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Report Highlights:

On March 27, 2020, the Russian Ministry of Agriculture finalized revised Methodological Guidance (MG) that establishes the procedures required to assess the biosafety of viable, non-viable GE organisms, as well as GE organisms of plant or animal origin used in feeds for animals and additives. This MG will allow registration to restart after a roughly 2-½ year hiatus. An unofficial translation of the document is provided at the end of this report

On March 27, 2020, Dmitry Patrushev, Minister of Agriculture of the Russian Federation, signed Order #160 "On approval of Methodological Guidelines for conducting assessments (studies) of the biological safety of genetically engineered/modified organisms used for production of animal feeds and feed additives". This document establishes new procedures for assessing the biosafety of genetically engineered (GE) organisms and stipulates that the expertise must be conducted by a laboratory accredited in the national accreditation system. The MG came into force on April 26, 2020.

In addition to the General Provision, the Guidelines outline three Assessments for animal feeds and additives: (1) derived from viable GE microorganisms, (2) derived from non-viable GE microorganisms, and (3) derived via the application of GE of plant or animal origin. The Guidelines also define the requirements for conducting studies and the safety conditions required to conduct studies of GE microorganisms. All testing of GMOs' biosafety is conducted on laboratory animals.

The final version of the Guidelines differs from its previous draft version (dated October 2019) in several aspects. For example, the requirement to provide information on non-viability of GE organisms, the requirement to conduct a specific study on GE non-viability, as well as the requirement to study the viability of GEs of plant and animal origin have been removed, which allows the import of not only processed GE products and meals, but also GE soybeans and corn.

However, the Methodology doesn't provide for guidance to allow for registration of stacks. This means that the Russian Federal Veterinary and Phytosanitary Service (VPSS) intends to conduct a full scope of studies on stacks which means that applicants will be obliged to repeat all the studies conducted on each single event contained in the stack.

The Methodology does not provide a list of laboratories accredited to conduct the studies. Only one Institute appears to be authorized to carry out the studies – the Federal State Budgetary Institution "The Russian State Center for Animal Feed and Drug Standardization Quality" (VGNKI) which is a subsidiary of VPSS. The Institute of Nutrition (ION) carries out studies for the Ministry of Health on GE for food use. It is unclear whether VGNKI or VPSS will accept the ION results already conducted.

Industry has expressed concern about the paragraph of the document related to toxicological studies and reproductive toxicity studies (Appendix 2, paragraph 1 g, 1 d of the draft). The final document stipulates that, in case of findings of any negative effects, the studies should be continued on generations F2 and F3 of GE organisms, whereas the previous draft (dated October 2019) provided that studies should continue on to just the third (F2) successive generations.

The unofficial translation of Order #160 is provided below.

BEGIN UNOFFICIAL TRANSLATION:

MINISTRY OF AGRICULTURE OF THE RUSSIAN FEDERATION

ORDER

of March 27, 2020, No. 160

On approval of Methodological Guidelines for conducting assessments (studies) of the biological safety of genetically engineered/modified organisms used for production of animal feeds and feed additives

In accordance with paragraph 13 of the Rules for the state registration of genetically engineered/modified organisms intended for release into the environment, as well as products derived by using such organisms or containing such organisms, including the above products imported into the territory of the Russian Federation, approved by Decree of the RF Government of September 23, 2013, No. 839 (Collected Legislation of the Russian Federation, 2013, No. 39, Article 4991), I hereby order:

To approve the attached Guidelines for conducting assessments (studies) of the biological safety of genetically engineered/modified organism used for the production of animal feed and feed additives.

Minister
D. N. PATRUSHEV

Approved by
Order of the RF Minister of Agriculture
of March 27, 2020 No. 160

Methodological Guidelines for conducting assessments (studies) of the biological safety of genetically engineered/modified organisms used for the production of animal feeds and feed additives

I. General Provisions

1. These Methodological Guidelines establish a procedure for conducting the biological safety assessments (studies) of genetically engineered/modified organisms used for the production of animal feed and feed additives (hereinafter – assessment, GMO, respectively).

