Short course of lectures
on the discipline of risk management of transgenes

Lecture 1. *Introduction. Definitions of genetically modified organisms and a genetically engineered organism (GEO). The purpose of their creation.*

With the development of genetic engineering, not only its active supporters appeared, but also opponents, whose actions are aimed at stirring up public opinion against the introduction of genetic technologies. In this regard, in 1996, the Federation of European Microbiological Societies (FEMO) published a memorandum aimed at informing the public about the benefits and potential dangers of large-scale application of genetic engineering in biotechnology.

The memorandum is intended for scientists and the general public.

First, since the temporary moratorium on genetic engineering (1975-1985), public opinion has been suspicious and sometimes hostile towards genetic technology. The accusations against microbiologists engaged in genetic engineering are very diverse: "genetic engineers will clone thousands of Hitlers or Einsteins," "a genetic bomb will kill all living things or turn everyone into freaks," and so on.

The second reason for public concern is that in the last decade such transgenic organisms have been intensively created, the benefits of which should be manifested only after their introduction into the environment. On the contrary, modern safety rules for the use of genetic technologies, adopted in most countries, are aimed at preventing viable transgenic organisms from entering the environment.

It is necessary to develop reasonable, adequate and flexible rules for the safety of genetic technologies, a calm and welcoming public atmosphere, especially in the market for the supply of bread, cheese and beer prepared with the help of transgenic microbes, the sale of transgenic tomatoes and corn, and when field operations are already underway. tests of transgenic soil microbes.

Genetic technologies have led to the development of powerful methods for the analysis of genes and genomes, and this, in turn, to synthesis, i.e. to the design of new, genetically modified microorganisms.

By 1996, the nucleotide sequences of 11 different microorganisms were established, starting from the smallest autonomously multiplying mycoplasma (only 580 thousand nucleotide pairs).

Knowledge of the nucleotide sequences of the genomes of industrial strains will allow them to be "programmed" to generate a large income.

Cloning eukaryotic, i.e. nuclear, genes in microbes is that fundamental method that led to the rapid development of microbiology. Fragments of the genomes of animals and plants for their analysis are cloned in microorganisms. To do this, artificially created plasmids are used as molecular vectors - gene carriers, as well as many other molecular devices designed to isolate and clone eukaryotic genes.

The key to developing rules and laws governing the application of gene technologies is to create rational concepts of risk assessment.

The first step in this direction is to establish exactly what dangers may arise and how to avoid them. The next step is to assess the degree of risk. The risk can be reduced by identifying the hazard categories of pathogens and using appropriate protective equipment to handle them. Assessments should be refined as specific knowledge is gained about specific hazards.

Lecture 2 The goals of creating genetically modified organisms.

The Food and Agriculture Organization of the United Nations (FAO) considers the use of genetic engineering techniques to create transgenic varieties of plants or other organisms as an integral part of agricultural biotechnology.

Direct transfer of genes responsible for useful traits is a natural development of work on animal and plant breeding, which expanded the possibilities of breeders in terms of controllability of the process of creating new varieties and expanding its capabilities, in particular, the transfer of useful traits between non-breeding species.

The use of both individual genes of various species and their combinations in the creation of new transgenic varieties and lines is part of the FAO strategy for the characterization, conservation and use of genetic resources in agriculture and the food industry.

A 2012 study (based on reports from seed companies) of the use of transgenic soybeans, corn, cotton and canola showed that herbicide-tolerant crops are cheaper to grow and, in some cases, more productive.

Crops containing the insecticide produced higher yields, especially in developing countries, where previously used pesticides were ineffective. Insect-resistant crops have also been found to be cheaper to grow in developed countries. According to a meta-analysis carried out in 2014, the yield of GMO crops is 21.6% higher than that of unmodified crops due to the reduction of losses from pests, while the consumption of pesticides is 36.9% lower, the cost of pesticides is reduced by 39, 2%, and the income of agricultural producers increased by 68.2%.

The gene synthesis process is currently very well developed and even to a large extent automated. There are special devices equipped with computers, in the memory of which programs for the synthesis of various nucleotide sequences are laid. This apparatus synthesizes DNA segments up to 100-120 nitrogenous bases (oligonucleotides).

To insert a gene into a vector, restriction enzymes and ligases are used. With their help, a gene can be "glued", connected in a different combination, constructing a new gene or enclosing it in a vector.

The technique of introducing genes into bacteria was developed after Frederick Griffith discovered the phenomenon of bacterial transformation. This phenomenon is based on a primitive sexual process, which in bacteria is accompanied by the exchange of small fragments of non-chromosomal DNA, plasmids. Plasmid technologies formed the basis for the introduction of artificial genes into bacterial cells. Popular methods for introducing a vector into a plant cell is the use of the soil bacterium Agrobacterium tume-faciens or a gene gun [31]. For genetic engineering of animals, transfection, vectors, based on retroviruses and other methods are used.

If unicellular organisms or cultures of multicellular cells undergo modifications, then at this stage cloning begins, that is, the selection of those organisms and their descendants (clones) that have undergone modification. When the task is set to obtain multicellular organisms, then cells with a changed genotype are used for vegetative propagation of plants or injected into the blastocysts of a surrogate mother when it comes to animals. As a result, cubs are born with an altered or unchanged genotype, among which only those that show the expected changes are selected and crossed with each other.

The goals of creating genetically modified organisms

Methods for creating GMOs

The main stages of creating GMOs:

1. Obtaining an isolated gene.
2. Introduction of a gene into a vector for transfer into an organism.
3. Transfer of the vector with the gene into the modified organism.
4. Transformation of body cells.
5. Selecting genetically modified organisms and eliminating those that have not been successfully modified.

Methods for carrying out each of these stages make up the methods of genetic engineering in the aggregate.

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Significant difficulties were associated with the introduction of a ready-made gene into the hereditary apparatus of plant and animal cells. However, in nature there are cases when a foreign DNA (of a virus or bacteriophage) is included in the genetic apparatus of a cell and, with the help of its metabolic mechanisms,

begins to synthesize its “own” protein. Scientists investigated the features of the introduction of foreign DNA and used it as a principle for introducing genetic material into the cell. This process is called transfection.

Lecture 3. Application of GMOs and genetic engineering methods in research

In p.t. time genetically modified organisms are widely used in fundamental and applied scientific research. With the help of genetically modified organisms, the patterns of development of some diseases (Alzheimer's disease, cancer), aging and regeneration processes are investigated, the functioning of the nervous system is studied, and a number of other urgent problems of biology and modern medicine are being solved.

In medicine and pharmaceutical industry]

Genetically modified organisms have been used in applied medicine since 1982. This year, genetically engineered human insulin obtained with the help of genetically modified bacteria was registered as a medicine.

