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Draft Regulations for GE Feeds and Other Products

Report Categories:
Biotechnology - GE Plants and Animals

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Report Highlights:
The Russian Ministry of Agriculture drafted nine regulatory documents for safety assessment and testing of GE ingredients for feeds, feed additives, veterinary pharmaceuticals, GE animals, GE microorganisms and also regulatory document that outlined the functions of the Federal Service for Veterinary and Phytosanitary Surveillance (VPSS) at the Ministry of Agriculture in these assessments and testing. These documents, as stated, were drafted in accordance with the Government Resolution No. 839 of September 23, 2013 that required Ministry of Agriculture to develop mechanism for registration of feeds. The Resolution No. 839 is scheduled to come to force on July 1, 2017. All drafts were posted on the portal of the draft government documents (www.regulation.gov.ru). These documents, if adopted, will have great impact on the development of Russian agricultural biotechnology and on trade in agricultural products and veterinary pharmaceuticals. However, these draft documents were not notified to WTO. The GAIN report contains unofficial translations of these nine draft documents.

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Background information

The list of all drafts with reference to the government site where these drafts have been posted (in Russian) is the following:

1. Draft 55324 “The Procedure for Monitoring by the Federal Service for Veterinary and Phytosanitary Surveillance of the Effects on Humans and the Environment of GE Organisms and Products Obtained Using Such Organisms or Containing Such Organisms and for Control over the Release of Such Organisms into the Environment: http://regulation.gov.ru/projects#npa=55324
2. Draft 64520 “Procedure for Assessing the Biological Safety of GE Organisms of Plant Origin for the Production of Feeds”: http://regulation.gov.ru/projects#npa=64520
3. Draft 64512 “Procedure for Carrying out Molecular Genetic Studies of GE Organisms of Plant Origin for the Production of Feeds”: http://regulation.gov.ru/projects#npa=64512
4. Draft 64522 “Procedure for Assessing the Biological Safety of the Use of GE Organisms for the Production of Feed Additives”: http://regulation.gov.ru/projects#npa=64522
5. Draft 64513 “Procedure for Carrying out Molecular Genetic Studies of GE organisms for the Production of Feed Additives”: http://regulation.gov.ru/projects#npa=64513
6. Draft 64525 “Procedure for Assessing the Biological Safety of GE Organisms for the Production of Veterinary Pharmaceuticals”: http://regulation.gov.ru/projects#npa=64525

7. Draft 64521 “Procedure for Carrying out Molecular Genetic Studies of GE Organisms for the Production of Veterinary Pharmaceuticals”: http://regulation.gov.ru/projects#npa=64521
8. Draft 63060 “Procedure for Risk Assessment and Risk Management when Using (Releasing) GE Animals in Open Systems”: http://regulation.gov.ru/projects#npa=63060
9. Draft 63139 “Procedure for the Examination and Registration of GE Microorganisms Intended for Use in Technologies Necessary for the Development of Agriculture” http://regulation.gov.ru/projects#npa=63139

The present GAIN report contains unofficial translation of these nine draft documents (Annexes 1-9).

(Note 1: All Russian legislative and regulatory documents use the term “GMO” (genetically modified organisms) or “GMM” (genetically modified microorganisms) instead of genetically engineered (GE) organisms/microorganisms. Therefore, throughout the unofficial translation of nine documents the terms are used as in the original text.
Note 2. The format of the translated texts and the numbers of paragraphs are as in the Russian original text)

Annex 1. Draft 55324 - Government Resolution Pertaining to Control Functions of the VPSS over GE organisms

GOR Resolution

Draft

GOVERNMENT OF THE RUSSIAN FEDERATION
RESOLUTION
Dated___ 2017, No. ___
MOSCOW

On approval of the rules on monitoring of the effects of genetically-engineered-modified organisms and products derived from such organisms or containing such organisms on humans and the environment and control over the release of such organisms into the environment to be conducted by the Federal Service for Veterinary and Phytosanitary Surveillance

In accordance with Article 7 of Federal Law dated July 05, 1996, No. 86-FZ “On the state regulation in the sphere of genetic engineering activities,” the Government of the Russian Federation hereby **resolves**:

1. To approve the attached Rules for monitoring of the effects of genetically-engineered-modified organisms and products derived from such organisms or containing such organisms on humans and the environment and control over the release of such organisms into the environment to be conducted by the Federal Service for Veterinary and Phytosanitary Surveillance.
2. The authorities envisaged in this Resolution shall be exercised by the Federal Service for Veterinary and Phytosanitary Surveillance within the limits established by the RF Government on the numbers of employees in the headquarters and local bodies of the above Service, as well as the budget appropriations provided to it in the federal budget for supervision and management in the sphere of

designated functions.

Chairman of the Government of the
Russian Federation D. Medvedev

Rules for VPSS for monitoring GE organisms and products

Approved by Resolution of the Government of the Russian Federation

Dated ____No.

Rules for monitoring of the effects of genetically-engineered-modified organisms and products derived from such organisms or containing such organisms on humans and the environment and control over the release of such organisms into the environment to be conducted by the Federal Service for Veterinary and Phytosanitary Surveillance

1. These Rules establish a procedure of monitoring of the effects of genetically-engineered-modified plants and animals intended for breeding and growing on the territory of the Russian Federation; genetically-engineered-modified microorganisms for agricultural applications; genetically-engineered-modified organisms used for the production of feeds and feed additives for animals; genetically-engineered-modified organisms used for the production of veterinary drugs (hereinafter - genetically-engineered-modified organisms); grains, animal feeds and feed additives derived with the use of genetically-engineered-modified organisms or containing such organisms (hereinafter – products, monitoring, respectively), as well as control over the release of such organisms into the environment to be conducted by the Federal Service for Veterinary and Phytosanitary Surveillance.

Monitoring of the veterinary drugs derived with the use of genetically-engineered-modified organisms or containing such organisms is conducted within the framework of monitoring of the efficacy and safety of veterinary drugs in accordance with Federal Law dated April 12, 2010, No. 61-FZ "On the circulation of medicines" (Collected Acts of the Russian Federation, 2010, No. 16, Article 1815; No. 31, Article 4161; No. 42, Article 5293; No. 49, Article 6409; 2011, No. 50, Article 7351; 2012, No. 26, Article 3446; No. 53, Article 7587; 2013, No. 27, Article 3477; No. 48, Article 6165; 2014, No. 11, Article 1098; No. 43, Article 5797; No. 52, Article 7540; 2015, No. 10, Article 1404; No. 27, Article 3951; No. 29, Articles 4359, 4367, 4388; № 51, Article 7245; 2016, No. 1, Article 9; No. 23, Article 3287; No. 27, Articles 4194, 4238, 4283).

2. Monitoring includes the following actions:

a) to collect information on the effects of genetically-engineered-modified organisms and/or products on human health and the environment;

b) to process and review information on the effects of genetically-engineered-modified organisms and/or products on human health and the environment;

c) to provide public outreach of information on the effects of genetically-engineered-modified organisms and/or products on human health and the environment.

3. Monitoring shall be conducted by the Federal Service for Veterinary and Phytosanitary Surveillance (hereinafter – the Rosselkhoznadzor) within its established competence.

4. To conduct monitoring the following data are used:

a) data on the state registration of genetically-engineered-modified organisms and/or products;

b) data of the foreign states and international organizations on genetically-engineered-modified organisms and/or products;

c) research findings on the impact of genetically-engineered-modified organisms and/or products on humans and the environment;

d) information of the legal entities performing genetically engineered activities.

5. Based on the information specified in item 4 of these Rules, in order to confirm or deny the statements contained in this information, the Rosselkhoznadzor will request data from a legal entity performing genetically engineered activities or manufacturing (shipment) of products on the territory of the Russian Federation, who holds the issued Certificate on the state registration of genetically-engineered-modified organism intended for the release into the environment, or the Certificate on the state registration of products (hereinafter – the Certificates) in accordance with Article 10 of Federal Law dated July 05, 1996, No. 86-FZ "On the state regulation in the sphere of genetic engineering activities," (Collected Acts of the Russian Federation, 1996, No. 28, Article 3348; 2000, No. 29, Article 3005; No. 1, Article 21; 2010, No. 41, Article 5191; 2011, No. 30, Article 4596; 2016, No. 15, Article 2066; No. 27, Article 4291).

6. In case where the results of monitoring conducted by the Rosselkhoznadzor have demonstrated the negative impact of genetically-engineered-modified organisms and/or products on humans and/or the environment, a decision can be made:

a) to cancel the Certificates issued by the Rosselkhoznadzor;

b) to make amendments to the Certificates as regards the establishing of special conditions for the use of modified organisms and/or products;

c) to submit to the Government of the Russian Federation a proposal on banning the import of genetically-engineered-modified organisms and/or products to the territory of the Russian Federation.

7. The Rosselkhoznadzor will post on its official website in the "Internet," the information on the results of conducted monitoring and forward it to the Ministry of Health of the Russian Federation for its entry into the Consolidated state register of genetically-engineered-modified organisms and products derived from such organisms or containing such organisms.

8. Control over the release of genetically-engineered-modified organisms into the environment is aimed at identifying and restraining violations from actions or inaction that have resulted in entering genetically-engineered-modified organisms into the environment, as well as eliminating effects of the identified violations.

9. Relations associated with the exercise of control over the release of genetically-engineered-modified organisms into the environment; making arrangements for and performing inspections of legal entities and individual entrepreneurs; and, conducting monitoring are subject to the provisions of Federal Law dated December 26, 2008, No. 294-FZ "On the Protection of the Rights of Legal Entities and Individual Entrepreneurs during the Exercise of State Control (Supervision) and Municipal Control" (Collected Acts of the Russian Federation, 2008, No. 52, Article 6249; 2009, No. 18, Article 2140; No. 29, Article 3601; No. 52, Article 6441; 2010, No. 17, Article 1988; No. 18, Article 2142; No. 31, Articles 4160, 4193, 4196; No. 32, Article 4298; 2011, No. 1, Article 20; No. 17, Article 2310; No. 23, Article 3263;

No. 27, Article 3880; No. 30, Article 4590; No. 48, Article 6728; 2012, No. 19, Article 2281; No. 26, Article 3446; No. 31, Articles 4320, 4322; No. 47, Article 6402; 2013, No. 9, Article 874; No. 27, Article 3477; No. 30, Article 4041; No. 44, Article 5633; No. 48, Article 6165; No. 49, Article 6338; No. 52, Articles 6961, 6979, 6981; 2014, No. 11, Articles 1092, 1098; No. 26, Article 3366; No. 30, Articles 4220, 4235, 4243, 4256; No. 42, Article 5615; No. 48, Article 6659; 2015, No. 1, Articles 53, 64, 72, 85; No. 14, Article 2022; No. 18, Article 2614; No. 27, Article 3950; No. 29, Articles 4339, 4362, 4372, 4389; No. 48, Article 6707; 2016, No. 11, Article 1495; No. 18, Article 2503; No. 27, Articles 4160, 4164, 4187, 4210, 4287; No. 50, Article 6975).

Annex 2. Draft 64520 - Procedure for Biological Safety Assessment of GE Organisms of Plant Origin for Production of Feeds.

Draft

Procedure for Biological Safety Assessment of Genetically Modified Organisms of Plant Origin for Production of Feeds

Introduction

The procedure was developed in accordance with the resolution of the Government of the Russian Federation # 839 dated September 23, 2013 "On the state registration of genetically modified organisms intended for release into the environment as well as products derived with the use of such organisms or containing such organisms" with respect to the safety assessment of GMOs of plant origin for the purpose of feed production.

GM plants are widely used in agriculture, namely as animal and poultry feed components. One of the main objectives of plant selection is to improve quality and quantity of synthesized proteins, fats, polysaccharides and other substances that determine nutritional values of plants. Gene engineering methods used in developing new varieties allow to insert beneficial traits into genomes, without linkage to negative traits, and to create plants resistant to environmental stress, herbicides, insects, and diseases of different etiologies.

Biological safety assessment of GMOs used for feed production shall be carried out to identify GMO potential harmful impact on animal health and the severity of such impact. The assessment shall include comparison of the modified plant with its conventional analogue that has a history of safe use. Common features and differences shall be taken into consideration. During the assessment, it is necessary to establish safety of every effect resulting from genetic modifications; safety of new proteins resulting from genetic modifications (toxicity, allergenicity, etc.); compositional equivalence, and no reduction in biological value of feed containing GMO.

During the risk assessment of GMO use, it is necessary to take into consideration its potential threat to the environment, and as such to evaluate the likelihood of gene transfer into related and/or other wild plant species; potential changes in susceptibility of plants to diseases or pests.

1. General provisions and scope of application

1.1 The present procedure establishes requirements to the biological safety assessment of GMOs of plant origin for feed production, *including feed containing GMOs*.

1.2. Requirements set forth in this procedure must be implemented during the state registration of GM lines of plant origin and feed containing GMOs of plant origin, as well as monitoring and random quality control sampling of products designed for veterinary applications containing or manufactured with the use of GMOs.

1.3. The procedure was developed to establish a common scientifically based GMO safety assessment system, and takes into account new methodological approaches either developed in Russia or recommended by the international organizations (WHO, FAO, OIE, etc.)

2. Expert analysis and evaluation of information about GMO of plant origin for feed production.

General characteristics of GMO of plant origin and feed containing GMO involve the review of documentation submitted by the applicant including:

2.1. Description of properties the organism obtained through modification;

- description of gene construct structure (inserted or removed) and its localization, characteristics of inserted or modified gene expression,

- assessment of GMO differences from its parent organism including description of its propagation and dissemination modes, incubation protocols, and new phenotypic characteristics.

- GMO genetic and phenotypic stability profile specifying differences from its parent organism. It is necessary to provide information about the analysis of several generations of GMO, evaluation of genes ability to transfer to other organisms (plants, microorganisms);

- characteristics of inserted gene expression in the process of plant ontogenesis, levels of expression in structural components of the plant, and variations from its parent organism;

2.2. Information shall be reviewed about cultivation techniques to grow this GMO crop, modes of reproduction, ability to cross-pollinate, stress resistance, etc., specifying differences from the parent organism.

2.3. Information about the original parent organism (taxonomic identification, propagation and dissemination modes; data on toxicity, allergenicity and other adverse properties).

2.4 Information about donor organisms of inserted genes (taxonomic identification, toxicity or allergenicity, history of use, etc.).

2.5. Information about genetic modification method (description of modification method, vector structure, and insert structure).

2.6. Information about GMO genetic safety and stability study.

2.7. Evaluation of compositional equivalence shall be based on the information submitted by the applicant, about comparison of GMO chemical composition with chemical composition of its conventional analogue against the following parameters: protein content; amino acid composition; fat content; fatty acid profile; carbohydrate composition; vitamin content; macro- and micronutrient content; bioactive substance content; allergen content; levels of anthropogenic and naturally occurring contaminants (toxic elements, mycotoxins, pesticides, radionuclides, harmful impurities, etc.); antinutrients and other substances characteristic of the plant species.

The checklist shall vary depending on properties of the plant organism under examination. Evaluation of compositional equivalence of the GMO and its conventional analogue shall take into consideration biological variations in values specific to the plant species.

2.8. Toxicology test results shall be reviewed based on the information submitted by the applicant including:

- safety assessment of one or several proteins that define the development of desirable traits in the GMO

(protein molecular and biochemical characteristics; presence or absence of homology with toxins of protein nature or proteins with pharmacological or other biological activities (when using PIR, EMBL, SwissProt, GenBank or other data bases); protein stability studies during treatment, storage, technological processing; temperature and pH impact, possible modifications and/or formation of stable protein fragments triggered by various factors; protein resistance to proteolytic enzymes during *in vitro* experiments; protein acute oral toxicity study on rodents; etc.);

- safety assessment of native product: 90 days worth of data from a rodent trial; studies of young fast growing animals (broiler chicks, lambs, etc.), if such studies have taken place;

- other toxicological test results.

2.9. Allergenicity test results shall be reviewed based on the information submitted by the applicant, including:

- allergenic properties of one or several proteins that define the development of desirable trait in the GMO (comparison with known allergens using databases containing information on 3D structure and functions of known allergens and related proteins); determination of protein potential allergenicity via *in vitro* immunochemical analysis using IgE isolated from serum of patients suffering from allergies; determination of resistance to proteolytic enzymes (pepsin); screening tests with serum of patients suffering from allergies; additional tests (including *in vivo*);

- allergenicity tests of native product must be performed if there is information available regarding the donor organism suggesting that it has allergic properties (comparison of allergen sets of the test GMO and its conventional analogue, etc.).

2.10. Impurity content of GMO crops and feed containing GMO must not exceed maximum permissible levels effective in the Russian Federation.

2.11. Analysis of other test results (if such tests have been performed) shall be carried out based on the information provided by the applicant, including: determination of nutritional value (as the effects of both desirable and undesirable genetic modifications can alter macro- and micronutrient balance and therefore cause changes in the product nutritive value); outcomes of the latest analytical methods such as industry-specific technologies, etc.

2.12. Post-registration monitoring data on GMO and feed containing it from the applicant country and other countries shall be reviewed to identify any undesirable genetic modification effects, which could not have been found during the registration studies.

3. A minimum list of tests for registration of GMOs of plant origin for feed production required for the biological safety assessment.

3.1. Expert evaluation of methods of GMO detection, identification and quantitative determination, verification of presence of declared foreign genes in GMO genome, tests for potential undeclared genetic modifications, and GMO genetic stability study.

3.2. GMO hygienic studies including determination of quality and safety parameters. Test parameters: levels of toxic substances, mycotoxins, pesticides, radionuclides, harmful impurities; microbiological parameters.

3.3. Evaluation of compositional equivalence of GMOs of plant origin and their conventional analogues to eliminate the risk that their biological full value was reduced. The list of quality parameters for testing shall be defined based on properties of the relevant plant organism and review of materials submitted by

the applicant. Provided that there may be biological variations in parameters typical of the plant species, feed values of new and conventional products shall be compared (protein content, amino acid profile, fat content, fatty acid content, carbohydrate composition, vitamin content, macro- and micronutrient content; bioactive substance content; antinutrients, allergens; levels of anthropogenic and naturally occurring contaminants (toxic elements, mycotoxins, pesticides, radionuclides, harmful impurities) and other substances characteristic of the plant species); intake levels; applications; bioavailability and digestibility; impact on intestinal microflora.

3.4. Toxicology studies of GMOs of plant origin for reproductive toxicity with 5 generations of lab animals. Wistar rats are used in the experiment (males, 40-50 days old, baseline BW is 70-80 g). Animals are divided into 2 groups, the control receives a diet supplemented with a conventional analogue of the test GMO; the experimental group receives a diet supplemented with the test GMO. Number of animals per group in the beginning of trial is a minimum of 50 rats per group. The test GMO and its conventional analogue are included in the feed formulation in the maximum possible quantity not to disturb the balance of macronutrients. Animals get access to feed and water *ad libitum* and are kept in heated and ventilated premises.

The following parameters shall be analyzed: general health status (appearance, mobility, coat quality) – every 2 days; feed intake – daily, body weight – every 7 days. Internal organ weight (brain, heart, spleen, lungs, thymus, pituitary, liver, kidneys, adrenals, testes) shall be evaluated on days 30 and 180 of the experiment.

10 animals per group shall be sampled for hematological, biochemical and morphological tests on days 30 and 180 of the experiment.