2. The assessment is conducted by an institution (testing laboratory) accredited with the national accreditation system in accordance with the scope of accreditation corresponding to the studies indicated in these Guidelines.

3. Requirements for conducting studies and indicators (conditions) of the GMO safety are specified in Sections V and VI of these Guidelines.

4. The assessment results are documented in the report of assessment results based on the GMO study protocols, which should include the following data:

Title of the report;

Full name and address of the institution (testing laboratory) where the assessment was carried out;

Name of the GMO and its taxonomic status;

Full name, address, taxpayer identification number (INN) of the legal entity conducting genetic engineering activities in the Russian Federation for the purpose of GMO development (hereinafter - the applicant);

Full name and address of the legal entity, or the last name, first name and patronymic (if any), residence address of the individual entrepreneur - manufacturer of GMO samples provided for the assessment;

Type of potential intended use of the GMO;

Information on the transformation event presented as a code generated according to the All-Russian Classifier of Transformation Events;

Place of deposition and collection number (indicated for the deposited GMO strains);

Registration number of the certificate of state registration of the GMO(s) intended for the release into environment from which the assessed GMO(s) was (were) derived (in case where the GMO was derived from other GMO(s));

Registration number of the certificate of state registration of the GMO for another intended use (if any);

Information on the GMO registration in foreign countries (if available);

Review of the completeness of the documents and data submitted by the applicant;

Summary of the documentation and data submitted by the applicant for conducting the assessment;

List of GMO studies with their results;

Description of the GMO and original recipient organism samples provided by the applicant for the assessment, specifying their quantity, and the evaluation of their suitability for conducting studies;

Conclusions based on the assessment findings: the presence or absence of adverse GMO impact on the environment;

Specific conditions for the GMO use (if any);

Period of validity of the certificate of state registration of the GMO (if no data is available on the adverse impact of the GMO on the environment);

Last names, first names and patronymic names (if any) of the researchers who carried out the assessment, academic ranks (titles) (if any), their places of work and positions;

Date and number of the assessment report;

Signature of the head of the institution (testing laboratory) that carried out the assessment;

5. The report on the assessment results should be accompanied by the GMO study protocols, supporting the compiled report, and signed by persons who conducted the studies.

6. In case where the applicant has not submitted documents or data envisaged in p.p. 7, 11, 15 of the Guidelines, and (or) has not provided the required samples suitable for conducting studies, as well as in case where study protocols have not been submitted by the applicant, the assessment is not performed. The applicant should be issued a substantiated refusal to conduct assessment, signed by the head of the institution (testing laboratory), accredited with the national accreditation system in accordance with the scope of accreditation corresponding to the studies indicated in these Guidelines, which the applicant contacted for performing the assessment.

II. Assessment of the GMO used for the production of animal feed and feed additives derived from viable GMO which is a microorganism or contains such organism

7. The assessment of the GMO for producing animal feed and feed additives derived from viable GMO which is a microorganism or contains such organism includes the review of documents and data submitted by the applicant:

a) name of the GMO, indicating its taxonomic status; full name, address, taxpayer identification number (INN) of the applicant; full name and address of the legal entity or last name, first name and patronymic (if any), place of residence of the individual entrepreneur - manufacturer of GMO samples provided for the assessment; type of intended use of the GMO; registration number of the certificate of state registration of the GMO(s) intended for release into the environment, that are used for deriving the GMO(s) to be assessed (in case where the GMO is derived from another (other) GMO(s)); registration number of the certificate of state registration of the GMO for another intended use (if any), or information about the absence of such certificate;

b) information on the original recipient organism (taxonomic characteristic indicating the method of identification; source of strain isolation: substrate, geographical site, date of isolation; methods of identification of the strain, identified by (last name, first name, patronymic (if any)), reference to the determinants used);

c) description of the genetic construct structure (inserted or deleted) and its site; expression characteristics of the inserted or modified genes;