Currently, the pharmaceutical industry produces a large number of drugs based on human recombinant proteins: such proteins are produced by genetically modified microorganisms or genetically modified animal cell lines. Genetic modification in this case consists in the fact that a human protein gene is introduced into the cell (for example, the insulin gene, the interferon gene, the beta-follitropin gene). This technology makes it possible to isolate proteins not from donor blood, but from GM organisms, which reduces the risk of drug infection and increases the purity of the isolated proteins. Work is underway to create genetically modified plants that produce components of vaccines and drugs against dangerous infections (plague, HIV).

Proinsulin obtained from genetically modified safflower is at the stage of clinical trials []. A drug against thrombosis based on protein from the milk of transgenic goats has been successfully tested and approved for use.

Lecture 4. Application of GMOs and genetic engineering methods in agriculture [

Genetic engineering is used to create new varieties of plants that are resistant to adverse environmental conditions and pests [44], with better growth and taste qualities.

Genetically modified varieties of forest species with a significant cellulose content in wood and rapid growth are undergoing testing [45].

However, some companies place restrictions on the use of genetically modified seeds they sell, prohibiting the sowing of self-obtained seeds. This is done using legal restrictions such as contracts, patents or seed licensing. Also, for such restrictions, restrictive technologies (GURT) were at one time worked out, which were never used in commercially available GM lines. GURT technologies either make the grown seeds sterile (V-GURT), or require special chemicals to manifest the added by means of modification of the property (T-GURT). It should be noted that F1 hybrids are widely used in agriculture, which, like GMO varieties, require an annual purchase of seed material. Some foods contain a gene that makes pollen sterile [49], for example, the barnase gene derived from the bacterium Bacillus amyloliquefaciens.

Lecture 5. The use of GMOs in animal husbandry.

Genetic engineering methods in agricultural livestock breeding

Animal • Animals provide a number of products we use in every day life: –Milk –Leather –Meat –Wool –Egg –Enzymes –And many more e-g medicine 24

1- Animal Biotech a) Improve animals or the products they produce ♣ Animals may be used to produce products that promote human health ♣ Increase milk productivity , Example Transgenic organisms are organisms that are injected with foreign DNA from another organism ♣ Cows engineered to produce human hemoglobin 25

- Animal Biotech b) Animal Cloning • Cloning is the copying animal gene into many copies, • Start with Embryo Twinning (splitting embryos in half) • Advantage of cloning: preservation of endangered animals, studying the effect of drugs etc on duplicates, improve agricultural production 26 Dolly and her surrogate mother.

- Animal Biotech c) Improvement animal Health. • Animal health and well being have become increasingly important issues for animal producers and consumers. • Biotechnology can improve animal health by producing genetically engineered animal that resist disease. • The development of

genome resources and technologies allow for identification of several host resistance genes. • Aim: to prepare and present about genetic bases of disease resistance in the livestock sector 27
1- Animal D) Artificial Insemination (AI) • What AI? • Artificial insemination- the transfer of collected semen to a recipient female • Semen is collected from males of desired quality • Semen is graded and stored 28
1- Animal : Artificial insemination 29 • Female must be in estrus for conception • Hormone injections may be used to synchronize estrus • Semen is placed in the cervix near the horns of the uterus
1-Animal Biotech: Creating test tube baby E) What is test tube baby? • In vitro fertilization- fertilization of collected ova outside the reproductive tract; Usually in a test tube – Semen is collected from males of desired quality – Ova are removed from females – Sperm and ova are placed in a petri dish or test tube 30
1- Animal Biotech F) Embryo transfer • What is Embryo Transfer? • Embryo transfer- removing fertilized ova (embryos) from donor and implanting in a recipient – Surgical and nonsurgical methods are used to remove and implant – A quality donor female can produce more offspring 31
1- Animal biotech G) What is Multiple Ovulation • Multiple ovulation- promoting increased release of ova during estrus – Hormone injections administered prior to estrus – Used with embryo transfer – AI may be used to fertilize ova – After fertilization, embryos are removed and placed in recipients 32
2- Medical Biotech 33 2- Medical Biotechnology • is applied to medical processes. • Some examples are the designing of organisms to produce antibiotics, and the engineering of genetic cures through genomic manipulation. Example Genomic Manipulation: Gene Therapy Erythromycin Antibiotic

Lecture 6 Cellular Engineering

Cell engineering is the process of adding, deleting or modifying genetic sequences in living cells. Individually isolated cells that are engineered to contain genetic modifications may be cultured to produce clonal cell lines. Clonal cell lines are referred to as stable cell lines when the genetic properties for which they were selected are reliably transmitted to progeny cells.

Fluorogenic oligonucleotide signaling probes also known as molecular beacons may be used to track genetic modifications in individual cells that that can be clonal expanded into a genetically engineered cell line

Engineering cellular environments at the micrometer scale is critical for tissue engineering. The primary strategy for engineering tissue constructs uses a combination of cells and artificial scaffolds. Obtaining an adequate source of cells is a major challenge, since many of the cell types taken from adult tissue have a limited capacity for expansion. Recent developments in stem cell biology suggest that these cells might provide a key source of cells because they have the capacity for self-renewal and differentiation into multiple lineages. While promising, these cells alone cannot form a tissue. Cells must be combined with a scaffold, which provides the initial structural support onto which the cells adhere and organize into a functioning tissue. While simple in concept, forming complex tissues such as liver, which contain many different cell types and a defined tissue architecture, is a formidable task. When cells are removed from their natural *in vivo* environment, and placed in an artificial environment they often lose their tissue-specific functions. Hepatocytes, for example, are normally rounded and do not proliferate, but when removed from the body and cultured on a plastic culture dish, they spread, dedifferentiate, and reduce their liver-specific functions.

Mesenchymal stem cells (MSCs), which are derived from the bone marrow, differentiate into osteoblasts or adipocytes depending on their adhesive environment. Engineering a functional cellular phenotype in an artificial environment has become a major effort in tissue engineering. A greater understanding of the extracellular cues that control the behavior of cells, stem cells or others, may lead to smarter design of scaffold materials.

Lecture 7. Human genetic engineering

Human genetic modification is the direct manipulation of the genome using molecular engineering techniques. Recently developed techniques for modifying genes are often called "gene editing." Genetic modification can be applied in two very different ways: *somatic* genetic modification and *germline* genetic modification.

Somatic genetic modification adds, cuts, or changes the genes in some of the cells of an existing person, typically to alleviate a medical condition. These gene therapy techniques are approaching clinical practice, but only for a few conditions, and at a very high cost.

Germline genetic modification would change the genes in eggs, sperm, or early embryos. Often referred to as "inheritable genetic modification" or "gene editing for reproduction," these alterations would appear in every cell of the person who developed from that gamete or embryo, and also in all subsequent generations.