The following parameters shall be analyzed:

- blood hematology: hemoglobin concentration; hematocrit; total RBC count; mean cell volume (MCV); mean cell hemoglobin (MCH); mean cell hemoglobin concentration (MCHC); total thrombocyte count; total leukocyte count; differential white blood cell count (neutrophils, lymphocytes, eosinophils, monocytes, basophils);
- blood biochemistry: alanine aminotransferase (ALT); aspartate aminotransferase (AST); bile acids; alkaline phosphatase; total bilirubin; direct bilirubin; total protein; albumin; globulin; creatinin; glucose; alpha amylase; lipase; lactate dehydrogenase; total lipids; triglycerides; cholesterol; cholinesterase; urea; chlorides; sodium; phosphorus; potassium;
- common urine analysis; tests parameters: daily urine; color and transparency; relative density; pH; protein; glucose; creatinin;
- antioxidant defense biomarkers (test material: erythrocytes); test parameters: antioxidant defense enzyme activity: glutathione reductase; glutathione peroxidase; superoxide dismutase; catalase;
- antioxidant defense biomarkers (test material: blood, liver); test parameters: levels of lipid peroxidation products: malondialdehyde;
- xenobiotic metabolism enzyme system (test material: liver); test parameters: enzyme activity of xenobiotic metabolism at phase 1 and 2; total cytochrome P-450 content; 7-ethoxyresorufine-O-deethylase; 7-pentoxyresorufine-O-deethylase; UDP-glucuronosyl transferase; glutathione transferase;
- apoptosis regulation system, lysosome membrane stability (test material: liver); test parameters: total and unsedimentary lysosome enzyme activity, galactosidase, glucoronidase, arylsulfatase A

and B.

Morphological tests shall be performed on skin, brain, heart, aorta, spleen, lungs. Macroscopic and microscopic tests, general histological, morphometric studies shall be performed on days 30 and 180 of the experiment (scheduled sampling). Microscopic (electron microscopic), histochemical; immuno-histochemical tests shall be performed on pancreatic gland; kidneys; testes.

Animals perished in the course of trial (unscheduled sampling) shall be additionally tested for morphology of lymph nodes, thymus, thyroid gland; pituitary; stomach, small and large intestines; and liver to establish the cause of death.

Parameters characterizing the generative function shall be evaluated:

- testicular morphology (index of spermatogenesis, average number of unimpaired spermatogones in each tubule, relative number of tubules with 12 stage meiosis);

- ovarian morphology (primordial follicles, follicles with two or more follicular cell layers, tertiary follicles, interstitial glands, yellow bodies, total number of generative forms).

Parameters characterizing offspring pre-natal development:

- a minimum of 7 pregnant females per group must be slaughtered and autopsied;

- visual examination of uterus, placenta, fetuses; identification of live and dead fetuses, yellow body count, implantation sites, number of resorptions on left or right horn (followed by calculating pre- and post-implantation mortality rates) on 19-20 days of pregnancy;

- Analysis of fetal material (at least 5 fetuses per rat)

Parameters characterizing offspring post-natal development:

- Birth control on 20-22 days of pregnancy;

- Records of litter size on day of birth, number of live and dead infant rats, number of species of different sex, identification of external deformities, body weights, craniocaudal size on day 1;

- Records of physiological development of baby rats: pinna detachment age, first lanugo hair, incisor teething, eyes opening, testes dropping, vaginal opening; offspring survival rates on days 1-30;

- Body weights and heights of baby rats on days 1, 4, 7, 14, 21 and 25.

GMO reproductive toxicity study report must include digital data in spreadsheets containing basic information required to make conclusions whether or not the test GMO can have an adverse impact on embryofetal development and reproduction.

3.5. Immunology testing of GMO of plant origin and its conventional analogue using lab animals.

Test trials shall be done with CBA line rats (having high sensitivity to erythrocytes and insensitivity to histamine and *Salmonella typhimurium*) and C57Bl/6 (having low sensitivity to sheep erythrocytes and insensitivity to histamine and *Salmonella typhimurium*). Immune modulating and sensitizing properties shall be determined in four tests:

- effect on humoral component of immune system (assessment of hemagglutinin levels to sheep erythrocytes);
- effect on cellular component of immune system in delayed-type hypersensitivity test (DTH) to sheep erythrocytes;

- effect as a sensitizing agent in histamine sensitivity test;
- effect on natural resistance of mice to *Salmonella typhimurium*.

3.6. Allergenicity studies of GMO of plant origin using lab animals.

Potential allergenicity shall be estimated by severity of generalized anaphylaxis and blood levels of sensitizing antibodies (subclasses IgG + IgG) in mice receiving the test GMO in diets (experimental group) and its conventional analogue. Method is based on qualitative comparison of severity of generalized anaphylaxis caused by intraperitoneal (i/p) sensitization of adult rats by food antigen — chicken egg albumin (CEA), followed by intravenous (i/v) introduction of the recall dose of same protein to the sensitized animal.

Wistar rats (adult males, baseline weight of 150–180 g) shall be used in the experiment. Animals shall be divided into two groups; control group receives a diet with conventional equivalent of the test GMO; experimental group receives a diet with the test GMO. A minimum of 25 animals must be included in each group at the beginning of trial. Animals are fed with standard vivarium diets throughout the experiment. The test GMO and its conventional equivalent are incorparated in feed in the maximum possible quantity not to disturb the balance of main nutrients. Levels of proteins, fats, and carbohydrates in the added product must be considered when substituting the diet ingredients: number of calories should not be changed. Nutritional and biological value of the diet should fully satisfy the animals physiological requirements. The diet must be free of egg protein. Animals have free access to food and water and are kept in heated and ventilated premises.

Rats are sensitized with CEA i/p on days 1, 3, and 5. Additional ("booster") dose of antigen that is 10 times smaller than the initial dose is injected on day 21. Rats are fed with the diets until morning of day 29 when they receive CEA i/v. Severity of the anaphylactic reaction is assessed during the next 24 hours by a number of deaths, total number of seizure reactions, and anaphylactic index value. 0.1–0.2 ml of blood is taken from tail veins just before injection of the recall dose to determine specific antibodies levels. Levels of circulating specific antibodies to CEA are estimated by enzyme immunoassay.

Severity of anaphylactic shock in each animal group shall be estimated by anaphylactic index that shows severity of anaphylactic reaction, and by percent of fatal anaphylactic reactions.

Based on expert evaluation of documents and materials submitted, molecular genetic test results and biological risk assessment of use, conclusion statement shall be issued on whether the product can be used for production of feed or feed additives on the territory of the Russian Federation.

Annex 3. Draft 64512 - Procedure for Molecular Genetic Testing of GE Organisms of Plant Origin for Production of Feeds

Draft

Procedure for molecular genetic testing of genetically modified organisms of plant origin for production of feeds

The procedure was developed in accordance with the resolution of the Government of the Russian Federation # 839 dated September 23, 2013 "On the state registration of genetically modified organisms intended for release into the environment as well as products derived with the use of such organisms or containing such organisms" with respect to molecular genetic testing during safety assessment of GMO of plant origin for feed production.

1. General provisions and scope of application

1.1. The present procedure establishes methods of identification and quantification of GMO of plant origin in feed, as well as methods to examine the impact of genetic modification on functionalities of GM plant genome.

1.2. The procedure is designed for state registration of GM lines of plant origin and feed containing GM lines of plant origin, as well as monitoring and random quality control of products for veterinary applications containing or manufactured with the use of GMO.

1.3. The procedure is developed to establish a common scientifically based GMO safety assessment system, and takes into account new methodological approaches either developed in Russia or recommended by international organizations (WHO, FAO, etc.)

2. Expert analysis and evaluation of information about declared genetically modified organisms

2.1. General characteristics of GMOs of plant origin and feed containing GMOs involve the review of documentation submitted by the applicant including:

- description of properties the organism obtained through modification;
- description of the genetic construct structure (inserted or removed) and its localization site, characteristics of inserted or modified gene expression,
- assessment of GMO differences from the parent organism including propagation and dissemination modes, incubation protocols, and new phenotypic characteristics.
- GMO genetic and phenotypic stability characteristics specifying differences from the parent organism. It is required to provide information obtained through testing several generations of GMOs, and the assessment of genes ability to transfer to other organisms (plants, microorganisms);
- characteristics of inserted gene expression during plant ontogenesis, expression levels in the plant structural components, and differences from the parent organism.

2.2. Description of procedures that allow to identify the genetic modification (transformation event) and the taxonomic status of the organism (species, variety). In addition, test protocols, nucleotide sequences of primers used, reference materials of compositions and properties shall be reviewed.

2.3. Information about the original parent organism (taxonomic identification, propagation and dissemination modes; information about toxicity, allergenicity and other adverse properties).

2.4. Information about donor organisms of inserted genes (taxonomic identification, toxicity or allergenicity, history of use, etc.)

2.5 Information about the genetic modification method (description of modification method, vector structure, and insert structure).

2.6. Information about GMO genetic safety and stability study.

2.7. Post-registration monitoring data on the GMO and feed containing it from the applicant country and other countries shall be reviewed to identify any undesirable genetic modification effects, which could not have been found during the registration studies.

3. Molecular genetic testing of GMO of plant origin for feed production.

Molecular genetic expert analysis shall include verification of information submitted by the applicant, a test for potential undeclared genetic modifications of the GMO crop, and also an evaluation of the

impact of genetic modifications on functionalities of GM plant genome.

3.1. The applicant shall submit reference materials of compositions and properties including samples of the GMO crop and its conventional analogue in the amount required to carry out comprehensive tests.

3.2. PCR-based procedures for detection, identification and quantification of GMOs in the feed submitted by the applicant shall be tested (validated), sensitivity and specificity shall be evaluated.

Test protocols presented by the applicant shall include the description of PCR primers and the thermal cycling mode.

Oligonucleotides used as PCR primers shall be synthesized in accordance with their nucleotide sequence provided.

Special equipment required for PCR-based diagnostics is described in ISO 20837:2006 and ISO20838:2006. Test consists of sequential processes: sample preparations, DNA extraction from a product sample, DNA amplification, detection of amplification products, analysis and interpretation of results.

DNA extraction from samples of plant origin shall be performed by CETAB or sorption methods in accordance with GOST R 52173. For the sorption method, it is recommended to use a commercially available DNA extractor kit in accordance with technical requirements stipulated by the manufacturer.

For PCR test, it is advisable to use heat-stable DNA polymerase, mixture of deoxyribonucleotide triphosphate, and tenfold buffers. Depending on the method, amplification products can be detected by electrophoretic separation of PCR products in agarose gel based on whether or not specific bands of the amplified DNA are visible on the electrophoregram or by recording the fluorescence signal accumulation in the course of PCR with hybridization fluorescent detection method.

PCR procedures must have 100% specificity (absence of cross-reactions with non-specific DNAs in concentrations up to $1x10^6$ genomic equivalents/ml) and high specificity (no less than $1x10^4$ genomic equivalents/ml).

The analysis period: no longer than 30 days.

3.3. Transgenes declared in the GMO genome shall be confirmed, their localization site and regulatory and/or marker vector sequences shall be established. GMO analysis for the presence of modifications declared shall be performed based on information provided by the applicant containing the description of molecular structure of gene constructs (nucleotide sequence, localization inside a recipient genome) with the use of validated identification methods (see para. 3.2) submitted by the applicant/manufacturer or obtained from reputable sources.

For detection of DNA regulatory elements in the declared genetic construct, validated PCR methods (see para. 3.2) or methods described in ISO/FDIS 21569:2005 standards shall be used. Examples for PCR primer pairs are given in Table 1.

For detection of DNA of the most common regulatory elements, p-CaMV35S, p-FMV promoters and t-NOS terminator, ready-made PCR kits shall be used.

Table 1. PCR primers for detection of regulatory elements of genetically modified constructs of GMOs of plant origin

Element	Soybean, corn and rapeseed	5' — 3' primer and probe sequences

	GM lines containing this element	
p-CaMV35S promoter	GTS 40-3-2, A2704-12, A5547-127, BT 176, BT11, MON810, GA21, MON88017, NK603, MON863, T25, T45, etc.	PCR with fluorescent hybridization detection: CGTCTTCAAAGCAAGTGGATTG TCTTGCGAAGGATAGTGGGATT FAM-TCTCCACTGACGTAAGGGATGACGCA-TAMRA PCR with electrophoretic detection GCTCCTACAAATGCCATCA GATAGTGGGATTGTGCGTCA Product PCR size is 195 base pairs
t-NOS terminator	GTS 40-3-2, MON863, MON531, MON757, MON1076, MON1445, MON1698, MON15985, BT11, GA21, NK603, MON802, 3272, MIR604, MON88017, etc.	PCR with fluorescent hybridization detection: CATgTAATgCATgACgTTATTTATg TTgTTTTCTATCgCgTATTAAATgT (YY)–ATgggTTTTTATgATTAgAgTCCCgCAA–(BHQ1) PCR with electrophoretic detection GAATCCTGTTGCCGGTCTTG TTATCCTAGTTTGCGCGCTA Product PCR size is 180 base pairs
p-FMV promoter	MON89788, MON89034, GT73, etc.	AAGCCTCAACAAGGTCAG CTGCTCGATGTTGACAAG Product PCR size is 196 base pairs
p-SSuAra promoter	MS1, RF1, RF2, MS8xRF3, etc.	PCR with fluorescent hybridization detection: GGCCTAAGGAGAGGTGTTGAGA CTCATAGATAACGATAAGATTCATGGAATT FAM-CCTTATCGGCTTGAACCGCTGGAATAA-TAMRA
p-TA29 promoter	MS1, RF1, RF2, MS8xRF3, etc.	PCR with fluorescent hybridization detection: GAAGCTGTGCTAGAGAAGATGTTTATTC GCTCGAAGTATGCACATTTAGCAA FAM-AGTCCAGCCACCCACCTTATGCAAGTC-TAMRA
p-NOS promoter	MS1, RF1, RF2, Topas 19-2, etc.	PCR with fluorescent hybridization detection: GTGACCTTAGGCGACTTTTGAAC CGCGGGTTTCTGGAGTTTAA FAM-CGCAATAATGGTTTCTGACGTATGTGCTTAGC-TAMRA
t-E9 terminator	MON89788, etc.	PCR with fluorescent hybridization detection: TGAGAATGAACAAAAGGACCATATCA TTTTTATTCGGTTTTCGCTATCG

		FAM- TCATTAACTCTTCTCCATCCATTTCCATTTCACAGT- TAMRA
t-35S terminator	BT 176, T14, T25, 3272, A2704-12, A5547-127, T45, Topas19/2, etc.	PCR with electrophoretic detection GAAACCCTTAGTATGTATTTGTATTTGTAAAATACTTC TTTTAGTACTGGATTTTGGTTTTAGGAATTAG
t-OCS terminator	MS1, RF1, RF2, etc.	PCR with fluorescent hybridization detection: CGGTCAAACCTAAAAGACTGATTACA CGCTCGGTGTCGTAGATACT FAM-TCTTATTCAAATTTCAAAAGTGCCCCAGGG- TAMRA
t-g7 terminator	MS1, RF1, RF2, MS8xRF, etc.	PCR with fluorescent hybridization detection: ATGCAAGTTTAAATTCAGAAATATTTCAA R ATGTATTACACATAATATCGCACTCAGTCT FAM- ACTGATTATATCAGCTGGTACATTGCCGTAGATGA- TAMRA

Data submitted by the applicant and/or validated procedures from reputable sources shall be used to identify and confirm the GM lines (transformation event) (see para. 3.2.). Test protocol submitted by the applicant shall include the description of PCR primers and the thermal cycling mode.

Several primers suggested for PCR identification of several GMO lines are given in Table 2.

Table 2. Primers for detection of several corn, soybean and rapeseed GM lines in accordance with procedures from reputable scientific sources.

Target DNA	5' — 3' primer and probe sequences
Corn line DAS-40278-9	CACGAACCATTGAGTTACAATC FAM-CGTAGCTAACCTTCATTGTATTCCG-TAMRA TGGTTCATTGTATTCTGGCTTTG
Corn line MON87460	CACGTTGAAGGAAAATGGATTG FAM- AGGGAGTATGTAGATAAATTTTCAAAGCGTTAGACGGC- TAMRA TCGCGATCCTCCTCAAAGAC
Corn line Bt11	GCGGAACCCCTATTTGTTTA FAM-AAATACATTCAAATATGTATCCGCTCA-TAMRA TCCAAGAATCCCTCCATGAG
Corn line GA21	CTTATCGTTATGCTATTTGCAACTTTAGA FAM-CATATACTAACTCATATCTCTTTCTCAACAGCAGGTGGGT- TAMRA TGGCTCGCGATCCTCCT

Corn line NK603	ATGAATGACCTCGAGTAAGCTTGTTAA FAM-TGGTACCACGCGACACACTTCCACTC-TAMRA AAGAGATAACAGGATCCACTCAAACACT
Corn line MON863	TGTTACGGCCTAAATGCTGAACT FAM-TGAACACCCATCCGAACAAGTAGGGTCA-TAMRA GTAGGATCGGAAAGCTTGGTAC
Corn line TC1507	TAGTCTTCGGCCAGAATGG FAM-TAACTCAAGGCCCTCACTCCG-TAMRA CTTTGCCAAGATCAAGCG
Corn line T25	ACAAGCGTGTCGTGCTCCAC FAM-TCATTGAGTCGTTCCGCCATTGTCG-TAMRA GACATGATACTCCTTCCACCG
Corn line 59122	GGGATAAGCAAGTAAAAGCGCTC FAM-TTTAAACTGAAGGCGGGAAACGACAA-TAMRA CCTTAATTCTCCGCTCATGATCAG
Corn line MIR604	GCGCACGCAATTCAACAG FAM-AGGCGGGAAACGACAATCTGATCATG-TAMRA GGTCATAACGTGACTCCCTTAATTCT
Corn line MON88017	GAGCAGGACCTGCAGAAGCT FAM-TCCCGCCTTCAGTTTAAACAGAGTCGGGT-TAMRA TCCGGAGTTGACCATCCA
Corn line LY038	TGGGTTCAGTCTGCGAATGTT FAM-CGAGCGGAGTTTATGGGTCGACGG-TAMRA AGGAATTCGATATCAAGCTTATCGA
Corn line MON89034	TTCTCCATATTGACCATCATACTCATT FAM-ATCCCCGGAAATTATGTT-MGBNFQ CGGTATCTATAATACCGTGGTTTTTAAA
Corn line 3272	TCATCAGACCAGATTCTCTTTTATGG FAM-ACTGCTGACGCGGCCAAACACTG-TAMRA CGTTTCCCGCCTTCAGTTTA
Corn line MON810	TCGAAGGACGAAGGACTCTAACGT FAM-AACATCCTTTGCCATTGCCCAGC-TAMRA GCCACCTTCCTTTTCCACTATCTT
Corn line 98140	GTGTGTATGTCTCTTTGCTTGGTCTT FAM-CTCTATCGATCCCCCTCTTTGATAGTTTAAACT-TAMRA GATTGTCGTTTCCCGCCTTC
Corn line MIR162	GCGCGGTGTCATCTATGTTACTAG FAM-TCTAGACAATTCAGTACATTAAAAACGTCCGCCA- TAMRA TGCCTTATCTGTTGCCTTCAGA
Corn LY038	TGGGTTCAGTCTGCGAATGTT AGGAATTCGATATCAAGCTTATCGA