d) data on the transformation event presented as a code generated according to the All-Russian Classifier of Transformation Events;

e) information on the method of genetic modification (description of the modification method, vector structure, insert structure);

f) information on the place of deposition and collection number of the GMO strain, passport of the GMO strain (for the deposited GMO strains), or the following information (if information on the place of deposition, collection number of the GMO strain, passport of the GMO strain is not provided):

on the cultural and morphological, physiological and biochemical (enzymatic), antigenic, biological properties and genetic features of the GMO strain;

on the cultivation conditions: names of nutrient media, pH of the medium, cultivation temperature and duration, shelf life and frequency of re-inoculation of the GMO strain culture in native form;

on the used method and storage conditions of the GMO strain: in case of lyophilization, the duration of cultivation on the nutrient medium (culture age), composition of the protective medium, cell suspension titer, drying mode, storage temperature, and the shelf life are specified; in case of cryopreservation, the duration of cultivation on the nutrient medium (culture age), composition of the protective environment, titer of the cell suspension, freezing rate (deg/min), storage temperature, and the shelf life are specified;

on the dissociation of culture depending on the storage method (description of the morphological types of colonies on a specific medium with detailed description of the type, preserving useful or diagnostic trait);

on the medium used by the applicant for providing the GMO strain;

on the quantity, date of preparation and shelf life of the GMO strain samples;

g) information on the inserted genes (for donor organisms the following information is given: taxonomic status, data on virulent, allergenic and pathogenic properties);

h) description of properties acquired by the GMO as a result of modification;

i) characteristics of GMO differences from the original recipient organism, including description of the ways of reproduction, spread, virulence, cultivation techniques, new phenotypic properties, and the GMO biological advantages vs. the original organism;

j) description of techniques for confirming the GMO taxonomic status and genetic modification characteristic, describing nucleotide sequences, used primers, probes, composition and properties of the reference samples;

k) the results of the GMO stability evaluation, e.g. in animals (rats or mice) and the assessment of their capability to transfer genes inserted in the original recipient organism by genetic engineering techniques, including genes responsible for antibiotic resistance, to other organisms;

l) information on the potential GMO colonization in the gastrointestinal tract of animals and its effect on the natural microflora;

m) information on the registration of GMO, animal feed and feed additives derived from viable GMO which is a microorganism or which contains such organism in member states of the Eurasian Economic Union, other states, as well as on the presence or absence of facts proving the adverse effects of the application of the GMO, animal feed and feed additives produced with the use of viable GMO which is a microorganism or which contains such organism;

n) copies of the reports on the GMO molecular genetic study results;

o) data received from the specialized databases, such as bioinformatics analysis, search for homology of the recombinant protein with the amino acid sequences of toxic proteins, and proteins possessing pharmacological or another biological activity;

p) information on the GMO commercial cultivation conditions;

q) study protocols (see p. 8 of these Guidelines) conducted in the institutions (testing laboratories) accredited with the national accreditation system with the accreditation scope corresponding to the studies indicated in these Guidelines (if available).

8. With respect to GMO intended for the production of animal feed and feed additives derived from viable GMO which is a microorganism or which contains such organism, the following studies should be carried out in comparison with the original recipient organism:

a) toxicological studies in laboratory animals;

b) GMO allergenicity studies in laboratory animals;

c) GMO virulence studies in laboratory animals;

d) studies of microbiological properties, susceptibility to antibiotics and bacteriophages, hemolytic activity in animal erythrocytes (for GMO bacteria);

e) studies to evaluate stability of the properties of GMO bacteria, protozoa, fungi;

f) study of the GMO viability in the environmental media, including but not limited to wet wood chips, cotton wool tampons, and after the exposure to heat treatment;

g) GMO invasiveness study using the corneal and conjunctival test;

h) study of the GMO antagonistic activity with the use of the resident intestinal microflora species;

i) GMO immunological studies in laboratory animals;

9. If the applicant does not have study protocols mentioned in p. 7, item c), of these Guidelines, the applicant should provide samples of the GMO and original recipient organism. The required studies of these samples will be conducted by the institution (testing laboratory) that conducts the assessment.