For safety, ethical, and social reasons, there is broad agreement among many scientists, ethicists, policymakers, and the public that germline editing is a red line that should not be crossed. Using germline editing for reproduction is prohibited by law in more than 40 countries and by a binding international treaty of the Council of Europe. However, in November 2108, a scientist named He Jiankui announced he had edited the genes of twin baby girls who had subsequently been brought to term. His reckless experimentation has been nearly universally condemned. This development has sparked new debate around human germline modification, particularly between parties who desire to push the technology forward and those who fear it could open the door to a new market-based form of eugenics.

https://www.geneticsandsociety.org/topics/human-genetic-modification

Lecture 8. Fundamentals of the safety of genetic engineering

The practical application of the achievements of genetic engineering has two important aspects. On the one hand, it is obvious that it can significantly contribute to solving the world's problems of human well-being, primarily related to the urgent needs for food, efficient agriculture and health improvement. On the other hand, genetic engineering is a revolutionary technology that opens up previously unthinkable possibilities of directed modification of genetic material. In this regard, the question arises, how safe are genetically modified organisms for human health and the environment?

Taking into account the second aspect, when using the achievements of modern biotechnology, the principle of taking precautions, which appeared in the 1970s, became defining. as a skeptical reaction of the environmental social movement to the possibility of scientific risk assessment and prevention of harmful consequences of the use of complex technologies. In essence, the principle means that in the face of scientific uncertainty or lack of necessary knowledge, it is better to err in the direction of redundancy of safety measures than to err in risk assessment. Currently, this principle is contained in more than 20 international laws, treaties, protocols and conventions, including the main international treaty governing relations related to GMOs - the Cartagena Protocol on Biosafety to the Convention on Biological Diversity. The formulations of the principle of taking precautions given in it do not require proof of the absolute safety of the technology, but suggest its limitation if the level of scientific uncertainty about the potential risk is significant, and the risk management capabilities are insufficient.

If there are sound scientific assumptions that a new process or product may be hazardous, it should not be implemented until there is evidence that the risk is small, manageable, and the benefits of the technology outweigh it. Application of the precautionary principle in this sense must demonstrate, not in an absolute manner, but above the level of reasonable doubt, that the genetic engineering activity proposed by the applicant is safe.

Thus, one of the main international requirements related to the development and application of modern biotechnology in science and production is to ensure, in accordance with the principle of taking precautionary measures, the safety of any genetic engineering activity (conducting research, field and other tests GMO) and

the safety of GM products placed on the market. In this context, biosafety is understood as a system of measures aimed at preventing or reducing to a safe level the adverse effects of GMOs on human health and the environment in the course of genetic engineering activities. The basis of biosecurity is a scientifically grounded, comprehensive and adequate assessment of the risk of possible harmful effects of GMOs on human health and the state of the environment, and the development of measures to prevent it.

The concepts of "risk", "risk factor", "risk assessment"

In a broad sense, risk is the likelihood of an unwanted event. The risk of an unwanted event is associated with some specific features of the substances used by the person or the actions performed. Every substance and activity is a potential risk factor. Some substances and activities can immediately cause a number of adverse events of various kinds, while others can cause single or few types of such events. A risk factor is a potential ability inherent in a substance or any activity (process) to cause harm (cause an undesirable event). The risk factor is a function of the unfavorable properties of an object (activity, process) and the conditions for their manifestation. If the term “risk factor” denotes only the cause (essence) of a potential adverse event, then the term “risk” denotes the estimated probability of this event occurring with one or another scale of its consequences. Accordingly, the risk can be defined as the following mathematical expression:

risk = the likelihood of a negative impact of the risk factor x the magnitude of the consequences of the impact.

The risk assessment procedure in this context should answer three questions:

1. What is the hazard (potential for harmful effects - identification of risk factors)?
2. How likely is it that this will happen (the likelihood that the impact will take place)?
3. What will be the magnitude of the consequences if this event occurs (the magnitude of the consequences of this harmful effect)?

In genetic engineering, the term "risk factor" is used to determine the potentially possible direct and indirect adverse effects of GMOs or products made from GMOs (including GMO components) on human health and (or) the environment due to the effect of insertion of recombinant DNA , the functioning of transgenes and the transfer of transgenes from GMOs to other organisms. Direct exposure refers to the impact of GMOs on human health and the environment, which does not require an analysis of a chain of interrelated events. Indirect is the indirect impact of GMOs on human health and the environment, which is carried out through a chain of interdependent events. In particular, it can manifest itself as a result of the interaction of GMOs with other organisms, the transfer of genetic material from GMOs to other organisms, changes in the procedure for the operation of objects of economic activity and their management due to the release of GMOs, etc. Immediate impact of GMOs on human health and the environment are observed directly during the period of genetic engineering activities. It can also be direct or indirect. Long-term impact becomes apparent in the form of direct or indirect impact after the end of this genetic engineering activity.

Lecture 9 The Nature of Risks to Human Health and the Environment Associated with Genetically Modified Organisms

To better understand the nature of the risks associated with GMOs, it is necessary to remember the differences between genetically modified organisms and conventional, unmodified ones.

The Cartagena Protocol on Biosafety contains the following definition: living modified organism means any living organism that possesses a new combination of genetic material obtained through the use of modern biotechnology (Cartagena Protocol on Biosafety, Art. 3).

Thus, any transgenic plant variety differs from the original one in that a relatively small DNA fragment is added to its genome to the existing genes, which contains information about one or two new genes and their regulatory elements. The activity of these added genes in the body is expressed in the biosynthesis of one or two new proteins for the body (enzymes or structural proteins). Since genetic engineering can operate with any genes that exist in nature, and not only genes from organisms that are evolutionarily related to certain species of cultivated plants, as is done in traditional breeding, the products of introduced genes (enzymes, proteins) can look like genetically modified organism as unusual, unusual, alien for a given species, which are not found in nature in it. Accordingly, it is the transgenic products that are the most significant risk factors associated with GMOs.

The added DNA fragment is not considered a risk factor: there are no scientifically substantiated indications of the toxicity of DNA transgenes to humans perse. People eat an average of 0.1-1 g of DNA daily in various foods. Therefore, the DNA of the transgene is not a new, special component in the human diet and is present in it in extremely small quantities. Decades of research have not revealed the toxic effects of transgenic DNA on humans and other mammals. There is no reliable information about the cases of insertion of transgenic DNA, which entered the body with food, into the human genome. Defense mechanisms of mammals (hydrolytic destruction of DNA during digestion, exclusion of foreign DNA from the recipient's genome during reparative processes**,**
hindering the expression of inserted genes due to their targeted methylation, etc.) counteract the insertion of foreign DNA into the human genome and its expression.

Thus, the likelihood of harmful effects on human health due to the consumption of transgenic DNA is minimal.

According to recombinant proteins, not all GMOs contain absolutely foreign compounds, unusual for a certain plant species. First, there is a fairly large group of transgenic plant varieties that are obtained through genetic manipulations with their own genes (see Ch. 9, Table 9.2; Ch. 10).