	FAM-CGAGCGGAGTTTATGGGTCGACGG-TAMRA
Corn line Bt176	GGCCGTGAACGAGCTGTT FAM-AGCAACCAGATCGGCCGACACC-TAMRA GGGAAGAAGCCTACATGTTTTCTAA
Soybean line FG72	AGATTTGATCGGGCTGCAGG FAM-AATGTGGTTCATCCGTCTT-MGBNFQ GCACGTATTGATGACCGCATTA
Soybean line MON87769	CATACTCATTGCTGATCCATGTAGATT FAM-CCCGGACATGAAGCCATTTACAATTGAC-TAMRA GCAAGTTGCTCGTGAAGTTTTG-3’
Soybean line MON 87705	TTCCCGGACATGAAGCCATTTAC FAM-AAGAGACTCAGGGTGTTGTTATCACTGCGG- TAMRA ACAACGGTGCCTTGGCCCAAAG-3’
Soybean line A2704-12	GCAAAAAAGCGGTTAGCTCCT FAM-CGGTCCTCCGATCGCCCTTCC-TAMRA ATTCAGGCTGCGCAACTGTT
Soybean line GTS-40-3-2	TTCATTCAAAATAAGATCATACATACAGGTT FAM-CCTTTTCCATTTGGG-MGB-NFQ GGCATTTGTAGGAGCCACCTT
Soybean line MON89788	TCCCGCTCTAGCGCTTCAAT-3’ FAM-CTGAAGGCGGGAAACGACAATCTG-TAMRA TCGAGCAGGACCTGCAGAA
Soybean line A5547-127	GCTATTTGGTGGCATTTTTCCA FAM-CCGCAATGTCATACCGTCATCGTTGT-TAMRA CACTGCGGCCAACTTACTTCT
Soybean line 305423	CGTGTTCTCTTTTTGGCTAGC FAM-TGACACAAATGATTTTCATACAAAAGTCGAGA-TAMRA GTGACCAATGAATACATAACACAAACTA
Soybean line DP-356043-5	GTCGAATAGGCTAGGTTTACGAAAAA FAM-CTCTAGAGATCCGTCAACATGGTGGAGCAC-TAMRA TTTGATATTCTTGGAGTAGACGAGAGTGT
Soybean line MON87701	TGGTGATATGAAGATACATGCTTAGCAT 6-FAM-TCAGTGTTTGACACACACACTAAGCGTGCC- TAMRA CGTTTCCCGCCTTCAGTTTAAA
Soybean line CV127	5’-AACAGAAGTTTCCGTTGAGCTTTAAGAC 6-FAM-TTTGGGGAAGCTGTCCCATGCCC-TAMRA 5’-CATTCGTAGCTCGGATCGTGTAC
Rapeseed line T45	CA ATGGACACATGA ATTATGC GACTCTGTATGAACTGTTCGC FAM-TAGAGGACCTAACAGAACTCGCCGT-TAMRA

Rapeseed line Ms8	GTTAGAAAAAGTAAACAATTAATATAGCCGG- GGAGGGTGTTTTTGGTTATC FAM-AATATAATCGACGGATCCCCGGGAATTC-TAMRA
Rapeseed line Rf3	CATAAAGGAAGATGGAGACTTGAG 5’-AGCATTTAGCATGTACCATCAGACA FAM-CGCACGCTTATCGACCATAAGCCCA-TAMRA’
Rapeseed line GT73	5’-CCATATTGACCATCATACTCATTGCT 5’-GCTTATACGAAGGCAAGAAAAGGA FAM-TTCCCGGACATGAAGATCATCCTCCTT-TAMRA
Rapeseed line Ms1	ACGCTGCGGACATCTACATT CTAGATCGGAAGCTGAAGATGG FAM-CTCATTGCTGATCCACCTAGCCGACTT-TAMRA
Rapeseed line RF1	CTAAGGGAGGTCAAGATGTAGC CGGGCCTAACTTTTGGTGTG FAM-CTCATCATCCTCACCCAGTCAGCATCA-TAMRA
Rapeseed line RF2	GGGTGAGACAATATATCGACG GGGCATCGCACCGGTGAG’ FAM-CACCGGCCAAATTCGCTCTTAGCCGT-TAMRA
Rapeseed. Topas 19/2	GTTGCGGTTCTGTCAGTTCC CGACCGGCGCTGATATATGA FAM-TCCCGCGTCATCGGCGG-TAMRA

Test samples shall be observed for regulatory elements declared for the GMO line of plant origin and the declared transformation event.

The analysis period: no longer than 45 days.

3.4. Test samples shall be screened to check for undeclared GM lines of plant origin:
- regulatory and/or marker sequences inserted into the plant genome during genetic modifications
- target transgenes or other elements most frequently inserted to obtain desirable traits.

GMO regulatory elements shall be detected in accordance with para. 3.3. (Table 1).

Examples of PCR-primer pairs designed to identify target transgenes most frequently inserted to obtain desirable traits are given in Table 3. Primer pairs can help in identifying the key genes characteristic of GM crops: genes pat and bar promoting resistance to phosphinothricin (ammonium glufosinate), gene cp4 epsp promoting resistance to glyphosate, and gene cryIA(b) promoting resistance to corn worm.

Table 3. PCR primers for detection of target transgenes most commonly inserted into the plant genome to obtain desirable traits.

Target gene	GMO	PCR primers/probes, 5' 3'

gene pat phosphinothricin-N-acetyltransferase *Streptomyces viridochromogenes*	Corn lines 3272, Bt10, Bt11, GA21, MIR604, 4114, 5307, TC1507, MIR162, 59122, MON810, NK603, MON88017, 676, 678, 680, 98140, DAS40278, T25, and their hybrids.	TTGAGGGTGTTGTGGCTGGTA FAM-CTTCCAGGGCCCAGCGTAAGCA-TAMRA TGTCCAATCGTAAGCGTTCCT
gene bar phosphinothricin-N-acetyltransferase *Streptomyces hygroscopicus*	Corn lines Bt176, CBH-351, DBT418, DLL25, MS3, MS6, TC6275, soybean lines W62, W98, etc.	ACAAGCACGGTCAACTTCC GAGGTCGTCCGTCCACTC FAM-TACCGAGCCGC AGGA ACC-TAMR A
gene *cp4 epsp* enolpyruvylshikimate-3-phosphate synthase from CP4 strain *Agrobacterium tumefaciens*	Corn lines MON802, MON809,MON81, NK603, soybean lines MON87708, MON87769, wheat lines MON87769, etc.	GGGATGACGTTAATTGGCTCTG GGCTGCTTGCACCGTGAAG FAM-CACGCCGTGGAAACAGAAGACATGACCTAMRA
Synthetic gene cryIA(b) of toxin protein *Bacillus thuringiensis*	Corn lines BT-176, MON802, MON809, MON810	CCCATCGACATCAGCCTGAGC FAM-ATGTCCACCAGGCCCAGCACG-TAMRA CAGGAAGGCGTCCCACTGGC

GMO soybean line CV127 does not contain regulatory sequences (35S, NOS and FMV) or common transgenes. Therefore, PCR with primers for a transgenic event shall be used to detect this line during screening tests (Table 2).

The analysis period: no longer than 45 days.

3.5. If no registered GMO lines were detected in samples with the GMO crop during screening tests while at the same time CaMV 35S promoter was found (and no other transgenic sequences identified), such samples shall be tested for DNA of *Cauliflower mosaic virus* (CamV) that affects tissues of Brassicaceae plants (rape, horseradish, cabbage, radish, mustard, garden cress, turnip, etc.). A ready-made kit for detection of the virus DNA can be used.

The analysis period: no longer than 10 days.

3.6. If any undeclared GM lines are detected, identification shall be carried out in accordance with para. 3.3. (Table 2) using validated procedures from reputable sources.

The analysis period: no longer than 30 days.

3.7. Whenever necessary, PCR test results shall be reconfirmed through determination of nucleotide sequence by Sanger sequencing method using terminating dideoxynucleoside triphosphates. For the sequencing, it is recommended to use systems based on the above principle that ensures reliable determination of DNA nucleotide sequence up to 700-800 bp long per one read.

The analysis period: no longer than 30 days.

4. Molecular genetic testing of feed containing GMOs of plant origin.

Molecular genetic expert analysis shall include verification of data submitted by the applicant and a test for potential undeclared GMO components in the feed.

4.1. The applicant shall submit reference materials of compositions and properties including feed samples containing GMOs to carry out comprehensive tests.

4.2. Presence of declared transgenes in the GMO genome shall be verified, their localization sites shall be established, regulatory and/or marker vector sequences shall be identified (pursuant to para. 3.3).

Regulatory elements declared in the GMO line of plant origin and the declared transformation event must be detected in the test sample.

The analysis period: no longer than 45 days.

4.3. Screening tests shall be carried out to identify the undeclared GM lines of plant origin in the test sample (pursuant to para. 3.4):

The analysis period: no longer than 45 days.

4.4. If no registered GM lines were detected in feed samples during screening tests while at the same time CaMV 35S promoter was found (and no other transgenic sequences identified), such samples shall be tested in accordance with para. 3.5.

The analysis period: no longer than 10 days.

4.5. The plant DNA shall be identified in feed using species-specific primers in accordance with validated methods approved for testing.

PCR primers for DNA detection in soya lectin gene (le1) using electrophoretic detection method ISO/FDIS 21570:2005.

5' GCC CTC TAC TCC ACC CCC ATC C 3'

5' GCC CAT CTG CAA GCC TTT TTG TG 3';

Product PCR size is 118 bp.

PCR primers for DNA detection in corn invertase gene (ivr1) using electrophoretic detection method ISO/FDIS 21570:2005.

5’-CCGCTGTATCACAAGGGCTGGTACC-3’

5’-GGAGCCCGTGTAGAGCATGACGATC-3’

Product PCR size is 225 bp.

For Corn DNA detection, primers in zein gene region can be also used, PCR with electrophoretic detection method.

5’-AGTGCGACCCATATTCCAG-3’

5’-GACATTGTGGCATCATCATTT-3’

Product PCR size is 277 bp.

Validated method (ISO/FDIS 21570:2005) for DNA detection in Corn alcohol dehydrogenase gene 1 (adh1) with fluorescent hybridization detection method

5’-CGTCGTTTCCCATCTCTTCCTCC-3

5’-CCACTCCGAGACCCTCAGTC-3’

5’-(FAM)-AATCAGGGCTCATTTTCTCGCTCCTCA-(TAMRA)-3’

For rapeseed DNA detection, it is recommended to use species-specific primers in cruA gene region, cruciferin reserve protein.

5'-GGCCAGGGTTTCCGTGAT-3’

5’-CCGTCGTTGTAGAACCATTGG-3’

5’-(VIC)-AGTCCTTATGTGCTCCACTTTCTGGTGCA-(TAMRA)-3'

Available kits can be used to detect the presence of Corn DNA and soybean DNA in feed or feed additives.

The analysis period: no longer than 10 days.

4.6. If undeclared GMO is detected in feed samples, GM lines shall be identified in accordance with para. 3.3. (Table 2) using validated methods from reputable sources.

The analysis period: no longer than 30 days.

4.7. For the quantification of GMOs of plant origin, procedures shall be used based on calculations of the percentage of the amount of DNA of a specific GM line relative to the total amount of DNA of the test crop. Simultaneously, two independent tests shall be performed in one test tube. One test allows to detect DNA of the test crop (soybean, Corn, etc.). The other test allows to detect a sequence specific to a particular GM plant line. Behavior of the reactions can be detected via two specific fluorescent labeled probes. One probe can be used in the DNA detection test to identify DNA in the test crop (soybean, Corn), and the other can be used to detect a genetic insertion (GM line).

Determination of GMO percentages shall be done using calibration samples representing DNA blends of a conventional variety (0% GMO) and DNA of a GM line (100% GMO) in certain percentages. Difference in threshold cycle values of two reactions with calibration samples shall be used to build a calibration line. Calibration line is used to calculate GMO DNA percentages in feed test samples.

GMO quantification in feed of plant origin includes the following steps: DNA extraction from the test sample, real-time PCR, data analysis using the device software, calculations and processing of results using the appropriate software programs.

For the quantification of GM soybean and Corn transformation events registered in the RF, it is recommended to use procedures presented by the GMO applicants/ manufactures, validated methods

from reputable scientific sources or ready-made PCR kits.

Standard certified samples containing between 0.1 and 5% of various GM Corn and soybean lines in the conventional crop variety must be obtained from official sources.

The analysis period: no longer than 30 days.

In the event that test results are contrary or not in compliance with specifications declared by the applicant/manufacturer, double arbitration retests shall be carried out.

Annex 4. Draft 64522 - Procedure for Assessing the Biological Safety of GE Organisms of Plant Origin for Production of Feed Additives

Draft

Procedure for assessing the biological safety of using genetically-engineered modified organisms for the production of feed additives

Introduction

This procedure was developed in accordance with Resolution of the Government of the Russian Federation # 839 dated September 23, 2013 "On the State Registration of Genetically-Engineered-Modified Organisms Intended for Release into the Environment as well as Products Derived from the Use of Such Organisms or Containing Such Organisms"; it applies to the assessment of the safety of using feed additives containing Genetically-Engineered-Modified Organisms or derived from the use of such organisms.

Feed additives are products of plant, animal, microbiological, mineral and synthetic origin or their mixes intended for including in feed formula or animal rations for purposes of providing physiological value, disease prevention (except medications), promotion of animal growth and productivity (except medications), preservation of ingredients, increase in nutrient availability, improvement of palatability and technological properties of the animal feed.

In accordance with the above definition, feed additives may contain GMO (Genetically Modified Organisms) of both plant and microbial origin. The use of GMM (Genetically Modified Microorganisms) in the production and application of feed additives should ensure the biosafety both for the animals and environment.

To assess the biosafety of feed additives derived from GMM based on the risk for the animals and environment the classification into the following categories is used:

Category 1: products containing live/viable microorganisms (fermentative cultures, *lactobacillus spp.*, etc.)

Category 2: products containing non-viable/inactivated GMM (heat-treated).

Category 3: products containing individual ingredients or additives synthesized by GMM (enzymes, vitamins, amino acids).

Category 4: products containing ingredients processed with GMM-synthesized enzymes, etc.

Potentially the most safe products are those where GMM are used only at the production stage as producer microorganisms (Categories 3 and 4). In this case, criterion for assessing the risks from the application of feed additives is the assurance that no viable GMM cells or recombinant DNA are present in feed additive, and that the substances derived from producer GMMs have no toxic properties.

For products belonging to Category 2, criterion for assessing the risks from the application of feed additives is the assurance that there are no viable (capable for self-reproduction) GMM cells and that there is no ability to transfer genetic information to other organisms.

For Category 1, the risk assessment criteria are the same as those for Category 2, and additional consideration is given to such factors as antagonistic and synergistic effects on the naturally occurring microorganisms; GMM impact on the animal health, potential colonization in the gastro-intestinal system and influence on the natural microflora; and the potential transfer of antibiotic-resistance genes.

Potential impact on the ecosystem

The application of plant-origin GMO in feed additives is also associated with certain risks, requiring mandatory assessment in the biosafety studies:

- the presence and the direct action of toxic and allergenic transgenic proteins of GMO,

- the pleiotropic action of transgenic proteins on the plant metabolism.

- the potential accumulation of herbicides and their metabolites in resistant varieties and species of crops.

- risks of the horizontal transfer of transgenic constructs, primarily into the genome of the symbiotic bacteria of humans and animals (*E. coli, Lactobacillus spp. (acidophillus, bifidus, bulgaricus, caucasicus), Streptococcus thermophilus, Bifidobacterium,* etc.).

1. General provisions and scope of application

1.1. This procedure establishes the requirements for conducting tests to assess the biological safety of feed additives containing GMO of plant origin and feed additives containing GMM or derived from GMM, as well as to evaluate their safety. 1.2. The requirements described in this procedure are mandatory for the process of the state registration of feed additives imported into the Russian Federation, as well as for the monitoring and random control of the quality of products for veterinary applications containing GMO or products manufactured with their use.

1.3. The procedure has been developed for the purpose of establishing a unified scientifically justified system for the assessment of GMO safety; and it is based on the latest guidelines either created in Russia or recommended by international organizations (WHO, FAO, OIE, etc.).

2. Expert analysis and assessment of the data characterizing genetically modified organisms for the production of feed additives

General characteristics of GMO and feed additives containing/derived from GMO involve the review of documentation submitted by the applicant including:

2.1 – Description of the properties obtained by the organism through modification;

- description of the gene construct structure (inserted or removed) and its localization site, characteristics of the inserted or modified gene expression,

- the characteristics of the GMO variations from the parental organism including the reproduction and dissemination mechanisms, cultivation techniques, and new phenotypic characteristics.

- characteristics of GMO genetic and phenotypic stability specifying variations from the parental organism. It is required to provide the data acquired through testing several generations of GMO, and the capability to transfer genes to other organisms (plants, microorganisms);

- for plant-origin GMO: characteristics of the inserted gene expression in the process of plant ontogenesis, the rate of expression in the structural plant components, and variations from the parental organism.

2.2. The description of procedures that enable to identify the genetic modification (transformation event for GMO of plant origin) and taxonomic status of the organism (species, variety for plants); in particular, the testing protocols, the description of nucleotide sequences of the primers used, the reference samples of the formulations and properties shall be reviewed.

2.3. For feed additives containing plant-origin GMO the following information is reviewed: cultivation techniques of the GMO crops; reproduction mechanisms; and capability to induce cross pollination and stress resistance. For feed additives containing GMM the information regarding the conditions of industrial cultivation of the GMM strains is reviewed, and conformity of the production conditions to the sanitary regulation requirements is assessed. For feed additives derived with the use of producer GMM, a process flowchart of the industrial production of substances is reviewed, including detailed description of the purification methods.

2.4. Information about the initial parental organism (taxonomic characteristics, the description of reproduction and dissemination mechanisms; data on toxic, allergenic and other adverse properties).

2.5. Information about the donor organisms of the inserted genes (taxonomic characteristics, data on toxicity and allergenicity, history of use, etc.)

2.6 Information about the genetic modification technique (description of the modification technique, the vector structure, and the insert structure).

2.7. Information about the studies of the GMO genetic safety and stability.

2.8. In assessing the risk associated with the use of GMM-derived feed additives of Categories 3 and 4, a safety criterion is the assurance that there are no viable GMM cells or recombinant DNA in the products.

In assessing the risk associated with the use of GMM-derived feed additives of Category 2, important safety criteria include the absence of viable GMM cells capable to have self-reproduction, as well as the absence of ability to transfer genetic information to other organisms.

For feed additives containing GMM of Categories 1 and 2 the ability to transfer genetic information to other organisms is reviewed. The data on recombinant characteristics and location (chromosome, plasmid or another mobile genetic element) are reviewed; consideration is given to the environmental media (gastrointestinal system of animals, manure, soil, water, air, etc.) where the recombinant DNA may enter; the recombinant DNA stability under the relevant environmental conditions; the presence of microorganisms – potential recombinant DNA recipients through the horizontal transfer.

For feed additives of Category 1 the following factors are also reviewed:

- competitive advantages of the GMM to which recombinant genes were transferred;
- potential antagonistic, synergetic or other effects on the naturally occurring microorganisms;
- potential impact of the GMM with transferred recombinant genes on human, animal and plant health;
- potential colonization in the gastrointestinal microflora and impact on its natural microflora;
- ability to transfer the antibiotic resistance gene in the gastrointestinal microflora; potential impact on the ecosystem.

Potential hazard posed by GMM entering the environment is estimated by the GMM ability to survive and reproduce in the environment, the GMM interaction with the surrounding biotic and abiotic systems, including the naturally occurring microorganisms, animals and plants.

2.9. As regards harmful impurities, their concentration in feed additives should not exceed the maximum allowed norms established in the Russian Federation.

