of nanobiotechnology will generally depend on the success of the planned R&D reforms. New state-of-the art infrastructures are necessary but first of all changes in the existing legislation and funding schemes have to be done to stimulate the formation of new, dynamic research units, overcoming the borders between the disciplines. The recruitment of scientific staff who are trained to work in interdisciplinary and industrial environments is another essential requirement.

The young and rapidly expanding market provides opportunities to a small country like Lithuania to find and occupy own niches. This is in contrast to the traditional biotech industries (pharmaceuticals, agriculture, and forestry), where Lithuania has failed to enter in the early phase and now it would meet much harder competition

from other countries. Noteworthy, laboratory based nanobiotechnologies would not create such a deep divide in public opinion like in the case of GMO crops. Thus, the specific R&D programs in nanobiotech have to be immediately launched according to the long-term economical goals of the country, e.g. increase in foreign green-field investments and start-ups in high-technologies, better international competitiveness of the traditional Lithuanian industries, etc. Moreover, nanotechnology is believed to play even more important role in sectors such as medicine, national security and others. Specific R&D programs have to be created to achieve and support high international standards in these sectors.

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