Secondly, many organisms that are very remote in evolutionary terms have a large number of identical metabolic pathways, and, accordingly, the composition and structure of the enzymes that ensure their implementation are also identical. An example is the EPSPS enzyme (see section 10.1), which is key in the biosynthesis of aromatic amino acids in all plants, fungi, and bacteria. Bacterial EPSPS, formed in transgenic soybeans tolerant to the herbicide glyphosate, successfully performs the corresponding functions in the plant organism after the treatment of plants with the herbicide, when the plant EPSPS of soybeans is deactivated. When assessing the safety of genes with similar functional activity, attention is paid not so much to the protein itself - the product of the transgene, but to the possible change in individual metabolic pathways of the transgenic plant due to an increase in the concentration of one of their components. In the case of EPSPS, when assessing the safety of genetically modified soybeans, it was taken into account that this enzyme catalyzes a reaction that does not limit the final rate of synthesis of aromatic amino acids; therefore, as expected, the indices of their synthesis in GMOs did not differ from those in the original plants.

Thirdly, the latest scientific data obtained as a result of sequencing the genomes of humans, some animals and plants, significantly expanded our understanding of the similarities and differences of genes of different systematic groups and the likelihood of their transfer from one distant systematic group to another (horizontal gene transfer). It turned out that the Arabidopsis genome contains about a hundred genes found in the human genome. Thus, in nature, there is an exchange of genetic information between distant species. Nevertheless, any scientist planning to add a new gene to a plant, microorganism or animal must carefully study the gene itself, as well as the product of its activity, and make sure that they are safe.

The second main group of risks is associated with the very fact of the insertion of transgenes into the genetic material of the organism. Along with the manifestation of target traits of genetic modification (intentional effect) as a result of the insertion of foreign DNA into GMOs, additional traits may appear or preexisting traits may undergo changes (unintended effect). Unintentional effects of genetic modification (GEM) can theoretically arise as a result of accidental insertion of DNA sequences into the plant genome, which causes the termination of expression or a change in the expression level of previously active genes, the onset of expression of previously "silent" genes. In addition, such effects may result from changes in the characteristics of metabolism in the recipient organism. Some UEGM can be partially predictable on the basis of knowledge about the transformation process, the place of insertion of the transgene, the functions of its products (including the effect on metabolism). Other UGEs are unpredictable (area of ??scientific uncertainty).

UEGM can be mediated by both the pleiotropic action of the inserted gene and the properties of the inserted construct itself, including its instability and possible action on neighboring genes. The insertion of foreign genetic material into the genome may be accompanied by rearrangements in the structure of the introduced genetic construct and the genome of the host plant. The expression of transgenes may differ depending on the copy number of the insert, its structure, the features of the region of insertion into the genome, etc. In this regard, each specific genetically modified plant is characterized by a certain level of expression and inheritance of the introduced genetic material, the likelihood of NEGM. It is no coincidence that one of the most important principles of biosafety is an individual approach: a thorough assessment of the safety for human health and the environment of each specific transgenic event (line) is carried out.

Finally, the third main group of risks associated with GMOs is based on the adverse effects caused by the transfer of transgenes to other organisms: vertical transfer of genes from GMOs to unmodified plants of the same species or wild relatives of a cultivated species, horizontal transfer of genes, for example, selective genes resistance to antibiotics, from genetically modified plants to microorganisms of the digestive tract. Genes and their products, which are harmless in GMOs, can turn out to be dangerous in a different genetic and ecological environment. Thus, the acquisition of antibiotic resistance by pathogenic bacteria of the digestive tract can significantly complicate the treatment of diseases that they can cause.

Lecture 10 Potential adverse effects of GM plants on human health, methods of their assessment and methods of prevention

Among the potential risks to human health associated with the use of GMOs, consider the following.

1. Synthesis of proteins new for the recipient organism - transgenic products, which may be toxic and / or allergenic. The toxic (allergenic) potential of the original host organism can increase as a result of genetic modification for a number of reasons. First, the transgene product itself can be toxic (allergenic) to humans (for example, if it belongs to proteins that are not components of organisms traditional for food production and have no history of safe consumption). Secondly, as a result of transgene incorporation, it is possible to increase the level of produced natural toxic substances and nutrient antagonists of the recipient plant, which in turn can lead to an increase in their consumption and to an increase in the likelihood of adverse effects on human health. Third, the toxic potential of the original plant can increase due to changes in its metabolism and the accumulation of toxic metabolites in it**.**

2. Changes in the activity of individual genes of living organisms under the influence of the insertion of foreign DNA, as a result of which the consumer properties of food products obtained from these organisms may deteriorate. For example, in genetically modified foods, there may be an increased level of any anti-nutritional substances in comparison with the recipient organisms, exceeding the established safety limits, or a reduced level of nutrients.

3. Horizontal transfer of transgenes to other organisms, in particular marker genes for antibiotic resistance from GMOs to microorganisms of the digestive tract. The presence and expression of antibiotic resistance genes in GMOs, which are necessary for the selection of transformed cells, raise serious concerns and are the subject of scientific discussions. If the horizontal transfer of selective genes occurs at a relatively high frequency, it can adversely affect the effectiveness of traditional antibiotic therapy in humans and pets.

As you can see, the risks to human health caused by GMOs are mainly associated with the consumption of products derived from them or produced by them.

The strategy and methods for assessing the quality of traditional food products and assessing the safety of new products (made using new technologies, using new food additives, etc.) are well developed and have shown high efficiency. Many of them can be used to assess the safety of GM foods.

Lecture 11. Methods for assessing the quality and safety of traditional food products

In most cases, the assessment of the quality and safety of traditional products consists in the analysis of possible adverse effects on human health of food additives (dyes, emulsifiers, preservatives, etc.) and food contaminants (pesticide residues, veterinary medicinal products, hormonal drugs, mi- cotoxins, etc.).

At the first (preparatory) stage, a preliminary assessment of the toxicity potential of pollutants is carried out. Potentially hazardous food contaminants assessed are identified by analytical laboratory methods; their physical and chemical properties are being investigated. The main component of this stage of risk assessment is the primary toxicological assessment of the investigated agents in an acute experiment on model animals (mainly rodents). Acute toxicity is determined by the adverse effects recorded in laboratory animals after a single forced feeding of the pollutants under study. For feeding, chemically pure evaluated substances are used in various dosages (maximum doses can be 2000-5000 mg / kg). According to the results of an acute experiment, the toxicity indicator of the pollutant LD50 is determined, which is a statistically calculated dose of a substance that, when fed once, can cause the death of 50% of animals. It is expressed in units of the mass of the evaluated substance in terms of the body weight of the animal (mg / kg) and determines whether the evaluated food component is capable of causing an acute toxic reaction of the body. In addition, in the test for acute toxicity of a substance, mandatory observations are carried out on animals, changes in body weight, skin, wool,

mucous membranes, the state of the circulatory, respiratory and central nervous systems, behavioral and somatomotor reactions are assessed**.**

At the second (main) stage of assessing the safety of traditional food products in a series of tests, the nature of the toxicodynamics of the identified agents (biological mechanism of toxic effects) and their toxicokinetics (the nature of chemical, biochemical transformation in the body) are determined. The "dose - effect" relationship experimentally established at the second stage of research determines the likelihood of adverse effects of the investigated pollutant at the usual level of its consumption for a given population of people (that is, the toxicological potential of the investigated agent). Assessment of the accepted level of consumption of a product includes measuring the average daily consumption volume, frequency of consumption and duration of consumption. Considering all of the above parameters, the risk of adverse effects of the toxic agent is assessed.