2.10. Analysis of the allergenicity study results shall be based on the data provided by the applicant, including:

- the results of the assessment of allergenic properties of one or more proteins determining the expression of intended indicators in the GMO (comparison with known allergens using the databases that include information on 3D (three-dimensional) structure and functions of the earlier characterized allergens and the related proteins);
- estimation of the protein allergenicity in immunochemical studies *in vitro*;
- estimation of resistance to the exposure to proteolytic enzymes (pepsin);

2.11. Analysis of the toxicology study results shall be based on the data provided by the applicant, including:

- the safety assessment results for one or more proteins determining the expression of intended indicators in the GMO (molecular and biochemical protein characteristics; the presence/absence of homology with the toxins of protein origin, as well as with the proteins possessing pharmacological or other biological activities (in cases where such databases as PIR, EMBL, SwissProt, GenBank, etc. are used); the investigation of protein stability through its treatment, storage and technical processing; temperature and pH effects, 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 the experiments *in vitro*; studies of the acute oral toxicity of protein in experimental rodents, etc.).
- the safety assessment results for the native product (90-day studies in rodents; the results of studies in young rapidly growing animals (broiler chickens, lambs, etc.) – in cases where such studies were carried out;
- the results of other toxicology studies.

2.12. For feed additives belonging to Categories 3 and 4, the analysis of potential toxicity of the non-protein substances derived as a result of the GMM expression implies that the risks should be assessed on a case-by-case basis, depending on the substance concentration and biological function. The investigation covers metabolism, toxicokinetics, chronic toxicity, carcinogenicity, effects on the reproductive function, and teratogenicity.

2.13. For feed additives belonging to Categories 1 and 2, the results of toxicology studies are reviewed using the following parameters: toxicokinetics; genotoxicity; potential allergenicity; the results of subchronic (90 days) toxicology experiments on the laboratory animals, and the animal productivity studies.

2.14. The results of other studies (if any) are reviewed on the basis of data provided by the applicant, including: the results of the evaluation of nutritional value (since intended and non-intended effects of the genetic modification may change the balance of macro- and micro-nutrients and, as a result, modify the product nutritional value); the results obtained through use of the latest analytical methods, such as dedicated technologies, etc.

2.15. The review covers the post-registration monitoring data on feed additives derived from a particular GMO, available in the applicant's country and other countries to identify any non-intended genetic modification effects that could not be detected at the stage of registration studies.

3. Testing of the probiotic GMM strains for the production of feed additives

3.1. Reference samples of the formulation and properties (GMM strain and parent strain) in the amount sufficient for comprehensive testing shall be provided by the applicant.

3.2. The GMM safety assessment takes into consideration the results of molecular genetic studies carried out by experts, e.g. the verification that the declared foreign genes are present in the GMM genome; studies for detecting the presence of potential non-declared genetic modifications; the GMM genetic stability; the expression of recombinant genes; taxonomy studies, etc.

Duration of the studies: no more than 250 days.

3.3. The studies shall cover microbiological and biochemical characteristics of the GMM and its parent strain; the susceptibility to antibiotics and bacteriophages; hemolytic activity for human and animal erythrocytes. Properties of the GMM strain should not differ significantly from the parent non-modified strain properties, e.g. the resistance of the GMM strain to antibiotics or its hemolytic activity should not be higher than those of its parent strain.

The biochemical identification of the GMM and parent strains is performed using diagnostic panels produced by domestic and international manufacturers and allowed for use in the established procedure.

The stability of phenotypical properties is verified through the passage of studied strains in liquid and solid nutrient media followed by testing biochemical properties after 3-5 passages. The agreement of the results obtained for multiple culture passages (no less than 10 passages) demonstrates the stability of phenotypical characteristics of the GMM and parent strains. The variability of the results in a large number of reactions is considered as a proof of the instability of GMM phenotypical characteristics.

The selection of antibacterial agents to be included in the study is based on the data on the natural resistance or susceptibility of the individual GMM types; the data on the parent strain resistance; and, the clinical efficacy of antibiotics. The serial dilution and diffusion methods are used to evaluate susceptibility to antibiotics.

Duration of the studies: no more than 100 days.

3.4. The GMM and parent strain adhesiveness is determined in hemagglutination tests using human and animal (mouse, rat, rabbit, sheep, etc.) erythrocytes. The adhesiveness is evaluated for the GMM, parent strain and the reference (non-modified probiotic analogue) strain of the same species without D-mannose (mannose-sensitive adhesion). In cases where a significant hemagglutinating activity is determined, the adhesion is evaluated in the presence of D-mannose for the purpose of detecting mannose-sensitive or mannose-resistant adhesion.

The quantitative adhesion assay is performed *in vivo*, using biological models (developing chicken embryos, enterally challenged suckling mice, the small intestinal loop of rabbits). Based on adhesion grade, the strains are categorized as follows: a) highly-adhesive strains with the adhesion coefficient of not lower than 3.0; b) adhesive – from 1 to 3; and c) low-adhesive strains – 0.001-0.9. As a rule, the low-adhesive strains are avirulent, while the adhesive and highly-adhesive strains usually possess certain virulent properties. A GMM strain should have an adhesion coefficient not above 0.9 and it should be equal to or lower than the adhesion coefficient of the parent strain.

Duration of the studies: no more than 60 days.

3.5. The evaluation of pathogenic and virulent GMM properties in the laboratory animals – SPF-category mice and chickens. LD50 in the SPF-mice is determined by the method of intraperitoneal injections of live daily cultures of the studied strain in the concentrations of $1x10^{(9)}$, $1x10^{(7)}$, $1x10^{(5)}$ and $1x10^{(3)}$ CFU/cm^3; every injection dose is 0.1 cm^3. Each of the animal groups is placed in an individual cage and for 10 days the animal deaths are recorded. Control groups are composed of the animals injected intraperitoneally with sterile saline solution or virulent strain with the determined LD50 dose. Upon completion of the experiment, the dead animals are counted in each of the groups and LD50 is determined. If LD50 is equal to 5X108 CFU/cm or higher, the strain is recognized avirulent. The GMM safety should be not inferior to that of the parent non-modified strain.

Duration of the studies: no more than 30 days.

3.6. The evaluation of GMM invasiveness. The invasiveness is estimated using a keratoconjunctival test. This test is based on the concept that causative agent penetrates the conjunctival epithelial cells and the cornea, where its reproduction occurs, destroying the tissues. The GMM, parent and reference (non-modified probiotic analogue) strains of the same species should not cause lesions of the conjunctiva or cornea. In cases where strains demonstrate considerable invasive properties, a refusal decision is made, stating that the GMM cannot be used as a probiotic agent.

Duration of the studies: no more than 30 days.

3.7. The evaluation of the GMM antagonistic or symbiotic activity in relation to some of the residential microflora of the intestinal tract. Control strains used in the test include a non-modified analogue of the probiotic strain of the same species, a parent strain and microbial strains representing the major species of the residential microflora of the intestinal tract of recipient animals (birds) (Gram-positive obligate anaerobes – *Lactobacillus spp., Bifidobacterium spp., Peptostreptococcus spp*.; Gram-negative obligate anaerobic bacteria, bacteroids; facultative anaerobic microorganisms, *E. coli, Staphylococcus spp., Streptococcus spp.*, yeast-like fungi of the *Candida* genus, etc.). The certified museum strains are used.

From the control strains cultivated on solid or liquid nutrient media, suspensions of microorganisms with the preset concentrations are prepared in the presence of GMM (parent strain or non-modified analogue of the probiotic strain of the same species). Every strain in the amount of 0.5 cm^3 $10^{(1)}$, $10^{(3)}$ and $10^{(5)}$ CFU/cm^3 is placed in each of the test tubes containing 3.0 cm^3 of the liquid nutrient medium suitable for the growth of all studied microorganisms (trypticase-soy broth or L-broth). In parallel, the control strains are inoculated onto selective nutrient media using the same dilutions to ensure an accurate cell count for each of the microorganism species. Six and 24 hours later, the microorganism mixtures are inoculated onto selective nutrient media and the number of cells of the residential microflora representative and the GMM is counted. The inoculations where a representative of the residential microflora or GMM are grown individually serve as controls. The number of cells of the residential microflora representative cultivated with/without the GMM is calculated. If the proportion of the individually grown cells and the cells grown in the GMM presence is almost the same, the GMM is considered as free of antagonistic action.

If the number of cells grown in the mixed cultivation is higher, a conclusion is made that the species are characterized by symbiotic action.

In cases where the cell counts of the residential microflora representative decrease by 5-fold or more in the mixture with the GMM, a conclusion is made that the GMM has antagonistic action against a

particular representative of the normal intestinal microflora of the recipient animals (birds). In case where such GMM antagonistic action is demonstrated against the majority of studied representatives of the animal normal intestinal microflora, a conclusion is made that the GMM cannot be used as a probiotic agent. In case where the GMM antagonistic action is found against one or two representatives of the normal intestinal microflora, the results of the studied antagonistic activity of the parent strain are reviewed; *in vivo* tests are performed; and, molecular genetic studies are conducted to identify genes responsible for this phenomenon.

Duration of the studies: no more than 90 days.

3.8. Immunological studies in the laboratory animals

Immunological studies of probiotic GMM are performed in CBA mice (characteristics: high sensitivity to the administration of sheep erythrocytes and no sensitivity to histamine or *Salmonella typhimurium*) and C57B1/6 mice (characteristics: low sensitivity to the administration of sheep erythrocytes; sensitivity to histamine and *Salmonella typhimurium*). The studies include the evaluation of immunomodulating and sensitizing properties in the following four tests:

- effects on the humoral component of the immune system in the test determining hemagglutinin levels following injections of sheep erythrocytes;
- effects on the cellular component of the immune system in the test of delayed-type hypersensitivity test following injections of sheep erythrocytes;
- sensitizing agent effects in the histamine sensitivity test;
- effects on the natural mouse resistance to *Salmonella typhimurium*.

Duration of the studies: no more than 40 days.

3.9. The following tests of probiotic agents shall be conducted, depending on species (genus) of the GMM probiotic strain:

GMM	Potential Risk Factor	Studies
Mould fungi	Production of mycotoxins; antibiotics	Detection: the detection of mycotoxins and antibiotics in the probiotic products
Yeasts-saccharomyces	Excessive ethanol production; allergenicity	The determination of ethanol concentration in the probiotic product; allergenicity studies
Streptomycetes	Production	The detection of antibiotics in the probiotic product;
Spore-forming bacilli	Excessive proteolytic and hemolytic activities; the production of antibiotic substances	Acute toxicity testing of the probiotic product; hemolysis of the red blood cells exposed to GMM; the detection of antibiotics in the probiotic product;
Enterococci	Production of N-nitrosamines, histamine; resistance to antibiotics	The detection of histamine, N-nitrosamines; the identification of genes responsible for resistance to vancomycin and rifampin
Heterofermentative lactobacilli	Excessive production of D(-) lactic acid in the product	The determination of D(-) lactic acid concentration in the probiotic product;

Duration of the studies: no more than 90 days.

4 Testing of the feed additives containing non-viable GMM for the biological safety assessment

4.1. Reference samples of the formulation and properties, including samples of the feed additives containing non-viable GMM, live GMM cultures and parent strains in the amount sufficient for comprehensive testing are provided by the applicant.

4.2. The GMM safety assessment takes into consideration the results of molecular genetic studies carried out by experts, e.g. the verification that the declared foreign genes are present in the GMM genome; studies for detecting the presence of potential non-declared genetic modifications; the GMM genetic stability; the expression of recombinant genes; taxonomy studies, etc.

Duration of the studies: no more than 250 days.

4.3. Samples of the feed additives are tested for the absence of viable cells of the declared strain, using microbiological methods. Live cultures of the GMM and parent strains are used as control inoculations.

Duration of the studies: no more than 30 days.

4.4. Studies are conducted to assess the safety of the feed additives containing non-viable GMM for recipient animals and birds. During the study, a sample of animals receives a tenfold dose (compared with the dose proposed for practice) of the feed additive.

The follow-up period is one month. Animal mortality, clinical state, hematological and biochemical blood indicators are recorded. The consumption of GMM-containing feed additive should not cause visible clinical changes or adverse events; hematological indicators should not differ significantly from the normal values; and no pathological abnormalities should be found at the post-mortem examination.

Duration of the studies: no more than 90 days.

5 Testing of the feed additives containing individual ingredients synthesized by GMM or derived from them for the biological safety assessment

These products include vitamins; essential amino acids; antibiotics; substances with hormonal, immunomodulating action; and, enzymes derived from the GMM producers. The type of products where GMM are used only at the production stage as producers is supposed to have the highest safety, since in this case the technology implies that the producer strain will be removed completely from the final product.

The safety assessment of feed additives for veterinary applications takes into consideration the results of molecular genetic studies carried out by experts, including the verification of the absence of recombinant DNA in the product.

Duration of the studies: no more than 30 days.

6. Testing of the GMO of plant origin for the feed additive production and the biological safety assessment

6.1. The applicant shall provide the reference samples of the formulation and properties, including the GMO of plant origin and its conventional analogue in the amounts sufficient for the comprehensive testing.

6.2. The GMO safety assessment takes into consideration the results of molecular genetic tests conducted by experts, including the verification of the presence of declared foreign genes in the GMO genome; tests for the presence of potential non-declared genetic modifications; the GMO genetic stability; the expression of recombinant genes; taxonomic studies, etc.

Duration of the studies: no more than 45 days.

6.3. Hygienic studies of the GMO include the evaluation of quality and safety indicators. The following indicators are evaluated: concentration of toxic elements, mycotoxins, pesticides, radionuclides, hazardous impurities, and microbiological indicators.

The list of safety indicators is based on the following requirements:

GOST R 54319-2011 "Feed meal. Technical conditions. Veterinary and sanitary norms and requirements for the quality of feedstuffs for non-productive animals." No. 13-7-2/2010 of July 15, 1997.

GOST 2116-2000 "Feed meal from fish, marine mammals, crustaceans and vertebrates. Technical conditions."

GOST 25311-82 "Feed meal of animal origin. Methods of bacteriological analysis."

GOST 17536-82 "Feed meal of animal origin. Technical conditions."

GOST R 52337-2005 "Feedstuffs, raw materials for compound feed. Methods of general toxicity assessment."

GOST 17681-82 "Meal of animal origin. Testing methods."

Duration of the studies: no more than 45 days.

6.4. Composition equivalence of the plant-origin GMO and conventional analogue is assessed to avoid the risk of decrease in their biological wholeness. The feeding values of a new and conventional product (protein content, composition of amino acids, fat content, composition of fatty acids, composition of carbohydrates, contents of vitamins, macro- and trace elements, specific components, biologically active substances, anti-nutrients and other substances typical for a particular plant species); normal consumption values; methods of application; bioavailability and digestibility; impact on the gastrointestinal microflora are compared. In case where the applicant has provided comprehensive data, the tests may be limited by the determination of humidity, ash, and contents of protein, fat, carbohydrates and dietary fibers. If the composition equivalence comparison does not identify differences between the GMO products and conventional analogues, the GMO products are considered absolutely safe for animal health. In case where the genetic modification is targeted at changing the GMO chemical composition, it is necessary to perform tests for proving the declared changes.

6.5. Toxicological studies of the GMO of plant origin in laboratory animals

The experiment is performed in Wistar rats (males 40-50 days old, baseline body weight 70-80 g). The animals are divided into 2 groups; the control group receives the diet with a conventional analogue of the tested GMO; the experimental group receives the diet with the tested GMO.

At the beginning of the experiment, each group includes at least 50 animals. During the experiment the animals receive a semi-synthetic casein diet (SCD), SCD containing casein, Corn starch, unrefined sunflower oil, lard, mixture of salts (sodium chloride, potassium phosphate, magnesium sulfate, calcium carbonate, iron sulfate, potassium iodide, manganese sulfate, zinc sulfate, copper sulfate, cobalt

chloride, sodium fluoride, aluminum potassium sulfate), vitamins (B1, B2, B6, B12, nicotinic acid, calcium pantothenate, folic acid, cyancobalamin, Vicasol, L-methionine, retinyl acetate, ergocalciferol, tocoferol acetate), microcrystalline cellulose. The tested GMO and its conventional analogue are included in the feed formulation in the maximum possible amount which does not affect the balance of the essential nutrients. The diet ingredients should be substituted taking into consideration the amount of proteins, fats and carbohydrates in the tested product, while observing the principle of isocaloricity. The nutritional and biological value of the diet shall satisfy completely the physiological requirements of the animals. The animals shall have free access to feed and water, and shall be housed in heated and ventilated room.

The following indicators are evaluated: general condition of the animals (appearance, motor activity, hair coat condition) – every 2 days; feed intake – daily; body weight – every 7 days. The weight of internal organs (brain, heart, spleen, lungs, thymus, hypophysis, liver, kidneys, adrenal glands and testicles) is measured on days 30 and 180 of the experiment.

Samples are taken for the hematological, biochemical and morphological examinations from 10 animals of each of the groups on days 30 and 180 of the experiment.

The following indicators are evaluated:

- hematological parameters: hemoglobin level; hematocrit; total red blood cell count; mean cell volume (MCV); mean cell haemoglobin (MCH); mean cell hemoglobin concentration; total thrombocyte count; total white blood cell count; differential white blood cell count (neutrophils, lymphocytes, eosinophils, monocytes, basophils);
- clinical chemistry blood parameters: alaninaminotransferase (ALT); aspartataminotransferase (AST); bile acids; alkaline phosphotase; total bilirubin level; direct bilirubin; total protein; albumin; globulin; creatinine; glucose; alpha-amilase; lipase; lactate-dehydrogenase; total lipids; triglycerides; cholesterol; cholinesterase; urea; chlorides; sodium; phosphorus; potassium;
- clinical urine analysis; the following indicators are evaluated: daily urine volume; color and transparency; re;ative density; pH; protein, glucose; creatinin;
- biomarkers of the antioxidant defense system: (biomaterial for examination: erythrocytes); evaluated indicators of activity of antioxidant defense enzymes: glutathione reductase; glutathione peroxidase; superoxide dismutase; catalase;
- biomarkers of the antioxidant defense system: (biomaterial for examination: blood, liver); evaluated indicators: concentration of lipid peroxidation products: malondialdehyde;
- system of xenobiotic metabolism enzymes: (biomaterial for examination: liver); evaluated indicators: activity of the enzymes of xenobiotic metabolism, phases 1 and 2; total concentration of cytochrome P-450; 7-ethoxyresorufin-O-deethylase; 7-pentoxyresorufin-O-deethylase; UDP-glucuronosyl transferase; glutathione-transferase;
- apoptosis regulation system, lysosome membrane stability ((biomaterial for examination: liver); evaluated indicators: total and non-sedimented activity of lysosome enzymes, galactosidase; glucuronidase; arylsulfatase A and B.

Morphological examinations are conducted of the following organs: skin; brain; heart; aorta; spleen; lungs. Macro- and microscopic, and general histological examinations; morphometric analysis on days 30 and 180 of the experiment (scheduled sampling) are carried out. Microscopic (electron microscopy), histochemical and immunohistochemical examinations of the pancreas, kidneys and testicles are

performed.

For animals died during the experiment (non-scheduled sampling), morphological studies of the following organs are additionally performed: lymphatic nodes; thymus; thyroid; pituitary gland; stomach; large and small intestine; and liver, to find out the cause of death.

Duration of the studies: no more than 200 days.

6.6. Immunological testing of the GMO of plant origin in laboratory animals.

Immunological studies of the plant-origin GMO are conducted in CBAe mice (characteristics: highly sensitive to the administration of sheep erythrocytes and non-sensitive to histamine or *Salmonella typhimurium*). And C57B1/6 mice (characteristics: low sensitivity to the administration of sheep erythrocytes; sensitive to histamine and*Salmonella typhimurium*). The studies include the evaluation of immunomodulating and sensitizing properties using the following four tests:

- effects on the humoral component of the immune system in the test determining hemagglutinin levels following injections of sheep erythrocytes;
- effects on the cellular component of the immune system in the test of delayed-type hypersensitivity test following injections of sheep erythrocytes;
- sensitizing agent effects in the histamine sensitivity test;
- effects on the naturally occurring mouse resistance to *Salmonella typhimurium*.
- Duration of the studies: no more than 40 days.