10. If the GMO complies with the safety indicators (conditions) specified in p. 19 of these Guidelines, the applicant should be given a report stating that the GMO has no adverse environmental impact. The report on the absence of adverse environmental impact may establish specific conditions for using the GMO.

III. Assessment of the GMO used for the production of animal feed and feed additives derived from non-viable GMO which is a microorganism or contains such organism

11. Assessment of the GMO used for the production of animal feed and feed additives derived from non-viable GMO which is a microorganism or contains such organism includes the review of documents and data provided by the applicant, envisaged in p. 7, items a) – k), n), o), p) of these Guidelines, as well as:

a) information on the registration of GMO, animal feed and feed additives derived from non-viable GMO which is a microorganism or contains such organism in member states of the Eurasian Economic Union, other states, as well as on the presence or absence of facts proving the adverse effects of the consumption of animal feed and feed additives produced with the use of non-viable GMO which is a microorganism or which contains such organism;

b) protocols of the studies (see p.12 of these Guidelines) conducted by an institution (testing laboratory) accredited with the national accreditation system in accordance with the scope of accreditation corresponding to the studies indicated in these Guidelines (if available).

12. With respect to GMO intended for the production of animal feed and feed additives derived with the use of non-viable GMO which is a microorganism or which contains such organism, the following studies of samples of animal feed and/or feed additives derived from non-viable GMO which is a microorganism or which contains such organism, samples of viable GMO should be carried out in comparison with the original recipient organism:

a) GMO allergenicity studies in laboratory animals;

b) studies to prove the absence of viable GMO in the provided samples of animal feed and feed additives, using microbiological techniques;

c) toxicological studies in laboratory animals;

d) immunological studies in laboratory animals;

13. If the applicant does not have study protocols mentioned in p. 11, item b), of these Guidelines, the applicant should provide samples of the feed and/or feed additives, samples of the viable GMO and samples of the original recipient organism. Studies of these samples will be conducted by the institution (testing laboratory) that conducts the assessment.

14. If the GMO complies with the safety indicators (conditions) specified in p. 20 of these Guidelines, the applicant should be issued a report stating that the GMO has no adverse environmental impact. The report on the absence of adverse environmental impact may establish specific conditions for using the GMO.

IV. Assessment of the GMO used for the production of animal feed and feed additives derived with the application of GMO of plant or animal origin or containing such organism

15. The assessment of GMO for the production of animal feed and feed additives derived from or containing GMO of plant or animal origin should include the review of the documents and data submitted by the applicant that are specified in p. 7, items a) – e), g), h), i), j), n) – p), of these Guidelines, as well as:

a) information on the registration of GMO, animal feed and feed additives derived with the use of GMO of plant or animal origin or containing such organism, in member states of the Eurasian Economic Union, other states, or on the absence of such registration, as well as on the presence or absence of the facts proving the adverse effects from the application of the animal feed and feed additives derived with the use of GMO of plant or animal origin or containing such organism;

b) the results of the evaluation of recombinant protein stability during the processing, storage, reprocessing; effect of the temperature and pH, potential modifications and/or the formation of stable protein fragments as a result of exposure to different factors; protein resistance to the treatment with proteolytic enzymes in experiment;

c) the results of protein acute oral toxicity testing in experimental animals (mice or rats);

d) study protocols (see p. 16 of these Guidelines) conducted in the institutions (testing laboratories) accredited with the national accreditation system with the accreditation scope corresponding to the studies indicated in these Guidelines (if available).

16. With respect to the GMO intended for the production of animal feed and feed additives derived with the use of GMO of plant or animal origin or containing such organism, the following studies of GMO samples should be carried out in comparison with the original recipient organism:

a) assessment of the compositional equivalence of the GMO and the original recipient organism;

b) GMO toxicological studies, including reproductive toxicity, in laboratory animals

c) GMO immunological studies in laboratory animals;

d) GMO allergenicity studies in laboratory animals.