Assessment of the toxicity risk at this stage is carried out in the course of a subchronic experiment on laboratory animals. A subchronic experiment simulates a real situation of consumption of the evaluated agent by a person and therefore has the greatest value among other tests. It provides the researcher with extensive information about the points of application of toxicants (biological "targets"), the nature of their adverse effects. If necessary, the specific adverse effects of toxins are also investigated: their genotoxicity, carcinogenicity and mutagenicity, reproductive, ontogenetic toxicity and immunotoxicity.

In a subchronic experiment, a substance is forced to be fed to animals daily for 28-90 days. A number of doses are used, each group of animals receiving only one specific dose of the test substance. The dose level is selected based on the available preliminary toxicity data. The highest dosage should cause a clear toxic effect, but not lead to mass death of animals. By lowering the dosage, the test determines the threshold dose of a substance that does not cause a recorded adverse effect - the so-called indicator

NOAEL (no observed adverse effect level). The toxic effect of a substance is determined on the basis of a number of indicators: changes in the body weight of the animal, the level of food and water consumption, the nature of the toxic reaction depending on the dose of the substance, data from blood analysis and histological studies, the mortality rate, etc.

The results of testing the toxicity of food components in acute and chronic tests on animals are extrapolated further to humans (conditionally the third stage of assessment). The toxicity assessment ultimately focuses on determining the level of daily intake of the investigated agent by a person (taking into account body weight), which does not have a harmful effect on health (the so-called acceptable daily intake (ADI)). The ADI is calculated based on the experimentally determined NOAEL value, taking into account the safety factor. In most cases, when applied to humans, a coefficient of 100 (ADI = NOAEL / 100) is used to determine ADI: it is assumed that humans are approximately ten times more sensitive to toxins than animals, in addition, differences are allowed (up to ten times) in sensitivity between individuals.

It should be noted that the risk associated with food consumption is not solely determined by their contamination with toxic substances or their loss of quality (for example, due to improper storage). There are no absolutely safe food products. Even traditional products with a long history of eating may contain certain natural components that have adverse effects on human health (natural toxins, allergens, antagonists of nutrients (antinutrients), substances with potential mutagenic and carcinogenic action, etc.). The likelihood of their adverse effects on humans is determined by many factors: their content in food, interaction with other components of the product, the state of the individual's defense systems, exogenous environmental factors, the volume of the product in the diet, and many others. To date, both exogenous and many natural components of food products have been identified and sufficiently fully investigated, which have harmful or positive effects on human health. Substances that are natural for food and potentially hazardous to human health cannot be ignored when assessing food safety, as well as the effects of various kinds of contaminants.

Despite this, products and food raw materials that are obtained using traditional technologies with a long history of use do not represent the objects of a scrupulous and exhaustive study of their safety and nutritional value. For example, plant varieties created by traditional breeding methods are not subject to acute and chronic toxicity tests in laboratory animals. The exception is products intended for feeding infants and young children. The relative safety of traditional food products is guaranteed by a long history of safe human use.

Lecture 12 Application of the concept of substantial equivalence to assess the safety of GMOs and GM foods

Organisms obtained by traditional selection are not subject to a rigorous analysis of potential toxicity and allergenicity, although it is known that they may contain a number of harmful compounds. At the same time, on the basis of long historical experience, it is assumed that a new combination of genes, created in the process of traditional selection of organisms used for food production, and determining a number of new traits for them, will not pose a threat to human health. Taking into account this assumption, as well as the methodological problems arising in the study of new whole foods (GMOs as a whole), the basis for assessing their safety is the concept of significant equivalence, developed by OECD experts and supplemented by FAO / WHO. According to the OECD definition, the concept of substantial equivalence provides that traditional products or food raw materials can serve as a basis for comparison when assessing the safety or nutritional value of new products, including GMO derivatives. This comparative approach assumes that: 1) traditional products are not completely safe; 2) if new food products or their components are equivalent in essential characteristics to traditional products or food components, they can be used in the same way, without expecting harmful additional effects on human health.

Therefore, the safety of a new product should not be considered in general, but in relation to its traditional counterpart. The definition of "traditional analogue" as applied to GMOs or GM foods means a closely related organism, its component or similar foods for which there is considerable experience of safe consumption as food. The best analogue for determining significant equivalence is an organism that is initial for genetic modification (parental, practically isogenic line). However, such an analogue is not always available. In this case

The OECD recommends the use of several genetically similar control samples (other varieties of the same plant species, related strains of microorganisms, animal breeds) or similar products already on the market to determine if the differences found are due to natural variation or due to genetic modifications.

The essential equivalence of GMOs and analogs is established by demonstrating that the characteristics of GMOs and new products that are significant for assessing food safety are similar to those of traditional counterparts. Thus, the analysis of significant equivalence presupposes, first of all, the compositional analysis of GMO (new product) and its analogues. At the same time, the key components of the compared organisms (products), which are most important for human health, are investigated: nutrients and their antagonists; toxic substances, allergens, etc. Among the key nutrients, there are major (fats, proteins, carbohydrates) and minor (minerals, vitamins); nutrient antagonists are mainly inhibitors of certain enzymes. Key toxins mean known natural compounds and substances present in the evaluated raw materials or products that have toxic potency that is essential for human health (for example, glycoalkaloids in potatoes).

In order to identify in new products and raw materials different from analogs traits that affect the level of safety and nutritional value of food products, information regarding the characteristics of the parent organism from which the gene intended for transgenesis is taken, as well as the nature of the genetic -skoy modification. Next, a comparative analysis of the genetically modified organism and the original (unmodified) organism is carried out. For this, agronomic indicators, the products of embedded genes, the composition of key chemical components (including nutritional and anti-nutritional), the profile of the main metabolites, and the effects of processing of raw materials are compared.

A new product (plant variety) can be:

- equivalent (equivalent) in terms of essential characteristics to the selected analogue;
- equivalent to the analogue, with the exception of one (several) essential, well-defined feature;
- not equivalent to the analogue in terms of essential features.

In the latter two cases, a thorough safety assessment of GMO traits different from the initial analogue is carried out in terms of such indicators as potential toxicity, potential allergenicity, the possibility of transfer of antibiotic resistance genes to microorganisms of the digestive tract, the likelihood of a potential deterioration in nutritional value and absorption of nutrients.

Lecture 13. *Assessment of the potential toxicity of transgenic products new to the host organism*

With tz. human health, the phenomenon of toxicity can be characterized as the cause of the disease of chemical etiology (poisoning) and as a factor causing tissue damage (typical pathological process), as a result of which the corresponding pathological condition arises.