6.7. Allergological testing of the GMO of plant origin in laboratory animals.

Потенциальную аллергенность оценивают, определяя тяжесть протекания системной анафилаксии и уровня циркулирующих сенсибилизирующих антител (субклассов IgG + IgG) у крыс, получающих в составе рациона исследуемый ГМО (группа "опыт") и его традиционный аналог (группа "контроль"). Метод основан на количественной сравнительной оценке тяжести реакции системной анафилаксии, возникающей при внутрибрюшинной (в/б) сенсибилизации взрослых крыс пищевым антигеном - овальбумином куриного яйца (ОВА) с последующим внутривенным (в/в) введением сенсибилизированным животным разрешающей дозы того же белка.

Potential allergenicity is assessed by the severity of systemic anaphylactic reaction and the level of circulating sensitizing antibodies (IgG + IgG subclasses) in the rats that received the tested GMO (the experimental group) and its conventional analogue (the control group). The method is based on the comparative quantitative evaluation of the severity of systemic anaphylactic reaction induced by the intraperitoneal (i/p) sensitization of mature rats with a food antigen – ovalbumine (OVA) followed by the intravenous (i/v) injection of anaphylaxis-provoking dose of the same protein to the sensitized animals.

The experiment is conducted in Wistar rats (sexually mature males with a baseline body weight of 150-180 g). The animals are divided into 2 groups; the control group receives the diet with a conventional analogue of the tested GMO; the experimental group receives the diet with the tested GMO. At the beginning of the experiment, each group includes at least 25 animals. During the experiment the animals receive a standard diet used in the animal care and used facility (“vivarium”): daily intake per rat: oat meal 2.5 g; grain mixture 14.0 g; bread, Grade 2, 4.0 g; cottage cheese 2.0 g; fish meal 0.5 g; meat, Grade 2, 4.0 g; carrot 8.0 g; greens 8.0 g; fish oil 0.1 g; yeasts 0.1 g; NaCl 0.15 g; essential nutrients -

protein 3.69 g; fat 1.28 g; carbohydrates 12.42 g; energy 76.0 kcal). The tested GMO and its conventional analogue are included in the feed formulation in the maximum possible amount which does not affect the balance of the essential nutrients. The diet ingredients should be substituted taking in consideration the amount of proteins, fats and carbohydrates in the tested product, while observing the principle of isocaloricity. The nutritional and biological value of the diet shall satisfy completely the physiological requirements of the animals. The animals shall have free access to feed and water, and shall be housed in heated and ventilated room.

On days 1, 3 and 5 of the experiment the rats are sensitized i/p with OVA, and on day 21 of the experiment an additional (booster) dose of the antigen (10-fold lower than the initial dose) is injected. The animals receive the diet by the morning of Day 29 of the experiment. Then, the OVA solution is injected i/v and for the next 24 hours the severity of developing anaphylactic reaction is assessed using the following parameters: the number of lethal reactions; the total number of seizure reactions; and, the anaphylactic index. Immediately before the injection of the provoking dose, a blood sample of 0.1-0.2 ml is taken from the tail vein of the rats to measure the levels of circulating specific antibodies to OVA.

The severity of anaphylactic shock in the both animal groups is evaluated by the anaphylactic index which takes into account the severity of anaphylactic reactions and the percentage of lethal anaphylactic reactions.

Duration of the studies: no more than 40 days.

Based on the expert review of submitted documentation and materials; the results of completed molecular genetic tests; and the assessment of the biological risk associated with the use of a particular product, a report on the prospects of using this product for veterinary applications in the RF territory is issued.

Annex 5. Draft 64513 - Procedure for Molecular Genetic Testing of GE Organisms for Production of Feed Additives

Draft

Procedure for molecular genetic testing of genetically modified organisms for production of feed additives

Introduction.

The procedure was developed in accordance with the resolution of the Government of the Russian Federation # 839 dated September 23, 2013 "On the state registration of genetically modified organisms intended for release into the environment as well as products derived with the use of such organisms or containing such organisms" with respect to molecular genetic testing during GMO safety assessment for production of feed additives.

Feed additives are defined as products of plant, animal, microbiological, mineral and synthetic origin or their blends designed to be added as components into animal feed or diet formulations to ensure their physiological full value, disease prevention (excluding medicines), animal growth promotion and performance (excluding medicines), integrity of components, improve nutrient availability, feed palatability and machinability.

According to the definition, feed additives can contain GMOs of either plant and animal or microbial origin. Use of GMOs and GMMs in production of feed additives and feed additive applications must be biologically safe both for animals and the environment.

The following classification categories shall be used to conduct safety assessments of feed additives obtained with the use of GMMs based on their risk levels to animals and the environment:

Category 1 products containing live/viable microorganisms (fermentation cultures, lactic acid bacteria, etc.).

Category 2 products containing unviable/inactivated GMMs (heat treated).

Category 3 products containing individual GMM synthesized ingredients or additives (enzymes, vitamins, and amino acids).

Category 4 products containing ingredients treated with GMM synthesized enzymes, etc.

Products where GMMs are used only at production stage as producers (Category 3 and 4) are potentially the safest. In this case, risk assessment criterion for the use of feed additives is the guarantee that there are no GMM viable cells or recombinant DNA in the feed additive, and no toxicity in substances obtained with the use of producer GMMs.

Risk assessment criterion for the second product category is the guarantee that products contain no viable GMMs (capable of self-reproduction), and genetic information cannot be transferred to other organisms.

Same risk assessment criteria must be used with the first category as applied to the second. Moreover, other factors will be additionally considered such as the antagonistic and synergistic impact on naturally occurring microorganisms; GMM impact on animal health, potential colonization in gastro-intestinal tract and influence on natural microflora, likelihood of antibiotic resistance gene transfer; and potential impact on the eco-system.

The use of GMOs of plant origin in feed additives is also associated with certain risks that must be evaluated during the biosafety study, such as:

- presence and direct impact of toxic and allergenic GMO transgenic proteins,
- pleiotropic effects of transgenic proteins on plant metabolism,
- ability to accumulate herbicides and their metabolites in resistant crop varieties or species.
- risks related to the horizontal transfer of transgenic constructs, primarily into genomes of human and animal symbiotic bacteria (E.coli, Lactobacillus (acidophillus, bifidus, bulgaricus, caucasicus), Streptococcus thermophilus, Bifidobacterium, etc.).

1. General provisions and scope of application

1.1. The present procedure shall be applied during molecular genetic testing to control GMM strains and GMOs of plant origin used for production of feed additives, as well as feed additives containing (or obtained with the use of) GMOs and their safety assessment.

1.2. The procedure is designed for the state registration of GMM strains for their target use in production of feed additives, for the state registration, monitoring and random quality control of feed additives containing GMOs of plant origin, and also feed additives containing GMMs or obtained with the use of GMMs.

1.3. The procedure is developed to establish a common scientifically based GMO safety assessment system, and takes into account new methodological approaches either developed in Russia or recommended by the international organizations (WHO, FAO, OIE, etc.).

2. Expert analysis and evaluation of information about genetically modified organisms for production of feed additives.

General characteristics of GMOs and feed containing/ obtained with the use of GMOs involve the review of documentation submitted by the applicant including:

2.1. Description of properties the organism obtained through modification;

- description of the genetic construct structure (inserted or removed) and its localization site, characteristics of the inserted or modified gene expression,
- assessment of GMO differences from its parent organism including propagation and dissemination modes, incubation protocols, and new phenotypic characteristics;
- GMO genetic and phenotypic stability characteristics specifying differences from the parent organism. It is required to provide information obtained through testing several generations of GMOs, and the assessment of genes ability to transfer to other organisms (plants, microorganisms)
- for GMOs of plant origin: characteristics of inserted gene expression during plant ontogenesis, expression levels in the plant structural components, and differences from the parent organism.

2.2. Description of procedures that allow to identify the genetic modification (transformation event for GMOs of plant origin) and the taxonomic status of the organism (crop species, variety). In addition, test protocols, descriptions of nucleotide sequences of primers used, reference materials of compositions and properties shall be reviewed.

2.3. Information about the original parent organism (taxonomic identification, propagation and dissemination modes; information about toxicity, allergenicity and other adverse properties).

2.4. Information about donor organisms of inserted genes (taxonomic identification, toxicity or allergenicity, history of use, etc.)

2.5 Information about the genetic modification method (description of modification method, vector and insert structures).

2.6. Information about GMO genetic safety and stability study.

2.8. During the risk assessment of feed additives Category 3 and 4 obtained with the use of GMMs, the guarantee of the absence of recombinant DNAs in the product shall serve as a safety criterion.

2.9. For feed additives Category 1 and 2, ability to transfer genetic information to other organisms shall be evaluated. Parameters for evaluation shall include the characteristics and location of recombinant DNA (chromosome, plasmid, and any other mobile genetic element); environmental objects that recombinant DNA can enter (animal gastro-intestinal tract, manure, soil, water, air, etc.), recombinant DNA stability in relevant environments; presence of microorganisms – potential recipients of recombinant DNA as a result of horizontal transfer.

For feed additives Category 1, the following factors shall be additionally considered:

- competitive advantages of GMMs that received recombinant genes;
- potential antagonistic, synergistic or other impacts on naturally occurring microorganisms;
- potential impact of GMMs that received recombinant genes on human, animal and plant health;
- potential colonization in gastro-intestinal tract and impacts on its natural microflora;

- likelihood of antibiotic resistance gene transfer in GIT microflora; potential impact on the eco-system.

2.7. Post-registration monitoring data on the GMO and products containing it from the applicant country and other countries shall be reviewed to identify any undesirable genetic modification effects, which could not have been found during the registration studies.

3. Molecular genetic testing of probiotic GMM strains for production of feed additives for veterinary applications

Molecular genetic expert analysis shall include verification of information submitted by the applicant and a test for potential undeclared genetic modifications of the GMM strain.

3.1. The applicant shall submit reference materials of compositions and properties including samples of the GMM crop and its unmodified analogue in the amount required to carry out comprehensive tests.

3.2. GMM and parent strain genomes shall be sequenced to confirm that declared foreign genes are present in a specific location of the microorganism genome and to verify any potential undeclared genetic modifications.

Fluorescence detection of labeled nucleotides that are incorporated into superficial molecular clusters during *in situ* synthesis shall be used for full genome sequencing.

Genome DNAs shall be isolated from GMM and parent strains with the use of reagent kits according to manufacturer's recommendations. DNA library with GMM and parent strains shall be prepared using kits for sequencing from one end or both ends depending on which analyzer is used.

Read length shall consist of 36 nucleotides from one end of the fragment and 150 nucleotides from each end of the fragment depending on which analyzer is used. Analyzer-related software shall be used to analyze cluster images and convert them into DNA sequences. Coverage depth of genome reads across all regions should be at least 20 times. Localization sites of declared genetic modification, regulatory and marker vector GMM sequences shall be revealed during genome alignment. Information collected on the genetic construct structure and its site of localization shall be compared with the information submitted by the applicant.

Test strains of microorganisms (GMM and parent) must differ only in the region of the declared genetic modification, identity of nucleotide sequences in all other genome regions must be a minimum of 99%.

The analysis period: no longer than 180 days.

3.3. PCR-based procedure for identification of GMM lines submitted by the applicant shall be verified (validated), sensitivity and specificity shall be evaluated.

Test protocols presented by the applicant shall include descriptions of PCR primers and the thermal cycling mode.

Oligonucleotides used as PCR primers shall be synthesized in accordance with their nucleotide sequence provided.

Special equipment required for PCR-based diagnostics is described in ISO 20837:2006 and ISO 20838:2006. Test consists of sequential processes: sample preparations, DNA extraction from a product sample, DNA amplification, detection of amplification products, analysis and interpretation of results. When studying genetically modified bacteria, yeasts, and mycelial fungi, ready-made kits are recommended to extract DNA from the samples. For PCR tests, it is advisable to use heat-stable DNA

polymerase, mixture of deoxyribonucleotide triphosphates, tenfold buffers. Amplification products shall be detected by electrophoretic separation of PCR fragments in agarose gel. PCR results shall be recorded based on whether or not specific bands of the amplified DNA appear on electrophoregram.

PCR procedure must have 100% specificity (absence of cross-reactions with non-specific DNAs in concentrations up to $1x10^6$ genomic equivalents/ml) and high specificity (no less than $1x10^4$ genomic equivalents/ml).

The analysis period: no longer than 30 days.

3.4. Performance analysis of targeted insertions shall be carried out to identify target gene expression products and to establish:

- the amount of mRNA transcribed from the target gene by reverse transcription – polymerase chain reaction (RT-PCR);
- presence of recombinant protein by electrophoretic separation in polyacrylamide gel. Profiles of protein patterns shall be compared between the GMM and parent strains;
- specificity of recombinant protein by immunoblotting.

The analysis period: no longer than 60 days.

3.5. Genetic stability of GMM strains of bacteria, yeasts and mycelial fungi shall be determined by a minimum tenfold sub-culturing (passaging) on liquid and solid media followed by full genome sequencing of the microorganism.

GMM strain shall be considered genetically stable and safe if no genetic modifications are found in the genome including mutations, partial or complete loss of the modified fragment or its transfer to other genome regions after a minimum tenfold passaging.

The analysis period: no longer than 250 days.

3.6 Performance analysis of targeted insertions shall be carried out after tenfold passaging on liquid and solid media to evaluate the expression stability of a foreign gene of GMM strains of bacteria, yeasts and mycelial fungi (see para. 3.4). Expression level of the recombinant protein must be stable.

The analysis period: no longer than 150 days.

4. Molecular genetic testing of feed additives obtained from or with the use of GMMs.

Molecular genetic expert analysis of feed additives obtained from/ or with the use of GMMs shall include verification of information submitted by the applicant and a test for potential undeclared genetic modifications.

4.1. The applicant shall submit reference materials of compositions and properties including samples of feed additives obtained from/ or with the use of GMMs in the amount required to carry out comprehensive tests.

4.2. Ready-made kits shall be used to study enzyme preparations, starter cultures, food supplements, or to extract DNAs from the samples. Ready-made kits shall be used to study feed additives containing plant components and to extract DNAs from the samples.

For feed additives Category 1 and 2, it is required to confirm the presence of declared foreign genes in the GMM genome. Their localization sites, regulatory and/or marker vector sequences shall be detected. Validated (see para. 3.3) PCR identification procedure suggested by the applicant/manufacturer shall be

used to test GMMs for the presence of declared modifications. Validated procedures for the GMM identification can be also received from other reputable sources.

PCR test results shall be reconfirmed through determination of nucleotide sequence by Sanger sequencing method using terminating dideoxynucleoside triphosphates. For sequencing, it is recommended to use systems of any genetic analyzer or other similar units that ensure reliable determination of DNA nucleotide sequences up to 500-800 bp long per one read.

The analysis period: no longer than 45 days.

4.3. For feed additives Category 1 and 2, taxonomic status of GMMs shall be confirmed by defining nucleotide sequences of the recipient strain genomic fragments. If pure culture can be extracted GMM testing shall be carried out by amplification of DNA fragment with universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3’) and U1492R (5’-GGTTACCTTGTTACGACTT-3’), otherwise primers that are species-specific to the declared strain shall be used, followed by Sanger sequencing to determine the nucleotide sequence. Phylogenetic analysis shall be performed for the query sequence and similar sequences from available databases using the appropriate software. A microorganism genetically closest to the one under examination shall be established.

The analysis period: no longer than 70 days.

4.4. For feed additives Category 3 and 4, where GMM strains are used as producers of enzymes, amino acids, vitamins and other prebiotic substances, the production technology calls for complete removal of producer strains from the end product (purification). To rule out the risks associated with violations of the manufacturing process and incomplete removal of components (cell contents) of the producer strain during purification, or risks of viable GMMs still present, PCR test shall be performed to confirm that there are no GMM transgenic sequences contained in the end product.

DNA extraction from feed additives shall be done in accordance with para. 4.2. Validated procedures (see para. 3.3) submitted by the applicant shall be used for PCR identification of genetic modification lines of producer strains. Nucleic material of producer GMM strains shall be used as positive control.

The analysis period: no longer than 30 days.

4.5. For feed additives Category 1 containing viable GMMs, performance analysis of the targeted insertion shall be performed with detection of the target gene expression products:

- determination of mRNA transcribed from the target gene by reverse transcription – polymerase chain reaction method (RT-PCR);
- determination of recombinant protein by electrophoretic separation in polyacrylamide gel; profiles of protein patterns shall be compared between the GMM and parent strains;
- determination of recombinant protein specificity by immunoblotting.

The analysis period: no longer than 60 days.

4.6. For feed additives Category 1 containing viable GMM strains of bacteria, yeasts and mycelial fungi, genetic stability shall be evaluated by observing changes in genome nucleotide sequences after tenfold sub-culturing (passaging) on liquid and solid media.

Sanger sequencing and genome restriction analysis shall be used to establish genetic stability.

GMM strains shall be considered genetically stable and safe if no genetic modifications are found in the GMM genome including development of mutations, partial or complete loss of the modified fragment or

its transfer to other genome regions after a minimum tenfold passaging.

The analysis period: no longer than 180 days.

4.7. For feed additives Category 1 containing viable GMM strains of bacteria, yeasts and mycelial fungi, genetic stability of the foreign gene expression shall be evaluated to detect changes after tenfold passaging on liquid and solid media (see para. 4.5.).

Expression level of recombinant protein must be stable.

The analysis period: no longer than 150 days.

4.8. Feed additives Category 1 and 2 shall be screened for undeclared genetic modifications. Testing shall be based on recommendations of the international organizations (FAO/WHO/OIE) and involve the detection of DNA sequences of most frequently used plasmid vectors, through which genetic information is introduced into cells.

Multi-component PCR kits shall be used to detect most typical marker and selective genes (antibiotic resistance, bacteriocin, β-galactosidade, β-glukoronidase genes, RNA polymerase of T7 bacteriophage, regulatory sequences of promoters and terminators, polylinkers, sequences of migrating elements of transposable vectors) used in genetic constructions with recombinant DNA technology in various groups of microorganisms (bacteria, yeasts, and mycelial fungi). Detection of the listed sequences indicates potential presence of undeclared genetic modifications and requires additional testing. Information about sequences of marker and selective genes inherent to genetically modified constructions is available in the international databases. Specific oligonucleotide primers for identification of such sequences shall be selected with the help of programs in accordance with requirements to PCR primers. Specificity and sensitivity of selected primers shall be confirmed with DNA preparations from strains of various microorganisms (required sensitivity is no less than $1x10^4$ genomic equivalents/ml, absence of cross-reactions with non-specific DNAs in concentrations no less than $1x10^6$ genomic equivalents/ml). Examples of PCR-primer pairs designed for the detection of GMM marker and selective genes during PCR screening tests to monitor the presence of undeclared genetic modifications are given in Table 1.