17. If the applicant does not have study protocols mentioned in p. 15, item d), of these Guidelines, the applicant should provide samples of the GMO and original recipient organism. The required studies of these samples will be carried out by the institution (testing laboratory) that makes the assessment.

18. If the GMO complies with the safety indicators (conditions) specified in p. 21 of these Guidelines, the applicant should be issued a report stating that the GMO has no adverse environmental impact. The report on the absence of adverse environmental impact may establish specific conditions for using the GMO.

V. Requirements for conducting studies and safety indicators (conditions) of GMO that is a microorganism

19. Requirements for conducting studies and safety indicators (conditions) of GMO for the production of animal feed and feed additives derived from viable GMO which is a microorganism or contains such organism:

a) Toxicological studies in laboratory animals should be conducted in rat or mouse males and females (depending on the animal species described by the applicant in accordance with p. 7 of these Guidelines) within 90 calendar days with a 1:1 gender ratio.

Animals should be divided into 2 equal groups: a control group and experimental group. The control group will receive a diet including the original recipient organism; the experimental group will receive a diet including the studied GMO. Both groups of

animals should be given the same diet (as regards its amount and formula), except for the inclusion of the studied GMO or the original recipient organism. Equal amounts of the studied GMO and the original recipient organism should be included in feed formula. Animals have free access to feed and water and are housed in the room equipped with heating system and microclimate control.

The following indicators of animal status should be evaluated:

GMO persistence and excretion from the animal body;

general condition of the animals: external appearance, motion activities, hair coat condition – every 2 calendar days, feed intake – every day; body weight – every 7 calendar days;

hematological parameters: hemoglobin concentration; hematocrit; total red blood cell count; mean corpuscular volume (MCV); mean cell hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); total platelet count; total white blood cell count; differential leukocyte count (neutrophils, lymphocytes, eosinophils, monocytes, basophils);

blood chemistry parameters: alanine aminotransferase (ALT); aspartate aminotransferase (AST); bile acids; alkaline phosphatase; total bilirubin; direct bilirubin; total protein; albumen; globulin; creatinine; glucose; alpha amylase; lipase; lactate dehydrogenase; total lipids; triglycerides; cholesterol; cholinesterase; urea; chlorides; sodium; phosphorus; potassium;

general urine analysis; color and transparency; specific gravity; pH; protein; glucose; creatinine;

The examination of hematological and blood chemistry parameters, persistence, and urine parameters should be carried out on the 90-th calendar day of the experiment in 50% of animals from each group. At the end of the recovery period (calendar day 10 from the last day of receiving the GMO-containing diet; calendar day 101 of the experiment) the same tests should be performed in the other 50% of animals from each group.

Feeding the GMO-containing diet should not cause clinical changes in the animals (parameters of blood and urine tests; general condition of the animals in the experimental group: the GMO persistence and excretion should not differ from the parameters in the control group); pathological changes should not be found at the necropsy;

b) GMO allergenicity studies in laboratory animals should be conducted on Wistar rats.

Animals should be divided into 2 equal groups: a control group and experimental group. The control group will receive a diet including the original recipient organism; the experimental group will receive a diet including the studied GMO. Both groups of animals should be given the same diet (as regards its amount and formula) except for inclusion of the studied GMO or the original recipient organism. Equal amounts of the studied GMO and the original recipient organism should be included in feed formula. Egg white should not be contained in the diet. Animals have free access to feed and water and are housed in the room equipped with heating system and microclimate control.

On calendar days 1, 3, and 5 of the experiment, an intraperitoneal injection of dietary antigen – chicken egg ovalbumin (hereinafter – OVA) is made. On day 21 of the experiment an additional dose of OVA is injected which is 10 times lower than the earlier injected doses. Feeding diets should be continued by the 29-th calendar day of the experiment. Then, OVA should be injected intravenously and after the injection the severity of developing anaphylactic reaction is evaluated for 24 hours according to the anaphylactic index. Immediately before the intravenous injection of the challenge dose, 0.1-0.2 ml of blood is taken from the tail vein to determine the level of specific antibodies to OVA using enzyme immunoassay technique.