Alien genes are transferred into the transforming organism to transfer desirable traits to it. The phenotypic manifestation of these traits is due to the synthesis of protein and non-protein compounds new for the

recipient organism. Such compounds synthesized as a result of transgene insertion can be traditional food components: proteins, fats, carbohydrates, vitamins, which are new only in the context of certain initial organisms and GMOs. In addition, the products of transgenes can be target proteins that were not previously components of food and, accordingly, do not have a long history of safe use by humans. Metabolites formed in GMOs as a result of the activity of a transgenic enzyme can also be new in relation to the recipient. Assessment of the potential toxicity of transgenic products involves the following:•

determination of the chemical nature, functions of new synthesized compounds, as well as their concentration in a food product, taking into account natural variation;

• analysis of information on the nature of genetic modification and GMOs to obtain confidence that the donor's genes responsible for the synthesis of known toxic and anti-nutritional substances are not expressed in GMOs;

• if the new compound is a traditional food component with known biological functions (eg carotene in Golden Rice) and its concentration in the product does not exceed the usual range of variation, special toxicity tests may not be performed. In other cases, such tests are necessary;

• in the case of new proteins that did not have a history of eating proteins, the assessment of their potential toxicity is focused on determining the following characteristics: the level of similarity of their amino acid sequence with the amino acid sequence of known toxic proteins; the level of their physical and chemical stability. In some cases (see below), tests on model animals are required to assess the toxicity of proteins;•

the potential toxicity of non-protein compounds is assessed on an individual basis depending on their biological function and their proportion in the usual diet. In this case, it is necessary to study metabolism, toxicokinetics, subchronic toxicity, chronic toxicity, carcinogenicity, reproductive toxicity, etc.

The most common transgenic products of commercially used GMOs are certain proteins. Therefore, the procedure for assessing their potential toxicity is discussed in more detail below. It is known that there are a number of important differences in the toxic effects of proteins and industrial chemicals of a non-protein nature on humans. Proteins are usually non-toxic in acute experiments on model animals, and there are no known cases of them showing chronic toxicity, for example, having mutagenic, carcinogenic effects. Individual protein toxins are well studied and highly specific.

Proteins, unlike chemicals, are usually quickly digested in the human gastrointestinal tract and are deactivated. They also do not bioaccumulate (accumulate) like some harmful chemicals. Taking into account these features, the assessment of the toxic potential of transgenic proteins is somewhat different from the above procedure for assessing the toxicity of industrial and other food contaminants. It is designed to provide answers to the following questions:

• What is the expected amount of the estimated protein in the typical human diet?

• Does the new protein being assessed cause reported adverse (toxic) effects when consumed in amounts significantly in excess of the established intakes?

• Is the new assessed protein being digested in the human gastrointestinal tract?

• Is the new assessed protein degraded during food processing?

Chromas Pro Version 1.5. Степень идентичности полученных последовательностей последовательностям, включенным, например в базу данных NCBI (англ. National Centre for Biotechnology Information - Национального центра биотехнологической информации США), осуществляют при помощи пакета программ NCBI Standard Nucleotide BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/).

Lecture 14. *Assessment of the risk of potential allergenicity of GM foods*

Food allergy is an immunological reaction that develops in response to the ingestion of certain exogenous substances - allergen genes - into the body. Food allergen is a food component that stimulates the development of an adverse immune response in food allergic individuals. A product can contain from one to a number of allergens, which in the overwhelming majority of cases are proteins.

There are several types of adverse immune responses to dietary proteins. The most common type of food allergy is type I, which is accompanied by the production of antibodies in the body with a special cellular affinity (the ability to bind to the membranes of a number of specialized cells). Antibodies are specialized

immune effector proteins of blood serum (immunoglobulins) that specifically recognize macromolecules foreign to the body - antigens (mainly foreign proteins and protein complexes) and participate in their elimination. Antibodies are produced by specialized cells of the immune system in response to the penetration of foreign antigens into the body. There are 5 classes of antibodies, different in their structure, which, together with other specialized protein and cellular components of the immune system, are involved in protecting the body from genetically foreign substances.

An allergic reaction of type I is defined as an acquired hypersensitivity of the body of an immediate type to relatively harmless exogenous substances - allergens (including generally harmless food components). In contrast to the protective reactions of immunity, an allergic reaction of type I is unfavorable for humans and is associated with the production of an increased amount of a special class of antibodies - IgE, directed against specific allergens. Its symptoms can occur minutes after contact of the components of the immune system with the allergen.

The features of IgE-mediated allergic diseases include the following. First, only a small number of antigens with the potential to induce an immune response are allergens. Secondly, not all individuals in the same environmental conditions react to contact with an allergen. Although all people produce some amount of E antibodies, only a certain proportion of individuals become sensitive when foreign proteins from the external environment enter the body and develop an IgE-mediated allergic immune response. Acquisition of sensitivity is a complex process that depends on the nature of a particular person and the time of the first contact with an allergen.

Compared to other types of allergies, food IgE-dependent allergies are quite rare (in 0.3-8% of children, depending on age, and in 1-2% of the adult population). Children are more susceptible to food allergies due to the incomplete maturity of their IgE immune system and incomplete physiological maturity. They tend to "outgrow" food allergies, especially to milk, eggs and soybeans. Therefore, among the adult population, it is much less common than among children.

More than 90% of allergic reactions seen in children and adults occur with the consumption of eight staple foods or food groups. These are cow's milk, eggs, fish, sea crustaceans (shrimp, crabs), as well as shellfish, peanuts, soybeans, nuts (almonds, walnuts, etc.), wheat (Table 11.2). In addition, about 160 other products or food components cause an allergic reaction only in certain people. Among them, the majority of cereals, oilseeds and vegetable food crops, as well as industrially manufactured products: beer, chocolate, etc. are noted. In fact, all food allergens are proteins or glycoproteins. However, only a very low percentage of the many thousands of dietary proteins are allergens.