Table 1. PCR primers for detection of several marker and selective genes that are component parts of genetically engineered constructs

Target	Description	PCR primers, 5' — 3'
LacZ gene	Encodes enzyme b-galactosidase. Used in vector plasmid constructs (pCR2.1TOPO, pVIK112, pBSKSII, etc.), includes polylinker region for recombinant gene cloning. When the enzyme is present, colorless substrate X-gal turns into a colored product, bacterial colonies expressing LacZ have light-blue color.	LacZ-F: ATGCTTCCGGCTCGTATGTTGTGT LacZ-R: GCTGGCGAA AGGGGGATGTGCT
Gfp gene	Green fluorescent protein gene is used in a number of vector plazmids for genetic manipulations (pKEN-gfpmut2, pRK415-gfp, etc.) as a luminous mark.	Gfp-F: TTGTTGATATTAGATGGCGATGTTA Gfp-R: TTTGGAAAGGGCAGATTGTGT

Amp gene	Encodes resistance to ampicillin and is used in a number of vector plasmids for antibiotic resistance selection (pBR322, pUC18, pBSKSII, pET, pCR2.1 TOPO, etc.)	Amp-F: GTGGGCTATATCGAACTGGA Amp-R: CCCGTCGTGTAGATA ACTACG
Km gene	Encodes resistance to kanamicyn and is used in a number of vector plasmids for antibiotic resistance selection (pVIK112, pMC212, etc.)	Km-F: CGGAAGGAATGTCTCCTGC Km-R: CGACATACTGTTCTTCCCCG
tetO gene	Encodes resistance to tetracycline and is used in a number of vector plasmids for antibiotic resistance selection (pBR322, etc.)	TetO-F: CGCCTGCAGAGGGCGGCACGGATCA TetO-R: CGCCTGCAGGGCCAACGTTCCAACC
F1 ori	Plazmid origin of replication (pBSKSII, pKEN-gfpmut2, pCR2.1 TOPO, etc.)	f1-F: TAAGCGCGGCGGGTGTGGT f1-R: TTGGGGTCGAGGTGCCGTAAA
Tn7L transposon	The fragment ensures transposition of recombinant genes from plazmids into a genome (pRK415-gfp, etc.)	IncP-F: CAGAGCAGGATTCCCGTTGAG IncP-R:GGGCAGGATAGGTGAAGTAGG
R6K ori	Plazmid origin of replication (pVIK112, etc.)	R6K-F: GACAAA AAGGATCCAGCAGTT R6K-R: AGTACGTTAGCCATGAGGGTT
ermC gene	Encodes resistance of plasmid vectors to erythromycin	ErmC-F: AGTACAGAGGTGTAATTTCG ErmC-R :AATTCCTGCATGTTTTAAGG
Cat gene	Encodes resistance of plasmid vectors to chloramphenicol	Cat-F: ATGAACTTTAATAAAATTGATTTAGA Cat-R: AGCATTTTTCAGGTATAGGTG

When genetic modifications are detected, testing shall continue to identify a particular GMM genetic construct.

The analysis period: no longer than 90 days.

5. Molecular genetic testing of GMOs of plant origin for production of feed additives.

Molecular genetic expert analysis shall include verification of information submitted by the applicant, a test for potential undeclared genetic modifications of GMO crop, and an impact assessment of genetic modification on GM crop performance.

5.1. The applicant shall submit reference materials of compositions and properties including samples of

GMO crop and its conventional analogue in the amount required to carry out comprehensive tests.

5.2. PCR-based procedures for detection, identification and quantification of the GMO submitted by the applicant shall be verified (validated), sensitivity and specificity shall be evaluated.

Test protocols presented by the applicant shall include descriptions of PCR primers and the thermal cycling mode.

Oligonucleotides used as PCR primers shall be synthesized in accordance with their nucleotide sequence provided.

Special equipment required for PCR-based diagnostics is described in ISO 20837:2006 and ISO 20838:2006. Test consists of sequential processes: sample preparations, DNA extraction from a product sample, DNA amplification, detection of amplification products, analysis and interpretation of results.

DNA extraction from samples of plant origin shall be performed by CETAB or sorption methods in accordance with GOST R 52173. For DNA extraction by the sorption method, it is recommended to use commercially available reagent kits in accordance with manufacturer's recommendations.

For the PCR test, it is advisable to use heat-stable DNA polymerase, mixture of deoxyribonucleotide triphosphates, tenfold buffers. Depending on the method, amplification products can be detected by electrophoretic separation of PCR products in agarose gel based on whether or not specific bands of the amplified DNA are in place or by recording the fluorescence signal accumulation during PCR with hybridization fluorescent detection method.

PCR procedures must have 100% specificity (absence of cross-reactions with non-specific DNAs in concentrations up to $1x10^6$ genomic equivalents/ml) and high specificity (no less than $1x10^4$ genomic equivalents/ml).

The analysis period: no longer than 30 days.

5.3. Transgenes declared in the GMO genome shall be confirmed, their localization site and regulatory and/or marker vector sequences shall be established. GMO analysis for presence of declared modifications shall be performed based on information provided by the applicant, containing the description of molecular structure of gene constructs (nucleotide sequence, localization inside a recipient genome), using validated identification methods (see para. 3.2) submitted by the applicant/manufacturer or obtained from reputable sources.

For detection of DNA regulatory elements in the declared genetic construct, validated PCR methods (see para. 3.2) or methods described in ISO/FDIS 21569:2005 and other reputable scientific sources shall be used. Examples for PCR primer pairs are given in Table 2.

For detection of DNAs of the most common regulatory elements, p-CaMV35S, p-FMV promoters and t-NOS terminator, ready-made PCR kits shall be used.

Table 2. PCR-primers for detection of regulatory elements of genetically engineered constructs of GMO of plant origin

Element	Soybean, corn and rapeseed GM lines containing this element	5' — 3' primer and probe sequences
CaMV35S promoter	GTS 40-3-2, A2704-12,	PCR with fluorescent hybridization detection: CGTCTTCAAAGCAAGTGGATTG

	A5547-127, BT 176, BT11, MON810, GA21, MON88017, NK603, MON863, T25, T45, etc.	TCTTGCGAAGGATAGTGGGATT FAM-TCTCCACTGACGTAAGGGATGACGCA-TAMRA PCR with electrophoretic detection GCTCCTACAAATGCCATCA GATAGTGGGATTGTGCGTCA Product PCR size is 195 bp.
t-NOS terminator	GTS 40-3-2, MON863, MON531, MON757, MON1076, MON1445, MON1698, MON15985, BT11, GA21, NK603, MON802, 3272, MIR604, MON88017, etc.	PCR with fluorescent hybridization detection: CATgTAATgCATgACgTTATTTATg TTgTTTTCTATCgCgTATTAAATgT (YY)–ATgggTTTTTATgATTAgAgTCCCgCAA–(BHQ1) PCR with electrophoretic detection GAATCCTGTTGCCGGTCTTG TTATCCTAGTTTGCGCGCTA Product PCR size is 180 bp.
p-FMV promoter	MON89788, MON89034, GT73, etc.	AAGCCTCAACAAGGTCAG CTGCTCGATGTTGACAAG Product PCR size is 196 bp.
p-SSuAra promoter	MS1, RF1, RF2, MS8xRF3, etc.	PCR with fluorescent hybridization detection: GGCCTAAGGAGAGGTGTTGAGA CTCATAGATAACGATAAGATTCATGGAATT FAM-CCTTATCGGCTTGAACCGCTGGAATAA-TAMRA
p-TA29 promoter	MS1, RF1, RF2, MS8xRF3, etc.	PCR with fluorescent hybridization detection: GAAGCTGTGCTAGAGAAGATGTTTATTC GCTCGAAGTATGCACATTTAGCAA FAM-AGTCCAGCCACCCACCTTATGCAAGTC-TAMRA
p-NOS promoter	MS1, RF1, RF2, Topas 19-2, etc.	PCR with fluorescent hybridization detection: GTGACCTTAGGCGACTTTTGAAC CGCGGGTTTCTGGAGTTTAA FAM-CGCAATAATGGTTTCTGACGTATGTGCTTAGC-TAMRA
t-E9 terminator	MON89788, etc.	PCR with fluorescent hybridization detection: TGAGAATGAACAAAAGGACCATATCA TTTTTATTCGGTTTTCGCTATCG FAM-TCATTAACTCTTCTCCATCCATTTCCATTTCACAGT-TAMRA
t-35S terminator	BT 176, T14, T25, 3272, A2704-12, A5547-127, T45,	PCR with electrophoretic detection GAAACCCTTAGTATGTATTTGTATTTGTAAAATACTTC TTTTAGTACTGGATTTTGGTTTTAGGAATTAG

	Topas19/2, etc.	
t-OCS terminator	MS1, RF1, RF2, etc.	PCR with fluorescent hybridization detection: CGGTCAAACCTAAAAGACTGATTACA CGCTCGGTGTCGTAGATACT FAM-TCTTATTCAAATTTCAAAAGTGCCCCAGGG-TAMRA
t-g7 terminator	MS1, RF1, RF2, MS8xRF3, etc.	PCR with fluorescent hybridization detection: ATGCAAGTTTAAATTCAGAAATATTTCAA R ATGTATTACACATAATATCGCACTCAGTCT FAM- ACTGATTATATCAGCTGGTACATTGCCGTAGATGA-TAMRA

Information submitted by the applicant (see para. 5.2.) and/or validated procedures from reputable sources shall be used to identify and confirm the GM lines (transformation event). Test protocol submitted by the applicant shall include descriptions of PCR primers and the thermal cycling mode.

Primers for PCR identification of several GMO lines suggested by a number of reputable scientific sources are given in Table 3.

Table 3. Primers for detection of several Corn, soybean and rapeseed GM lines in accordance with procedures from a number of reputable scientific sources

Target DNA	5' — 3' primer and probe sequences
Corn, line DAS-40278-9	CACGAACCATTGAGTTACAATC FAM-CGTAGCTAACCTTCATTGTATTCCG-TAMRA TGGTTCATTGTATTCTGGCTTTG
Corn, line MON87460	CACGTTGAAGGAAAATGGATTG FAM- AGGGAGTATGTAGATAAATTTTCAAAGCGTTAGACGGC-TAMRA TCGCGATCCTCCTCAAAGAC
Corn, line Bt11	GCGGAACCCCTATTTGTTTA FAM-AAATACATTCAAATATGTATCCGCTCA-TAMRA TCCAAGAATCCCTCCATGAG
Corn, line GA21	CTTATCGTTATGCTATTTGCAACTTTAGA FAM-CATATACTAACTCATATCTCTTTCTCAACAGCAGGTGGGT-TAMRA TGGCTCGCGATCCTCCT
Corn, line NK603	ATGAATGACCTCGAGTAAGCTTGTTAA FAM-TGGTACCACGCGACACACTTCCACTC-TAMRA AAGAGATAACAGGATCCACTCAAACACT
Corn, line MON863	TGTTACGGCCTAAATGCTGAACT FAM-TGAACACCCATCCGAACAAGTAGGGTCA-TAMRA GTAGGATCGGAAAGCTTGGTAC
Corn, line TC1507	TAGTCTTCGGCCAGAATGG

	FAM-TAACTCAAGGCCCTCACTCCG-TAMRA CTTTGCCAAGATCAAGCG
Corn, line T25	ACAAGCGTGTCGTGCTCCAC FAM-TCATTGAGTCGTTCCGCCATTGTCG-TAMRA GACATGATACTCCTTCCACCG
Corn, line 59122	GGGATAAGCAAGTAAAAGCGCTC FAM-TTTAAACTGAAGGCGGGAAACGACAA-TAMRA CCTTAATTCTCCGCTCATGATCAG
Corn, line MIR604	GCGCACGCAATTCAACAG FAM-AGGCGGGAAACGACAATCTGATCATG-TAMRA GGTCATAACGTGACTCCCTTAATTCT
Corn, line MON88017	GAGCAGGACCTGCAGAAGCT FAM-TCCCGCCTTCAGTTTAAACAGAGTCGGGT-TAMRA TCCGGAGTTGACCATCCA
Corn, line LY038	TGGGTTCAGTCTGCGAATGTT FAM-CGAGCGGAGTTTATGGGTCGACGG-TAMRA AGGAATTCGATATCAAGCTTATCGA
Corn, line MON89034	TTCTCCATATTGACCATCATACTCATT FAM-ATCCCCGGAAATTATGTT-MGBNFQ CGGTATCTATAATACCGTGGTTTTTAAA
Corn, line 3272	TCATCAGACCAGATTCTCTTTTATGG FAM-ACTGCTGACGCGGCCAAACACTG-TAMRA CGTTTCCCGCCTTCAGTTTA
Corn, line MON810	TCGAAGGACGAAGGACTCTAACGT FAM-AACATCCTTTGCCATTGCCCAGC-TAMRA GCCACCTTCCTTTTCCACTATCTT
Corn, line 98140	GTGTGTATGTCTCTTTGCTTGGTCTT FAM-CTCTATCGATCCCCCTCTTTGATAGTTTAAACT-TAMRA GATTGTCGTTTCCCGCCTTC
Corn, line MIR162	GCGCGGTGTCATCTATGTTACTAG FAM-TCTAGACAATTCAGTACATTAAAAACGTCCGCCA- TAMRA TGCCTTATCTGTTGCCTTCAGA
Corn LY038	TGGGTTCAGTCTGCGAATGTT AGGAATTCGATATCAAGCTTATCGA FAM-CGAGCGGAGTTTATGGGTCGACGG-TAMRA
Corn, line Bt176	GGCCGTGAACGAGCTGTT FAM-AGCAACCAGATCGGCCGACACC-TAMRA GGGAAGAAGCCTACATGTTTTCTAA
Soybean line FG72	AGATTTGATCGGGCTGCAGG FAM-AATGTGGTTCATCCGTCTT-MGBNFQ GCACGTATTGATGACCGCATTA

Soybean line MON87769	CATACTCATTGCTGATCCATGTAGATT FAM-CCCGGACATGAAGCCATTTACAATTGAC-TAMRA GCAAGTTGCTCGTGAAGTTTTG-3’
Soybean line MON 87705	TTCCCGGACATGAAGCCATTTAC FAM-AAGAGACTCAGGGTGTTGTTATCACTGCGG- TAMRA ACAACGGTGCCTTGGCCCAAAG-3’
Soybean line A2704-12	GCAAAAAAGCGGTTAGCTCCT FAM-CGGTCCTCCGATCGCCCTTCC-TAMRA ATTCAGGCTGCGCAACTGTT
Soybean line GTS-40-3-2	TTCATTCAAAATAAGATCATACATACAGGTT FAM-CCTTTTCCATTTGGG-MGB-NFQ GGCATTTGTAGGAGCCACCTT
Soya, line MON89788	TCCCGCTCTAGCGCTTCAAT-3’ FAM-CTGAAGGCGGGAAACGACAATCTG-TAMRA TCGAGCAGGACCTGCAGAA
Soybean line A5547-127	GCTATTTGGTGGCATTTTTCCA FAM-CCGCAATGTCATACCGTCATCGTTGT-TAMRA CACTGCGGCCAACTTACTTCT
Soybean line 305423	CGTGTTCTCTTTTTGGCTAGC FAM-TGACACAAATGATTTTCATACAAAAGTCGAGA-TAMRA GTGACCAATGAATACATAACACAAACTA
Soybean line DP-356043-5	GTCGAATAGGCTAGGTTTACGAAAAA FAM-CTCTAGAGATCCGTCAACATGGTGGAGCAC-TAMRA TTTGATATTCTTGGAGTAGACGAGAGTGT
Soybean line MON87701	TGGTGATATGAAGATACATGCTTAGCAT 6-FAM-TCAGTGTTTGACACACACACTAAGCGTGCC- TAMRA CGTTTCCCGCCTTCAGTTTAAA
Soybean line CV127	5’-AACAGAAGTTTCCGTTGAGCTTTAAGAC 6-FAM-TTTGGGGAAGCTGTCCCATGCCC-TAMRA 5’-CATTCGTAGCTCGGATCGTGTAC
Rapeseed, line T45	CA ATGGACACATGA ATTATGC GACTCTGTATGAACTGTTCGC FAM-TAGAGGACCTAACAGAACTCGCCGT-TAMRA
Rapeseed, line Ms8	GTTAGAAAAAGTAAACAATTAATATAGCCGG- GGAGGGTGTTTTTGGTTATC FAM-AATATAATCGACGGATCCCCGGGAATTC-TAMRA
Rapeseed, line Rf3	CATAAAGGAAGATGGAGACTTGAG 5’-AGCATTTAGCATGTACCATCAGACA FAM-CGCACGCTTATCGACCATAAGCCCA-TAMRA’

Rapeseed, line GT73	5’-CCATATTGACCATCATACTCATTGCT 5’-GCTTATACGAAGGCAAGAAAAGGA FAM-TTCCCGGACATGAAGATCATCCTCCTT-TAMRA
Rapeseed, line Ms1	ACGCTGCGGACATCTACATT CTAGATCGGAAGCTGAAGATGG FAM-CTCATTGCTGATCCACCTAGCCGACTT-TAMRA
Rapeseed, line RF1	CTAAGGGAGGTCAAGATGTAGC CGGGCCTAACTTTTGGTGTG FAM-CTCATCATCCTCACCCAGTCAGCATCA-TAMRA
Rapeseed, line RF2	GGGTGAGACAATATATCGACG GGGCATCGCACCGGTGAG’ FAM-CACCGGCCAAATTCGCTCTTAGCCGT-TAMRA
Rapeseed Topas 19/2	GTTGCGGTTCTGTCAGTTCC CGACCGGCGCTGATATATGA FAM-TCCCGCGTCATCGGCGG-TAMRA

Regulatory elements declared for the GMO line of plant origin and the declared transformation event must be detected in the test sample.

The analysis period: no longer than 45 days.

5.4. Test samples shall be screened to check for undeclared GM lines of plant origin:

- regulatory and/or marker sequences inserted into the plant genome during genetic modifications;
- target transgenes most frequently inserted to obtain desirable traits.

GMO regulatory elements shall be detected in accordance with para. 5.3. (Table 2).

Examples of PCR primer pairs designed for the identification of target transgenes most frequently inserted to obtain desirable traits are given in Table 3. Use of those primer pairs can help in identifying the key genes specific to GM crops: pat and bar promoting resistance to phosphinotricin (ammonium glufosinate), cp4 epsp promoting resistance to glyphosate, and gene cryIA(b) promoting resistance to corn worm.

Table 4. PCR primers for detection of target transgenes most commonly inserted into the plant genome to obtain desirable traits.

Target gene	GMO	PCR- primers/probes, 5' 3'

pat gene phosphinothricin-N-acetyltransferase Streptomyces viridochromogenes	Corn lines 3272, Bt10, Bt11, GA21, MIR604, 4114, 5307, TC1507, MIR162, 59122, MON810, NK603, MON88017, 676, 678, 680, 98140, DAS40278, T25 and their hybrids.	TTGAGGGTGTTGTGGCTGGTA FAM-CTTCCAGGGCCCAGCGTAAGCA-TAMRA TGTCCAATCGTAAGCGTTCCT
bar gene phosphinothricin-N-acetyltransferase Streptomyces hygroscopicus	Corn Bt176, CBH-351, DBT418, DLL25, MS3, MS6, TC6275, soybean lines W62, W98, etc.	ACAAGCACGGTCAACTTCC GAGGTCGTCCGTCCACTC FAM-TACCGAGCCGC AGGA ACC-TAMR A
cp4 epsp gene enolpyruvylshikimate-3-phosphate synthase from CP4 strain Agrobacterium tumefaciens	Corn MON802, MON809,MON81, NK603, soybean lines MON87708, MON87769, wheat line MON87769, etc.	GGGATGACGTTAATTGGCTCTG GGCTGCTTGCACCGTGAAG FAM- CACGCCGTGGAAACAGAAGACATGACCTAMR
Synthetic gene cryIA(b) of toxin protein *Bacillus thuringiensis*	Corn lines BT-176, MON802, MON809, MON810	CCCATCGACATCAGCCTGAGC FAM-ATGTCCACCAGGCCCAGCACG-TAMRA CAGGAAGGCGTCCCACTGGC

GMO soybean line CV127 does not contain regulatory sequences (35S, NOS and FMV) or common transgenes. Therefore, PCR with primers for a transgenic event shall be used to detect the line during screening tests (Table 2).

The analysis period: no longer than 45 days.