The severity of response – anaphylactic shock in each of the animal groups should be evaluated by the anaphylactic index, taking into consideration the severity of anaphylactic response and the percentage of lethal anaphylactic reactions.

The following formula is used to calculate the anaphylactic index:

$$\frac{(N \times 4) + (N1 \times 3) + (N2 \times 2) + (N3 \times 1) + (N4 \times 0)}{N + N1 + N2 + N3 + N4},$$

where:

N – the number of animal deaths;

N1 – the number of animals that developed a severe anaphylactic shock (asphyxia, general seizures when animal loses ability to maintain balance while standing on the limbs, but does not die);

N2 – the number of animals that developed a moderate anaphylactic shock (seizures when animal does not loose ability to maintain balance while standing on the limbs, bronchial spasm);

N3 – the number of animals that developed a mild anaphylactic shock (anxiety, high respiratory rate, face scratching; involuntary urination and defecation, ruffled hair coat);

N4 – the number of animals that did not develop anaphylactic shock.

The anaphylactic index and percentage of lethal anaphylactic reactions in the experimental group should not be higher than those in the control group. The level of antibodies to OVA in the experimental group should not differ from the level of antibodies to OVA in the control group of animals;

c) GMO virulence studies in laboratory animals should be carried out in specific pathogen-free mice and chickens and include determining a lethal dose for 50% of laboratory animals (hereinafter – LD50). To determine LD50, an intraperitoneal injection of 0.1 cm3 of live daily culture of the studied strain at a concentration of 1x10 (9), 1x10 (7), 1x10 (5) and 1x10 (3) CFU/cm3 is performed. Each of the animal groups should be placed in individual cages, and for 10 calendar days the animals are monitored to record deaths. In control groups the animals receive intraperitoneal injections of the sterile saline solution or the virulent strain with determined LD50. At the end of the experiment, the number of deaths in each of the animal groups is calculated and LD50 is determined. If LD50 is equal to 5x10(8) CFU/cm3 or higher, the strain is considered avirulent. LD50 of the GMO strain should not be below LD50 of the original recipient organism;

d) Studies of microbiological properties, susceptibility to antibiotics and bacteriophages, hemolytic activity on animal erythrocytes should be carried out for GMO bacterial strains. To conduct experiments, it is necessary to have consumables (media, diagnostic panels, antibiotics, bacteriophages, etc.) and techniques in consistency with the information provided by the applicant according to p. 7 of these Guidelines. The assessment of GMO microbiological properties, susceptibility to antibiotics and bacteriophages, hemolytic activity should include the use of the original recipient organism as control. The experimental conditions should be the same for the original recipient organism and the GMO.

Microbiological properties, susceptibility to antibiotics and bacteriophages, hemolytic activity of GMO should not differ from properties of the original recipient organism in the portion not affected by genetic modification.

e) The stability of properties of GMO bacteria, protozoa, fungi should be evaluated by passaging the studied strains in liquid and solid nutrient media with testing phenotypical (biochemical) properties after 6 consecutive passages. The original GMO and the GMO after 6 passages should be tested using molecular genetic methods. Strains of GMO bacteria, protozoa, and fungi that have not changed phenotypical (biochemical) properties and maintained their genetic structure after 6 passages are considered stable. GMO bacteria, protozoa, and fungi should demonstrate stability of the phenotypical (biochemical) properties and genetic structure;

f) studies of GMO viability in the environmental objects, including but not limited to wet wood chips, cotton wool tampons, and after the heat treatment, should be conducted with the use of cultivation media, cell cultures or other test objects susceptible to the original recipient organism in consistency with the information provided by the applicant according to p. 7 of these Guidelines. Experiments shall include the use of the original recipient organism as control. The experimental conditions should be the same for the original recipient organism and the GMO. The GMO environmental viability should not be higher than that of the original recipient organism.