Таблица 11.2. Пищевые аллергены растительного и животного происхождения (по D. Metkalfe и др., 1996)

Видовое название растения или животного	Традиционноеназвание	Аллерген (систематическое и оригинальное название)	Молекулярный вес kDa
Аллергены растительного происхождения			
Arachis hypogea	Арахис	Ara h 1	
Bertholletia ecscelsa	Бразильский орех	Ber e 1 (2S альбумин)	
Brassica juncea	Горчица листовая	Braj 1; 2S альбумин	14
Sinapis alba	Горчица белая	Sin a 1 (2S альбумин)	14
Glycine max	Соя	Глицин (субъединица AlaBx, субъединица A2B1a, субъединица A3B4 и др.), β-конглицинин (α-субъединица, субъединица CG4), соевый лектин	

		(соевый агглютинин), ингибитор трипсина Кунница (Kunitz)	
Hordeum vulgare	Ячмень	Hor v 1; BMAI-1 (α-амилаза/ин- гибитор трипсина)	15
Oriza sativa	Рис	RAP (рисовый аллергенный белок), RAG1 (рисовый аллерген 1)	
Phaseolus vulgaris	Фасоль	PR-1 (белок, связанный с патогенезом - 1)	
Triticum aestivum	Пшеница мягкая	WGA (зародышевый агглютинин A, D пшеницы)	
Triticum durum	Пшеница твердая	WGA (зародышевый агглютинин пшеницы)	
Аллергены животного происхождения			
Bos taurus	Крупный рогатый скот	BSA (бычий сывороточный альбумин), β-лактоглобулин (белок молока), α-лактальбумин (белок молока), казеин (типы α-S1, M-S2, β-)	
Gadus callaria	Треска	Gad c1; allergen M, β-парваль- бумин	12
Аллергены животного происхождения			
Gallus domesticus	Домашние куры	Gal d1 (овомукоид),	28
		Gal d2 (овальбумин)	44
		Gal d4 (лизоцим)	14
Metapenaeus ensis	Креветки	Met el; тропомиозин	34

Usually allergens are readily soluble proteins (water-soluble albumin and salt-soluble globulins) with a molecular weight of 10-80 thousand daltons and an acidic isoelectric point. Most allergenic proteins are stable to digestion in the gastrointestinal tract and to various types of processing (including thermal). These properties allow them to maintain their structure until they enter the intestine and overcome the barrier of the intestinal mucosa in an immunologically intact form. The characteristic molecular weight and relative resistance to physicochemical destructive effects serve as indirect indicators of the allergenic potential of proteins (see below), but they do not have absolute reliability in assessing the risk of allergenicity. In particular, there are many thermolabile or partially thermolabile food allergens. Some allergenic proteins have a molecular weight below the characteristic (for example, lipid-transferring proteins of plants - 9 thousand daltons; soybean peel protein - 8 thousand daltons). In some cases, heat treatment may not reduce, but even increase the allergenicity of proteins, in particular as a result of their chemical glycosylation (for example, in the case of ß-lactoglobulin in cow's milk, some crustacean proteins).

The risk that allergenic potential may increase in a number of GM foods (made from GMOs, including GMOs, or being GMOs) is largely justified. It is known that many protein allergens have biological activity that can be used in transgenic organisms (may be the target effect of modification). For example, numerous proteins with potential antimicrobial, antifungal activity are known allergens. Important storage proteins of seeds of many dicotyledonous plants - 2S albumin - are simultaneously the main allergens of mustard, Brazil nut, walnut, and cotton seeds (see Table 11.2). The literature describes an attempt to transfer the gene responsible for the synthesis of 2S albumin from the Brazil nut to soybean plants in order to increase its content of the amino acid methionine and improve its feeding qualities. However, the 2S albumin produced in transgenic

soybean plants, which constituted a significant part of the total soy protein (6%), turned out to be allergenic for people sensitive to Brazil nuts. Although this transgenic soybean variety was intended exclusively for animal feed, it was not allowed for commercial use. This example is very indicative in terms of the fact that there is a real risk of transfer of genes responsible for the production of allergens from a donor organism with an allergenic potential to a recipient organism. Moreover, if the likelihood of introducing a known allergenic protein into GMOs can be relatively easily controlled, then it is more difficult to assess the likely allergenic potential of transgenic proteins new to the original organism that have not had a long history of ingestion (for example, GFP, Bt protein, etc. .).

In the process of genetically engineered modification, one or more transgenes are included in the original host organism, which are responsible for the production of a very small fraction (usually less than 0.4%) of the protein relative to the total GMO protein content. However, as noted above, this may be enough to trigger food allergies in people who are sensitive to it. So far, no cases of allergic reactions have been recorded in people from the use of new food products or their transgenic sources released for circulation on the commodity market. However, there is a certain likelihood that the allergenic potential of GMOs and related foods may be increased during the genetic modification process.

In theory, this increase in the allergenic potential of food can be due to two events. First, the expression of transgenes transferred to the original organism as a result of genetic modification can lead to the production of previously uncharacteristic allergenic proteins (i.e., the molecular products of transgenes can be allergens). Second, it is likely that the natural allergenic potential of the host organism may be increased due to unintended effects of genetic modification. Various food crops, such as peanuts, avocados, and wheat, are characterized by significant variability in the number of allergens, and their level may be subject to further change as a result of genetic modification. In addition, there is a possibility that previously non-allergenic proteins inherent in the host organism will become allergenic after genetic transformation (for example, due to glycosylation).

Эксперты ряда международных организаций (ILCI - Allergy and Immunology Institute; IFBC - International Food Biotechnology Council; FAO/WHO) разработали систему оценки риска аллергенности новых продуктов питания и исходных ГМО, включающую ряд связанных анализов. Ниже приведена процедура оценки аллергенности, принятая экспертами FAO/WHO(рис. 11.1).

Figure. 14.1. Assessment of the risk of allergenicity of GM foods. Sequence of tests and solutions proposed by experts FAO/WHO (FAO/WHO, 2001; http://www.fao.org/es/csn/gm/biotech-e.htm)

In addition to the comparative analysis of the amino acid sequence, at the first stages of the study, a physicochemical test is also carried out for the resistance of the tested proteins to proteases of the gastrointestinal tract (test for destruction by pepsin). It was already noted above that allergens are proteins that are generally resistant to destruction by reagents of the gastrointestinal tract (otherwise they cannot reach the reactive mast cells of the intestinal mucosa in the native state). The standard procedure for assessing the proteolytic activity of pepsin involves treating the target protein with a pepsin solution. A negative test result indicates that the protein under study can only be an allergen with a small (but requiring attention) probability. In contrast, the resistance of the evaluated proteins to degradation by pepsin under suitable conditions indicates that they may be allergens.

Taken together, preliminary indirect allergenicity tests make it possible, with a certain probability, to judge whether the evaluated protein is an allergen. A positive result indicates that the tested proteins are highly likely to be allergenic (but this is not necessarily the case).

A negative result from indirect tests does not provide absolute proof that the tested proteins have no allergenic potential. Therefore, after preliminary characterization of the structural and physicochemical features of the proteins under study, the risk assessment procedure continues. It provides for the conduct of specific immunological studies, which finally establish whether the tested proteins are allergens. For proteins originating from known allergenic sources or having structural homology with known allergens, the risk assessment procedure recommends a so-called specific serum screening. These are in vitro tests for the reactivity of the transgenic protein with specific IgE from the blood serum of people sensitive to the proteins of the donor organism (from whose DNA the transgene was isolated). These studies show whether the analyzed proteins are recognized by IgE antibodies from the blood serum of people sensitive to the donor's allergens. An in vitro analysis can establish the presence and amount of an allergenic protein in the studied food products and, to a certain extent, show a change in the allergenic properties of the protein.