5.5. If no registered GMO lines were detected in GMO crop samples during screening tests while at the same time CaMV 35S promoter was found (and no other transgenic sequences identified), such samples shall be tested for DNA of *Cauliflower mosaic virus* (CamV) that affects tissues of Brassicaceae plants (rape, horseradish, cabbage, radish, mustard, garden cress, turnip, etc.). A ready-made kit for detection of the DNA virus can be used.

The analysis period: no longer than 10 days.

5.6. If any undeclared GM lines are detected, identification shall be conducted in accordance with para. 3.3. (Table 2) using validated procedures from reputable scientific sources.

The analysis period: no longer than 30 days.

5.7. If necessary, PCR test results shall be reconfirmed by determining the nucleotide sequence using Sanger sequencing method with terminating dideoxynucleoside triphosphates. For sequencing, it is recommended to use any genetic analyzer systems or other similar units that ensure reliable determination of DNA nucleotide sequences up to 700-800 bp long per one read.

The analysis period: no longer than 30 days.

6. Molecular genetic testing of feed additives containing GMOs of plant origin.

Molecular genetic expert analysis shall include verification of information submitted by the applicant and a test for potential undeclared GMO components in the feed additive.

6.1. The applicant shall submit reference materials of compositions and properties including samples of feed additives containing GMO in the amount required to carry out comprehensive tests.

6.2. Presence of declared transgenes in the GMO genome shall be verified, their localization sites shall be established, regulatory and/or marker vector sequences shall be identified (pursuant to para. 5.3).

Regulatory elements declared for the GMO line of plant origin and the declared transformation event must be detected in the test sample.

The analysis period: no longer than 45 days.

6.3. Test sample shall be screened to identify undeclared GM lines of plant origin (pursuant to para. 5.4).

The analysis period: no longer than 45 days.

6.4. If registered GM lines were not detected in samples of feed additives during screening tests while at the same time CaMV 35S promoter was found (and no other transgenic sequences identified), such samples shall be tested in accordance with para. 5.5.

The analysis period: no longer than 10 days.

6.5. Plant DNA shall be identified in feed additives using species-specific primers in accordance with validated methods approved for testing.

PCR primers for detection of soya lectin gene DNA (le1) using electrophoretic detection method ISO/FDIS 21570:2005.

5' GCC CTC TAC TCC ACC CCC ATC C 3'

5' GCC CAT CTG CAA GCC TTT TTG TG 3';

Product PCR size is 118 bp.

PCR primers for detection of corn invertase gene (ivr1) using electrophoretic detection method ISO/FDIS 21570:2005.

5’-CCGCTGTATCACAAGGGCTGGTACC-3’

5’-GGAGCCCGTGTAGAGCATGACGATC-3’

Product PCR size is 225 bp

For Corn DNA detection, primers in zein gene region can be used, PCR with electrophoretic detection.

5’-AGTGCGACCCATATTCCAG-3’

5’-GACATTGTGGCATCATCATTT-3’

Product PCR size is 277 bp

Validated method (ISO/FDIS 21570:2005) for DNA detection in Corn alcohol dehydrogenase gene 1 (adh1) with fluorescent hybridization detection

5’-CGTCGTTTCCCATCTCTTCCTCC-3

5’-CCACTCCGAGACCCTCAGTC-3’

5’-(FAM)-AATCAGGGCTCATTTTCTCGCTCCTCA-(TAMRA)-3’

For rapeseed DNA detection, it is recommended to use species-specific primers in cruA gene region, cruciferin reserve protein.

5'-GGCCAGGGTTTCCGTGAT-3’

5’-CCGTCGTTGTAGAACCATTGG-3’

5’-(VIC)-AGTCCTTATGTGCTCCACTTTCTGGTGCA-(TAMRA)-3'

Available kits can be used to detect the presence of Corn DNA and soybean DNA in feed or feed additives.

The analysis period: no longer than 10 days.

6.6. If undeclared GMO is detected in samples of feed additives, GM lines shall be identified in accordance with para. 5.3. (Table 2) using validated methods from reputable scientific sources.

The analysis period: no longer than 30 days.

6.7. For quantification of GMOs of plant origin in feed additives, procedures shall be used based on calculations of the percentage of the amount of DNA of a specific GM line relative to the total amount of DNA of the test crop. Simultaneously, two independent tests shall be performed in one test tube. One test allows to detect the test crop DNA (soybean, Corn, etc.). The other test allows to detect a sequence specific to a particular GM crop line. Behavior of the reactions can be detected via two specific fluorescent labeled probes. One probe can be used in the DNA detection test to identify DNA in the test crop (soybean, Corn), and the other can be used to detect a genetic insertion (GM line).

Determination of GMO percentages shall be done using calibration samples representing DNA blends of a conventional variety (0% GMO) and DNA of a GM line (100% GMO) in certain percentages. Difference in threshold cycle values of two reactions for calibration samples shall be used to build a calibration line, which is required to calculate the GMO DNA percentage in test samples of feed additives.

GMO quantification in feed additives of plant origin includes the following steps: DNA extraction from the test sample, real-time PCR, data analysis using the device software, calculations and processing of results using the appropriate software programs.

For the quantification of GM soybean and Corn transformation events registered in the RF, it is recommended to use procedures presented by the GMO applicants/ manufactures, validated methods from reputable scientific sources or ready-made PCR kits.

Standard certified samples containing between 0.1 and 5% of various GM Corn and soybean lines in a conventional crop variety must be available from official sources.

The analysis period: no longer than 30 days.

In the event that test results are contrary or not in compliance with specifications declared by the applicant/manufacturer, double arbitration retests shall be carried out.

Annex 6. Draft 64525 - Procedure for Biological Safety Assessment of GE Organisms for Manufacturing of Medical Products for Veterinary Use

Procedure for biological safety evaluation of genetically modified organisms for manufacturing of medical products for veterinary use

Introduction

The procedure was developed in accordance with Decree of Government of the Russian Federation № 839 of September 23,, 2013 "On the state registration of genetically modified organisms, intended for release into the environment, as well as products obtained using such organisms or containing such organisms." It applies to the biological safety evaluation of the genetically modified organisms for manufacturing of medical products for veterinary use.

Medical products obtained using genetically modified microorganisms (GMM), can be divided into the following categories:

Category 1 - immunobiological and probiotic preparations containing living/viable microorganisms (viral vector vaccines, live bacterial vaccines, probiotic agents containing strains of bacteria, yeast, micellar fungi).

Category 2 - immunobiological preparations containing non-viable microorganisms (inactivated vaccines).

Category 3 – medical products containing components (enzymes, monoclonal antibodies, antibiotics, vitamins, amino acids, hormones) obtained using GMM producers.

The aim of the research is to assess the safety of the GMM for animals and the environment in accordance with their intended type of use.

1. **General provisions and scope of application**

1.1. This procedure establishes the requirements for conducting the biological safety evaluation of GMM strains used in the manufacture of medical products for veterinary use.

1.2. Requirements in this methodology are mandatory for the state registration of GMM strains for targeted using in the manufacture of medical products for veterinary use, as well as for state registration, monitoring and selective quality control of immunobiological, probiotic and medical products for veterinary use obtained from GMM strains or obtained using such organisms.

1.3. The methodology was developed to provide a universal, scientifically validated system for assessing the biological safety of the GMM and it considers the current methodological approaches developed in Russia and recommended by international organizations (OIE, WHO, FAO, etc.).

2. Expert review and evaluation of GMM biological safety for manufacture of medical products for veterinary use

The biological safety assessment of the GMM includes a review of the documentation provided by the applicant containing:

2.1. Description of properties acquired by the GMM strain, as a result of modification:

- A description of the genetic construct structure (introduced or removed) and its localization, the characteristics of the expression of the inserted or altered genes;

- Characteristics of the differences between the GMM and the parental organism, including a description of the reproduction and propagation method, growth patterns, new phenotypic properties;

- Information on the competitive advantages of the GMM strain;

- Information on the testing of genetic safety and stability of the GMM strain, including those in animal and bird organisms, and environmental objects (gastrointestinal tract of animals, manure, soil, water, air, etc.);

- An assessment of the ability to transfer transgenes including antibiotic resistance genes into other organisms and microorganisms;

2.2. Description of the methods allowing to identify the GMM strain (confirm its taxonomic status and the genetic modification characteristic) including analysis protocols, description of the primer nucleotide sequences, standard samples of the composition and properties.

2.3. For GMM strains for the production of live vaccines and probiotics, the information on the industrial cultivation conditions of GMM strains is studied. Assess the conditions of production in accordance with the requirements of sanitary regulations. For medical products obtained using GMM producers, a technological scheme of industrial manufacture of the preparation is studied including a detailed description of the purification methods.

2.4. Comprehensive information on the original parent microorganism (taxonomy, description of propagation and distribution methods, data on virulence, allergenic and other biological properties);

2.5. Information about donor organisms of introduced genes (taxonomy, data on virulence, allergenic and other properties);

2.6. Information about the method of genetic modification (description of the modification method, vector structure and insertion structure);

2.7. For GMM strains for the production of live vaccines and probiotic agents, reports on the safety of the GMM strain for recipients and other species of animals and birds are examined. Information on the tissue tropism of the GMM strain in comparison with the parental unmodified strain should be studied.

2.8. For medical products containing inactivated GMM strains, study the provided information on the non-viability of the GMM strain in this product.

2.9. For GMM strains for the manufacturing of live vaccines, the information about the ways and possibilities of GMM transmitting from vaccinated to unvaccinated animals is reviewed (contagiousness);

2.10. For probiotic GMM strains, the potential colonization of the GMM in the gastrointestinal tract of animals or birds of recipients and the effect on the natural flora are evaluated.

The report on GMM safety for the environment should be studied:

- an assessment of the GMM ability to survive and multiply in the environment;

- the presence of microorganisms in the environment that are the potential recipients of

recombinant DNA as a result of horizontal transfer;

- assessment of the possibility of transferring the recombinant genes from destroyed virus particles or plasmid DNA of GMM cells to other microorganisms;

- evaluation of the potential antagonistic, synergistic or other effects of GMM on natural microorganisms.

2.11. For GMM strains for the production of probiotic agents, the results of toxicological studies of the strain and recombinant proteins determining the manifestation of the specified characteristics in the GMM are evaluated:

- Study the results of a subchronic toxicological experiment on laboratory animals (rodents) and young fertile animals (broiler chickens, lambs, etc.);

- conduct the bioinformatic analysis, search for homology of the recombinant protein with amino acid sequences of toxic proteins, proteins having pharmacological or other biological activity using the PIR, EMBL, SwissProt, GenBank, and other databases;

- study the molecular and biochemical characteristics of recombinant proteins;

2.12. For medical products based on recombinant proteins (enzymes, antibodies) obtained using GMM producers, the potential toxicity of recombinant proteins is evaluated.

- Studies of the stability of recombinant protein during processing, storage, technical processing; influence of temperature and pH, possible modifications and/or formation of stable protein fragments because of various effects;

- The stability of the recombinant protein to proteolytic enzymes (pepsin, imitation of digestive fluids) in an in vitro experiment;

- Studies of acute oral toxicity of protein in a rodent experiment;

2.13. For medical products based on non-protein substances obtained using GMM producers, potential toxicity is assessed by a set of studies involving the study of metabolism, toxicokinetics, chronic toxicity, carcinogenicity, effects on reproductive function and teratogenicity.

2.14. Analyze the results of allergic studies, including:

- Study of the potential allergenicity of the GMM strain for the production of immunobiological and probiotic agents;

- Study of the potential allergenicity of recombinant proteins or nonprotein substances obtained using GMM producers in immunochemical studies in vitro;

2.15. Results of preclinical and field studies of medical products containing GMM strain or obtained with its use (including harmlessness, specific efficacy, duration of immunity, etc.);

2.16. The results of other molecular genetic studies, if such studies were performed;

2.17. Results of post-registration monitoring of GMM strains and products containing strains, in the applicant country and other countries, conducted to identify the unexpected effects of genetic modification that could not be detected at the registration research step. Information on the history of the safe use of the GMM strain / product obtained using GMM.

3. Testing of GMM strains for the manufacturing of vaccines for veterinary use

3.1. The reference standards of composition and properties (GMM and parental strain, or the samples of a vaccine product containing GMM) are provided by the applicant in the quantity necessary for the full extent of testing.

3.2. The GMM safety evaluation considers the results of molecular genetic testing conducted by experts, including confirmation of the presence of declared extraneous genes in the GMM genome and studies for possible undeclared genetic modifications, GMM genetic stability, expression of recombinant genes, taxonomic and other studies.

Research period: not more than 250 days.

3.3. For GMM strains of viruses intended for manufacturing of live vaccines, the cytopathic action on cell cultures (fibroblasts of chick embryos, CHO cultures, NIH, 3T3, HEK-293, etc.) is evaluated. The value of the TCID50 for the GMM should not significantly differ from the TCID50 of the parent unmodified strain.

Research period: not more than 30 days.

3.4. For the GMM bacterial strains, microbiological studies are conducted, characteristic biochemical properties; the sensitivity to antibiotics and bacteriophages, hemolytic activity on human and animal erythrocytes are studied. The properties of the GMM strain should not significantly differ from the properties of the unmodified parent strain; thus, the GMM strain should not be more resistant to antibiotics or have greater hemolytic activity.

For the biochemical identification of the GMM and the parent strain, testing is carried out using test panels produced by national and foreign manufacturers and authorized for use in accordance with the established procedure.

The stability of the phenotypic properties is checked by passaging the strains in liquid and solid growth media with further testing the biochemical properties after 3-5 passages. The similar results revealed by repeated passaging of cultures (at least 10 passages) indicate the stability of the phenotypic properties of GMM and the parent strain. The variability of the results for the greater number of reactions is evidence of the GMM phenotypic instability.

Basis for selection of antibacterial medical products to be included in the study is data on the natural resistance or sensitivity of certain GMM types, data on the parent strain resistance, on the distribution of acquired resistance this strain and on the clinical effectiveness of antibiotics. To determine the sensitivity, the serial dilution and the diffusion methods are used.

Research period: not more than 100 days.

3.5. Determine the presence of pathogenic properties (LD50) and GMM virulence in laboratory animals (SPF mice, chickens, etc.). GMM should be no less safe than the unmodified parent strain.

Research period: not more than 30 days.

3.6. Determine the safety of using the GMM strain in comparison with the unmodified parent strain in animals (birds), for which GMM is intended. A sample set of animals (birds) is given a ten-fold dose of the GMM strain (suggested for use and sufficient for vaccination). Observe the animals for a month. Mortality, clinical condition, hematological and biochemical blood parameters, local allergic reaction to the administration of medical product are recorded. Injection of GMM-based immunobiological preparation to the animal (bird) should not cause any visible clinical changes or negative reactions; blood parameters should not deviate significantly from the reference ranges; there should be no

pathologic anatomical changes at autopsy. The GMM should be no less safe than the unmodified parent strain.

Research period: not more than 90 days.

3.7. Assess the viability of the GMM on environmental objects (including wet filings, cotton swabs, at heat treatment). GMM stability in the environment should be similar to that of the parent microorganism.

Research period: not more than 100 days.

3.8. The tissue tropism of the GMM is evaluated in vaccinated animals (birds) by isolating the GMM from leukocytes, from spleen, thymus and other organs. The GMM should be isolated from the same tissues as the unmodified parent microorganism.

Research period: not more than 100 days.

3.9. The stability of GMM viral strains is evaluated at successive passages on cell culture or in living organisms in comparison with the parental unmodified strain. The strains of GMM that have not shown virulent properties (clinical changes, adverse reactions to administration) after at least six times passage are safe.

Research period: not more than 90 days.

4. Conducting the testing of probiotic GMM strains for veterinary use

4.1. Applicant provides the standard samples of composition and properties (GMM strain and parent strain or samples of probiotic preparation containing GMM) in the necessary amount to conduct the full extent of testing.

4.2. The GMM safety evaluation considers the results of molecular genetic studies carried out by experts including confirmation of the presence of declared extraneous genes in the GMM genome, studies of possible undeclared genetic modifications, GMM genetic stability, expression of recombinant genes, taxonomic and other studies.

Research period: not more than 250 days.

4.3. The characteristic microbiological and biochemical properties of GMM and the parent strain are studied, as well as sensitivity to antibiotics and bacteriophages, hemolytic activity on human and animal erythrocytes. The GMM strain properties should not significantly differ from the properties of the unmodified parent strain; the GMM strain should not be more resistant to antibiotics or have the greater hemolytic activity.

For the biochemical identification of GMM and the parent strain, testing is carried out using the test panels produced by national and foreign manufacturers and authorized for use in accordance with the established procedure.

The stability of the phenotypic properties is checked by passaging the strains in liquid and solid growth media with testing the biochemical properties after further 3-5 passages. The similarity of the results revealed by repeated passaging of cultures (at least 10 passages) indicates the stability of the phenotypic signs of GMM and the parent strain. The results variability for many reactions evidences the instability of GMM phenotypic traits.

Basis for the selection of antibacterial medical products to be included in the study is data on the natural resistance or sensitivity of certain GMM types, data on the parent strain resistance, on the distribution of

acquired resistance among these strains, and on the clinical effectiveness of antibiotics. To determine the sensitivity, the serial dilution and the diffusion methods are used.

Research period: not more than 100 days.

4.4. Determine the adhesiveness of GMM and the parent strain by hemagglutination of human and animal erythrocytes (mouse, rat, rabbit, sheep, etc.). Determine the adhesiveness of the GMM, the parent strain and the reference (unmodified probiotic analog) strain of the same species in the absence of D-mannose (sensitive adhesion). When the significant hemagglutination activity is detected, the adhesiveness in the presence of D-mannose is determined to determine mannose-sensitive or mannose-resistant adhesion.

Quantitation of adhesion is carried out in vivo using biological models (developing chick embryos, enterally infected suckling mice, a tied loop of the rabbit small intestine). According to the degree of adhesiveness, the strains are evaluated as follows: a) highly adhesive strains with an adhesion factor of 3.0 and higher, b) adhesive strains – those from 1 to 3 adhesion factor values, and c) weakly adhesive strains – 0.001-0.9 adhesion factor values. Low-adhesive strains, as a rule, are non-virulent, whereas adhesive and highly adhesive strains usually have certain virulent properties. The GMM strain should have an adhesion coefficient of not more than 0.9 and it should be equal to or less than the adhesion coefficient of the parent strain.

Research period: not more than 60 days.

4.5. Determination of GMM pathogenic and virulent properties in laboratory animals (SPF mice, chickens): The determination of LD50 on SPF mice is carried out by intraperitoneal injection of 0.1 cub.mm of live culture daily at concentrations of 1x10(9), 1x10(7), 1x10(5) and 1x10(3) CFU/cm3. Each group of animals is placed in a separate cage and observed for 10 days for the mortality. As controls, the groups of animals are used, which are intraperitoneally administered a sterile saline solution or a virulent strain with an established LD50 dose. At the end of the experiment, the animal mortality in each group is calculated and the LD50 is determined. If LD50 is equal to 5x10(8) CFU/cm and more, strain is considered non-virulent. The GMM should be no less safe than the unmodified parent strain.

Research period: not more than 30 days.

4.6. Determining the GMM invasiveness: Invasiveness is tested using cornea-conjunctival test. The essence of the method is that the pathogen penetrates the conjunctiva and cornea epithelial cells and multiplies in them causing the tissue destruction. GMM, parent strain and control (unmodified probiotic analog) strain of the same species, should not cause the damage to the conjunctiva or the cornea. In the case of significant invasive properties, a negative conclusion is made about the possibility of using GMM as a probiotic agent.