g) GMO invasiveness studies using corneal and conjunctival test are performed by introducing the GMO and original recipient organism (as control) into the corneal and conjunctival epithelial cells of laboratory animals to assess reproduction in these cells. The experimental conditions should be the same for the original recipient organism and the GMO. The GMO invasiveness should not be higher than that of the original recipient organism.

h) For assessing the GMO antagonistic activity, the original recipient organism and the test strains of microorganisms representing the main species of the resident intestinal microflora of recipient animals (gram-positive: obligate anaerobic bacteria – lactobacilli, bifidobacteria, peptostreptococci; gram-negative: obligate anaerobic bacteria, bacteroids, facultative anaerobic microorganisms, E.coli, staphylococci, streptococci, yeast-like fungi of the Candida genus) should be used as control.

The level of antagonistic activity against the resident intestinal microflora strains should be determined using the deferred-antagonism method on the solid medium by points of slow growth rates of the test strains. The GMO antagonistic activities should not affect the resident intestinal microflora species and should not be higher than the antagonistic activities of the original recipient organism.

i) GMO immunological studies should be conducted in CBA-strain mice that are highly susceptible to the sheep red blood cells and not susceptible to histamine and Salmonella typhimurium; and C57Bl/6-strain mice characterized by low susceptibility to the sheep red blood cells, susceptible to histamine and Salmonella typhimurium. Studies should include the assessment of immunomodulating and sensitizing properties in four tests: effect on the humoral immunity in the test for determining the level of hemagglutinins to sheep erythrocytes; effect on the cellular component of the immune system in the reaction of delayed-type hypersensitivity (DTH) to sheep erythrocytes; action as a sensitizing agent in the histamine sensitivity test; effect on the natural resistance of mice to Salmonella typhimurium. The original recipient organism should be used in experiments as control. The experimental conditions should be the same for the original

recipient organism and the GMO. The GMO safety should not be inferior to the safety of the original recipient organism.

20. Requirements for conducting studies and indicators (conditions) of the biological safety of GMO for the production of animal feed and feed additives derived from non-viable GMO which is a microorganism or contains such organism:

a) GMO allergenicity studies in laboratory animals should be conducted in Wistar rats according to the procedure established in p. 19, item b), of these Guidelines.

b) studies to prove the absence of viable GMO in the samples of animal feed and feed additives, should be conducted with the use of microbiological techniques. A live strain culture will be employed as control inoculations or passages.

c) toxicological studies in laboratory animals should be conducted according to p. 19, item a), of these Guidelines;

d) GMO immunological studies should be conducted according to p. 19, item i), of these Guidelines;

VI. Requirements for conducting studies and safety indicators (conditions) of GMO of plant or animal origin

21. Requirements for conducting studies and indicators (conditions) of the safety of GMO for the production of animal feed and feed additives derived from GMO of plant or animal origin or containing such organism:

a) evaluation of compositional equivalence of the GMO and the original recipient organism shall be carried out in order to eliminate the risk of decrease in the GMO biological value based on the results of comparison of the GMO chemical formulation with the chemical formulation of the original recipient organism by the following indicators:

protein content;

amino acid composition;

fat content;

fatty acid composition;

carbohydrate composition; -

content of vitamins;

content of macro-and micronutrients;

content of biologically active substances;

content of allergens;

content of anthropogenic and natural contaminants (toxic elements, mycotoxins, pesticides, radionuclides, harmful impurities);

content of anti-nutrients.

If the genetic modification is targeted at changing GMO chemical formulation, the GMO chemical formulation should conform to the data provided by the applicant in accordance with p. 15 of these Guidelines. If the genetic modification is not targeted at changing GMO chemical formulation, the GMO chemical formulation should not differ from chemical formulation of the original recipient organism;

b) GMO toxicological studies in laboratory animals for evaluating reproductive toxicity shall be carried out in two generations of Wistar rats during 180 days.