The standard tests used for in vitro immunological studies are solid-phase immunological diagnostic tests - radio-allergosorbent assay (RAST) and enzyme-linked immunosorbent assay (ELISA). For example, in the case of the RAST test, the serum of susceptible individuals is incubated with the test potential allergen (food extract) attached to a suitable solid phase carrier. Allergen-specific IgE binds to the carrier-attached allergen and can be detected using radioactive iodine-labeled anti-human IgE antibodies.

To obtain reliable risk assessments in in vitro tests, the availability of blood serum of a sufficient number of individuals sensitive to a certain allergen is critical. The analysis should, if possible, be carried out with 25 serum samples from people with food allergies. Tests using a minimum of eight appropriate sera are required to achieve 99% certainty that the test protein is not a major allergen. Accordingly, 24 sera are required to achieve the same reliability in the case of minor allergens. In the presence of at least 14 individual sera, a negative specific serum screening result with a probability of more than 99.9% indicates that the tested protein does not belong to the main allergens. Under similar conditions, a negative result with a probability of more than 95% indicates that the tested protein does not belong to minor allergens that cause an allergic reaction, at least in 20% of sensitive individuals. If the in vitro test is positive, the GMO or the corresponding product is considered allergenic. If the test result is negative (in combination with the data on the absence of structural homology of the tested protein with known allergens and the absence of resistance to proteases of the gastrointestinal tract), the transgenic protein will most likely not be an allergen.

For the clinical analysis of the allergenicity of transgenic proteins in vivo, a skin prick test (SPT) is usually used. Extracts of the required concentration, isolated from the recipient organism, donor and GMO (food products obtained on their basis), are introduced into the epidermal layer of the skin of sensitive individuals. Within 15 minutes after administration, a local allergic reaction is recorded, manifested by reddening of the test area (like a trace from a mosquito bite). If no reaction is observed within this period of time, the test products are likely to be free of food allergens. For this method, the production of extracts with an adequate content of the potential allergen is critical. SPT results are also influenced by the way the test products are prepared (for example, heating can both destroy and create new antigenic determinants in the allergen).

The procedure for assessing potential allergenicity is simpler and more reliable if the allergenic potential of the proteins in question - the products of transgenes - is already known at the time of testing. In this situation, serum from susceptible individuals is available for specific in vivo screening, a method with a good evidence base.

It is much more difficult to evaluate transgenic proteins if they have no history of consumption and, accordingly, there is no information about their allergenic potential. In this case, human serum containing IgE specific to the tested protein is not available. The above-mentioned indirect methods for assessing potential allergenicity come to the fore: a comparative analysis of the amino acid sequence of the tested proteins with known allergens and their physicochemical characteristics. At the same time, the results of such tests, which are not actually immunological, should be treated with some caution.

Usually, a complete risk assessment procedure is performed for proteins "new" from the point of view of human nutrition, including immunological tests on volunteers.

Lecture 15 *Risk assessment due to the possibility of horizontal transfer of marker genes of antibiotic resistance to microorganisms of the digestive tract*

As part of the assessment of the risk of GMOs for human health, along with other risk factors, an assessment of the risk of transfer of marker genes for resistance to antibiotics and herbicides (if they are present in GMOs) to microorganisms of the digestive tract is also carried out. This requirement and criteria for assessing the associated risk are included in the internationally accepted procedure for assessing the safety of GMOs and new foods (FAO / WHO, 2003).

Antibiotics are organic substances formed by living organisms (mainly bacteria, fungi) and possessing antimicrobial properties. This is one of the main components of modern drug therapy. Horizontal transfer of selective marker genes to pathogenic bacteria can cause the appearance of genotypes resistant to certain antibiotics and thereby reduce the effectiveness of treatment of diseases in humans and domestic animals.

The transfer of DNA fragments from transgenic plants to intestinal microorganisms due to the consumption of GM foods is theoretically an unlikely event, since in order for it to occur, a combination of the following factors is necessary:

• the GMO marker gene involved in the transformation of bacteria must be in the form of free linear DNA fragments;

• DNA of the marker gene should avoid degradation by nucleases of the gastrointestinal tract;

• DNA of the marker gene must avoid competition for transport into the bacterial cell with other DNA contained in food;

• recipient bacteria must be competent for transformation;

• DNA of the marker gene must avoid enzymatic restriction inside the bacterial cell;• маркерный ген может встроиться в ДНК реципиента вследствие редких репаративных или рекомбинационных событий. Для этого должна иметь место гомология маркерного гена или прилегающих к нему районов ДНК с ДНК хромосомы или плазмиды болезнетворной бактерии пищеварительного тракта;

• for the expression of the inserted marker gene in bacteria, it is necessary that the marker gene falls into the “sphere of action” of bacterial regulatory sequences (in eukaryotic GMOs, the transgene activity is regulated by eukaryotic promoters that do not function in prokaryotes).

Despite the theoretically low probability of lateral gene transfer, numerous experiments were carried out with the aim of assessing in practice the frequency of horizontal transfer of marker genes. The process of natural transformation of bacteria with the genetic material of higher plants was discovered exclusively under optimized laboratory conditions and with the indispensable requirement for homologous recombination. This means that the marker antibiotic resistance gene can be transferred from GMOs to bacterial cells if the same gene or genes containing fragments with identical nucleotide sequences are present in these cells in advance. In the case when DNA homology is not observed in living organisms, lateral gene transfer is unlikely even in optimized laboratory conditions.

The likelihood of PHI was also assessed in model systems simulating the conditions of the human gastrointestinal tract. It was shown that the DNA of GM plants is rapidly (within a few seconds) destroyed in the small intestine, but persists for a relatively long time (minutes) in the lower part of the ileum and in the large intestine, where HGP is theoretically possible. Calgene, an American agro-firm, presented the calculation of the probability of LHP kanamycin resistance from transgenic tomatoes to the microflora of the human large intestine. He showed that eating 250 g of transgenic tomatoes (3 mg of DNA, 10 copies of the nptII gene per genome) can increase the proportion of microorganisms carrying the nptII gene by only 2.4 x 10-15%. In this case, the fact of transformation does not mean yet the functioning of the gene, which requires additional processes of recombination and selection. The frequency of transformation of bacteria in the digestive tract with a linear fragment of transgenic DNA is estimated by various researchers as 1: 1017-1018.

In addition to the likelihood of an adverse effect of any factor, the parameter of the magnitude of its consequences is important for assessing the risk. It is known that a significant proportion of microorganisms of the human gastrointestinal tract is already resistant to certain antibiotics, regardless of genetic engineering activity. The proportion of resistant forms is growing along with the increase in the use of antibiotics in medical practice. For example, for kanamycin, there is evidence that out of 10^{14} bacteria of the digestive tract 102 already have a natural resistance to it. The widespread prevalence of bacteria resistant to kanamycin, neomycin, and other antibiotics, genes for resistance to which are used in genetic engineering research, limits the use of these drugs in medical practice. Currently, if they are used, it is mainly in veterinary medicine.