Toxicological studies shall include the investigation of reproductive function of F0 generation of rats; the investigation of embryotoxic and teratogenic effects recorded in the pre- and postnatal periods of development of F1 generation offspring, as well as the assessment of physiological and clinical chemical parameters of F0, F1 generations. The experiment is carried out in rat males and female rats (at the initial age of 25-30 days). Animals should be divided into 2 equal groups: a control group and experimental group. The control group shall receive a diet that includes the studied GMO. Both animal groups should receive the same diet (as regards its amount and formula), except for the inclusion of the studied GMO or the original recipient organism. The studied GMO or the original recipient organism should be included in feed formula in equal amounts. At the beginning of the experiment the number of animals should be 55 females and 25 males. The animals should have free access to feed and water and be housed in the room with heating system and microclimate control.

The following indicators of animal status should be evaluated:

general condition of the animals (external appearance, motion activities, hair coat condition) – every 2 calendar days, feed intake – every day; body weight – every 7 calendar days;

hematological parameters: hemoglobin concentration; hematocrit; total red blood cell count; mean corpuscular volume (MCV); mean cell hemoglobin (MCH); mean

corpuscular hemoglobin concentration (MCHC); total platelet count; total white blood cell count; differential leukocyte count (neutrophils, lymphocytes, eosinophils, monocytes, basophils);

blood chemistry parameters: alanine aminotransferase (ALT); aspartate aminotransferase (AST); bile acids; alkaline phosphatase; total bilirubin; direct bilirubin; total protein; albumen; globulin; creatinine; glucose; alpha amylase; lipase; lactate dehydrogenase; total lipids; triglycerides; cholesterol; cholinesterase; urea; chlorides; sodium; phosphorus; potassium;

general urine analysis; color and transparency; specific gravity; pH; protein; glucose; creatinine.

The examination of hematological and blood chemistry parameters, persistence and urine parameters should be carried out on the 90th calendar day of the experiment in 50% of animals from each group. At the end of the recovery period (calendar day 10 from the last day of receiving the GMO-containing diet; calendar day 101 of the experiment) the same tests should be performed in the other 50% of animals from each group.

The following studies should be carried out on calendar days 90 and 180 of the experiment (scheduled euthanasia of 15 rats per group): macro- and microscopic examinations, general-purpose histological examinations, morphometric analysis, weight measurement of the internal organs (brain, heart, spleen, lungs, thymus, pituitary gland, liver, kidneys, adrenal glands, uterus, ovaries, and testes). For those animals that died during the experiment the following studies should be carried out: morphological examinations of the skin, brain, heart, aorta, spleen, and lungs. In cases where the cause of death cannot be defined, it is necessary to conduct additional morphological examinations of the lymphatic nodes, thymus, thyroid gland, pituitary gland, stomach, large and small intestine, and liver.

Toxicological studies in laboratory animals, including reproductive toxicity in two generations of Wistar rats (F0 and F1) should be continued for the third generation (F2) and the fourth generation (F3) of the above mentioned laboratory animals in cases, if:

the study results demonstrate that differences between the experimental and control groups are found in the range between the experimental error and the twofold error (statistically significant difference) for the studied indicators;

differences between the experimental and control groups are found for the following indicators: pigmentation, behavioral deviations, appearance of non-typical physiological, anatomical, clinical chemistry signs);

in the course of the assessment (study), new data are found in scientific literature about adverse environmental effects of the studied GMO.

Feeding the GMO-containing diet should not cause changes of clinical signs in the animals (parameters of blood and urine tests; general condition of the animals: the GMO persistence and excretion should not differ from the parameters in the control group); pathological changes should not be found at the necropsy;

c) GMO immunological studies should be conducted according to the procedure established in p. 19, item i), of these Guidelines;

d) GMO allergenicity studies in laboratory animals should be conducted on Wistar rats according to p. 19, item b), of these Guidelines.

END UNOFFICIAL TRANSLATION.

Attachments:

No Attachments.