Research period: not more than 30 days

4.7. Determination of GMM antagonistic or symbiotic activity with representatives of resident intestinal flora: As control strains, unmodified analog of a probiotic strain of the same species, a parent strain and the strains that represent the main species of resident intestinal flora of recipient animals (birds) are used. The latter main species include gram-positive obligate anaerobic bacteria (lactobacilli, Bifidobacterium spp., Peptostreptococcus spp.) gram-negative obligate anaerobes (Bacteroides spp.), facultative-anaerobic microorganisms (E. coli, staphylococci, streptococci) Candida yeasts, etc. Use the characterized museum strains from international collections or collections of reference institutions of the

Russian Federation.

Suspensions of microorganisms with defined concentrations are prepared from control strains grown in solid or liquid media in the presence of GMM (a parent strain or an unmodified analog of a probiotic strain of the same species). 0,5 cm3, 10(1), 10(3) and 10(5) CFU/cm3 of each strain are introduced into tubes with 3.0 cm3liquid nutrient substrate, in which all the microorganisms under investigation can be grown (trypticase-soybean or L-broth). At the same time, control strains are inoculated into selective growth media in the same dilutions to count the cells of each species accurately. After 6 and 24 hours, the microbial mixture is inoculated into selective growth medium and the number of cells of a resident flora and GMM is counted. Only a resident microflora or GMM is grown in the controls. The number of representative cells of the resident flora grown in the presence and in the absence of the GMM is calculated. Approximately the same number of cells grown separately and in the presence of GMM is considered that the GMM does not have an antagonistic effect.

At higher indices of the number of grown cells in joint cultivation, the species are considered to exert a symbiotic effect.

When the number of cells of representative resident flora is reduced by 5 times or more in combination with the GMM, a conclusion is made about the antagonistic action of the GMM to a specific representative of the normal intestinal flora of the recipient animals (birds). If antagonistic effect of the GMM is manifested in the majority of the tested representatives of the normal intestine floral, a conclusion is made that GMM strain cannot be used as a probiotic preparation. When antagonistic action of GMM is revealed to one or two representatives of normal intestinal flora, the results of studies of the antagonistic activity of the parental strain, in vivo studies, and molecular genetic studies to identify genes that determine this phenomenon are evaluated.

Research period: not more than 90 days.

4.8. Immunological testing in laboratory animals.

Immunological studies of probiotic GMM are carried out in an experiment in CBA mice (animal strain characteristic: highly sensitive to the administration of sheep erythrocytes, not sensitive to histamine or Salmonella typhimurium) and C57B1/6 (animal strain characteristic: low sensitivity to the administration of sheep erythrocytes, sensitive to histamine and Salmonella typhimurium). Immunomodulation and sensitizing properties are studied in four tests:

- Effect on the humoral immunity in the test for determining the level of hemagglutinins to sheep erythrocytes;
- Effect on the cell immunity in the delayed type hypersensitivity (DTH) reaction to sheep erythrocytes;
- Sensitizer effects in the histamine sensitivity test;
- Effect on the natural immunity of mice to Salmonella typhimurium;

Research period: not more than 40 days

4.9. The following types of probiotic tests are conducted depending on GMM probiotic strain genus and species:

GMM	Potential risk factor	Tests
Molds	Products of mycotoxins; antibiotics	Determination of mycotoxins and antibiotics in a probiotic preparation

Yeasts (saccharomycetes)	Excessive production of ethanol; allergenic properties	Determination of the ethanol concentration in the probiotic preparation; allergenic properties studies
Streptomycetes	Antibiotic production	Determination of antibiotics in a probiotic preparation
Spore-forming bacilli	Excess proteolytic and hemolytic activity; formation of antibiotic substances	Tests for acute toxicity of a probiotic preparation; hemolysis under the influence of GMM; determination of antibiotics in a probiotic preparation
Enterococci	Formation of N-nitrosamines, histamine; antibiotic resistance	Determination of histamine, N-nitrosamines; identification of vancomycin and rifampicin resistance genes
Lactobacilli	Excess formation of D (-) lactic acid in the product	Determination of D (-) lactic acid concentration in a probiotic product

5. Conducting the testing of medical products containing non-viable GMM, for assessment of biological safety

5.1. Standard samples of composition and properties (GMM strain and parent strain or samples of probiotic preparation containing GMM) in the necessary amount to conduct the full extent of studies are provided by the applicant.

5.2. The GMM safety evaluation considers the results of molecular genetic studies carried out by experts, including confirmation of the presence of declared foreign genes in the GMM genome, studies of possible undeclared genetic modifications, genetic stability of GMM, expression of recombinant genes, taxonomic and other studies.

Research period: not more than 250 days.

5.3. The absence of viable cells (virus particles) of the claimed strain is tested in the product samples using microbiological (virological) methods. As control culture, live GMM cultures and parental strains are used.

Research period: not more than 30 days.

5.4. Testing is performed to study the safety of medical products containing non-viable GMM for animals or birds of recipients. In the study, a sample of animals is administered a ten-fold dose (relative to the dose intended to use) of the product. Observations are carried out for a month. The mortality, clinical condition of animals, hematologic and biochemical blood parameters, local allergic reaction to the product administration are recorded. GMM-based drug courses to the animals (birds) should not cause visible clinical changes and adverse reactions; blood parameters also should not deviate significantly from the standard; there should be no pathologic changes at the necropsy.

Research period: not more than 90 days

6. Testing of medical products containing individual ingredients synthesized by GMM or obtained with GMM use for assessing the biological safety

These products include vitamins, essential amino acids, antibiotics, substances that have a hormonal or immunomodulation effect, enzymes obtained using GMM producers. The product type where GMM is used only as a producer at the production stage is potentially the safest, since in this case the technology

provides for the complete removal of the producer strain from the final product.

In assessing the safety of these products for veterinary use, the results of molecular genetic testing conducted by experts are considered, including the confirmation of the recombinant DNA absence in the preparation.

Research period is not more than 30 days.

When receiving the results that contradict or do not correspond to the characteristics declared by the applicant/manufacturer, two repeated arbitration testing are carried out.

Annex 7. Draft 64521 - Procedure for Conducting Molecular Genetic Testing of GE Organisms for Manufacturing Medical Products for Veterinary Use

Draft

Procedure for conducting molecular genetic testing of genetically modified organisms for manufacturing of medical products for veterinary use

The procedure was developed in accordance with Decree of Government of the Russian Federation No. 839 of September 23, 2013 "On the state registration of genetically modified organisms, intended for release into the environment, as well as products obtained using such organisms or containing such organisms." It applies to molecular genetic testing with safety assessment of genetically modified organisms for manufacturing of medical products for veterinary use.

Medical products obtained using genetically modified microorganisms (GMM), can be divided into the following categories:

Category 1 - immunobiological and probiotic preparations containing living/viable microorganisms (viral vector vaccines, live bacterial vaccines, probiotic agents containing strains of bacteria, yeasts, micellar fungi);

Category 2 - immunobiological preparations containing non-viable microorganisms (inactivated vaccines);

Category 3 – medical products containing components (enzymes, monoclonal antibodies, antibiotics, vitamins, amino acids, hormones) obtained using GMM producers;

The aim of the research is to assess the GMM safety for animals and the environment in accordance with their intended type of use.

1. General provisions and application area

1.1. This procedure establishes the requirements for conducting molecular genetic testing in the control of GMM strains used in the manufacture of medical products for veterinary use.

1.2. Requirements in this procedure are mandatory for the state registration of GMM strains for targeted use in the manufacture of medical products for veterinary use, as well as for state registration, monitoring and selective quality control of immunobiological, probiotic and medical products for veterinary use obtained from GMM strains or obtained using such organisms.

1.3. The procedure was developed to provide a universal, scientifically validated system for assessing the GMM biological safety, and it considers the modern methodological approaches developed

in Russia and recommended by international organizations (OIE, WHO, FAO, etc.).

2. Expert review and evaluation of data characterizing the GMM for manufacture of medical products for veterinary use

General characteristics of the GMM include a review of the documentation submitted by the applicant, containing:

2.1 Description of properties obtained by the GMM strain because of modification:

- Description of the structure of the genetic construct (introduced or removed) and its localization, the characteristics of the expression of the inserted or altered genes;
- Characteristics of the differences between GMM and the parental organism including a description of the method of reproduction and propagation, growth patterns, new phenotypic properties;
- Characterization of GMM genetic and phenotypic stability including the ability to transfer transgenes to other organisms;

2.2. Description of procedures enabling to identify the GMM strain (to confirm its taxonomic status and the characteristics of the genetic modification) including testing protocols, description of the primer nucleotide sequences, standard samples of the composition and properties;

2.3 Comprehensive information on the original parent microorganism (taxonomic characteristics, description of propagation and distribution methods, data on virulent, allergenic and other biological properties);

2.4. Information about donor organisms of introduced genes (taxonomic characteristics, data on virulent, allergenic and other properties);

2.5. Information on the genetic modification method (description of the modification method, vector structure, insertion structure);

2.6. Information on the study of genetic safety and stability of the GMM strain;

2.7. The results of other molecular genetic studies, if such studies were performed;

2.8. Results of post-registration monitoring of GMM strains and products containing strains, in the applicant country and other countries conducted to identify the unidentified effects of genetic modification that could not be detected at the stage of registration research.

3. Conducting the molecular-genetic testing of GMM strains for the manufacture of medical product for veterinary use

An expert molecular genetic testing includes confirmation of the data provided by the applicant and a check for possible undeclared genetic modifications of the GMM strain.

3.1. Applicant provides the cultures of GMM and parental strains in the quantity necessary for conducting the full extent of research.

3.2. Sequencing of GMM genomes and parental strains is carried out to confirm the presence of the claimed foreign genes at a specific location in the microbial genome and to check for possible undeclared genetic modifications.

Method of detecting the fluorescent signals of labeled nucleotides incorporated into surface molecular

clusters during *in situ* synthesis is used for poly-genomic sequencing.

Genomic DNA (RNA) is isolated from the GMM and parental strains using reagent kits in accordance with the recommendations of the equipment manufacturer. DNA library of the GMM and the parent strains is prepared by using the sequencing kits for one end or from both ends.

The reading length is 36 nucleotides from one end of the fragment or 150 nucleotides from each end of the fragment. To analyze the resulting cluster images and to convert them into a DNA sequence, the software packages corresponding to the analyzer are used. The depth of the genome reading coverage should be at least 20 times in all areas. When the genomes are aligned, localization of the claimed genetic modification, regulatory and marker vector sequences of the GMM are revealed. The obtained data on the structure and its localization are compared with the information provided by the applicant.

The investigated strains of microorganisms (GMM and parental) should be different only in the area of the declared genetic modification, the matching of the nucleotide sequences should be at least 99% in the remaining regions of the genome.

Research period: not more than 180 days

3.3. The PCR technique for identification of the GMM line submitted by the applicant is subjected to validation, its sensitivity and specificity are assessed.

The analysis protocol provided by the applicant includes a description of the PCR primers and the thermal cycling regime.

Synthesis of oligonucleotides used as PCR primers is performed in accordance with the information provided on their nucleotide sequences.

The specialized equipment required for PCR diagnostics is described in ISO 20837:2006 and ISO 20838:2006. Testing includes the sequential processes: preparation of samples, isolation of a nucleic material (DNA or RNA) from the product sample, amplification of DNA (cDNA), detection of amplification products, analysis and interpretation of results. For the testing of genetically modified RNA viruses, the use of the "RNA SORB" kit produced by the Central Research Institute of Epidemiology (or equivalent) is recommended for the isolation of nucleic material. In the study of genetically modified DNA viruses, bacteria, yeast, micellar fungi, it is recommended to use a ready-made kit to extract DNA from the samples. For PCR and RT-PCR, the use of a thermostable DNA polymerase, reverse transcriptase, a mixture of deoxyribonucleotide triphosphates, and ten-fold buffers for reactions is recommended. Detection of amplification products is conducted by electrophoretic separation of PCR fragments in an agarose gel. The results of PCR analysis are recorded by the presence or absence of specific amplified DNA bands in the electrophoregram.

The PCR technique should have 100% specificity (no cross-reactions with nonspecific DNA at a concentration of up to $1x10^6$ genomic equivalents / ml) and high sensitivity (at least $1x10^4$ genomic equivalents / ml).

Research period: not more than 30 days.

3.4. An analysis is conducted of the functioning of the target insert with the detection of the target gene expression products, thus measuring:

- The amount of mRNA transcribed from the target gene, by reverse transcription - polymerase chain reaction (RT-PCR);
- The presence of recombinant protein by electrophoretic separation in a polyacrylamide gel; the

protein pattern profiles of the GMM strain and the parent strain are compared;

- The recombinant protein specificity by the immunoblot method;

Research period: not more than 60 days

3.5. To determine the genetic stability of GMM strains of viruses, full genome sequencing is used after six passages in a cell culture or in living organisms.

To determine the genetic stability of the GMM strains of bacteria, yeasts or molds, at least ten passages are performed on liquid and solid growth media, followed by complete sequencing of the microbial genome.

The GMM strain is considered genetically stable and safe if genetic changes in the genome are not detected, including no mutations, partial or complete loss of the modified fragment or its transfer to other genome areas after at least ten passages for GMM bacterial strains, yeast, micellar fungi and six passages for G virus MM strains.

Research period: not more than 250 days

3.6. To assess the stability of the foreign gene expression in GMM virus strains, an assessment of the change in the recombinant protein production is made after six passages on cell cultures (according to 3.4).

To assess the stability of the foreign gene expression of GMM strains of bacteria, yeasts or molds, the target insert is analyzed after ten passages on liquid and solid growth media (according to 3.4). The expression level of the recombinant protein should be stable.

Research period: not more than 150 days.

4. Conduction of molecular genetic testing of medical products obtained from/using GMM

An expert molecular genetic testing of medical products obtained from/using GMM includes the confirmation of the data provided by the applicant and verification of possible undeclared genetic modifications.

4.1. Standard samples of the composition and properties, including samples of the medical product obtained from/using the GMM, in the necessary amount to conduct the full extent of research are provided by the applicant.

4.2. For medical products containing viable and non-viable GMM, the presence of the claimed foreign genes is confirmed in the genome. The place of their localization, the regulatory and/or marker vector sequences are found. The GMM study for the presence of the claimed modifications is conducted using the validated PCR identification technique proposed by the applicant / manufacturer (see 3.3). The validated methodology for identifying this GMM can also be obtained from other authoritative sources.

The results of the PCR assay are further confirmed by determining the nucleotide sequence by Sanger's sequencing with dideoxynucleoside triphosphate termination. For sequencing, it is recommended to use analyzers, which reliably determine the nucleotide sequence of DNA up to 500-800 base pairs in one reading.

Research period: not more than 45 days.

4.3. For medical products containing viable and non-viable GMM, GMM taxonomy is confirmed by determining the nucleotide sequence of fragments of the recipient strain genome. In the case of

genetically modified bacteria, a sequence of the conserved region of the genome, the 16S rRNA fragment of the gene, is usually detected. The study is performed by amplifying a DNA fragment with the universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and U1492R (5'-GGTTACCTTGTTACGACTT-3') or species-specific primers for the claimed strain, followed by Sanger sequencing. In the case of genetically modified viruses, a region of the genome not affected by the genetic modification (genes for glycoproteins, capsid, matrix proteins, etc.) is chosen as the target. The specific oligonucleotide primers are selected using the specialized software in accordance with the requirements for PCR primers; the primer specificity is evaluated by matching with different genomes using the local match algorithm in the sequence databases. The sensitivity and specificity of the selected primers is confirmed on DNA/RNA preparations from strains of various microorganisms (the required sensitivity is not less than $1x10^4$ genomic equivalents/ml, with the absence of cross-reactions with nonspecific DNA at a concentration of up to $1x10^6$ genomic equivalents/ml). By sequencing the PCR fragments obtained using these primers, the genetic homogeneity of the GMM from the medical product is evaluated and the phylogenetic analysis of the target sequence with similar sequences from nucleotide base sequences is performed using the proper software.

If it is necessary to establish the GMM strain, additional studies are carried out by sequencing the extended regions of the genome obtained by PCR amplification with specific primers.

Research period: not more than 70 days.

4.4. In the case of medical products where GMM strains are used as producers (category 3), the production technology requires the complete removal of the producer strain from the final product (purification). To eliminate the risks associated with breaches of production technology and incomplete GMM removal during the purification of the (cellular) producer strain components, as well as the dangers of a viable GMM presence, PCR is conducted to confirm the absence of transgenic GMM sequences in the final product.

For the DNA isolation from medical products (enzymes, monoclonal antibodies, antibiotics, amino acids, vitamins, etc.) it is recommended to use a ready-made kit.

PCR diagnostics is conducted using the validated PCR identification method of the gene modification line of the producer strain presented by the applicant. As a positive control, the GMM nucleic material of the producer strain is used.

Research period: not more than 30 days.

4.5. For medical product containing viable GMM, an analysis of the function of the target insert is performed to identify the expression products of the target gene:

- The amount of mRNA transcribed from the target gene, by reverse transcription - polymerase chain reaction (RT-PCR);
- The presence of recombinant protein by electrophoretic separation in a polyacrylamide gel; the protein patterns profiles of the GMM strain and the parent strain are compared;
- The specificity of the recombinant protein by the immunoblot method;

Research period: not more than 60 days

4.6. For medical products containing viable GMM strains of viruses, genetic stability is assessed by the presence of changes in the nucleotide sequence of the genome after six passages of the virus on a cell culture or in living organisms.

For medical products containing viable GMM strains of bacteria, yeasts or molds, genetic stability is assessed by the presence of changes in the nucleotide sequence of the genome after ten passages on liquid and solid growth media.

Sanger sequencing and restriction analysis of the genome are used to determine the genetic stability.

GMM strains are recognized as genetically stable and safe if genetic changes in the GMM genome are not detected, including no mutations, partial or complete loss of the modified fragment or its transfer to other genome regions after at least six passages.

Research period: not more than 180 days.

4.7. For medical products containing viable GMM strains of viruses, an evaluation of the stability of the expression of a foreign gene is performed to detect changes after six passages on cell cultures (according to 4.5).

For medicinal preparations containing viable GMM strains of bacteria, yeasts or molds, the stability of a foreign gene expression is evaluated with the detection of changes after tenfold passaging on liquid and solid growth media (according to 4.5).

The expression level of the recombinant protein should be stable.

Research period: not more than 150 days.

4.8. For medical products containing viable and non-viable GMM, screening studies are conducted for the presence of undeclared genetic modifications. The study is based on the recommendations of international organizations (FAO / WHO / OIE) and involves the identification of DNA sequences of the most commonly used plasmid vectors, through which genetic information is introduced into the body.

The multicomponent reagents and primer sets (synthesized oligonucleotides complementary to respective portions of a target DNA) are used to identify the most typical marker and selective genes (antibiotic resistance genes, bacteriocins, β-galactosidase, β-glucoronidase, bacteriophage T7 RNA polymerase, regulatory sequences of promoters and terminators, poly-linkers, sequences of migrating elements of transposable vectors). Detection of these sequences indicates the possible presence of undeclared genetic modifications and requires further investigation. Sequence information on marker and selection genes that are an integral part of genetic engineering structures is available in international databases. Specific oligonucleotide primers for the identification of these sequences are selected using the appropriate software in accordance with the requirements for PCR primers. The specificity of the primers is estimated by equalization with different genomes using the local alignment algorithm in databases. The specificity and sensitivity of the selected primers is confirmed on DNA preparations from strains of various microorganisms (the required sensitivity is not less than $1x10^4$ genomic equivalents / ml, the absence of cross-reactions with nonspecific DNA at a concentration of at least $1x10^6$ genomic equivalents / ml). Examples of PCR primer pairs designed to identify marker and selective GMM genes in screening PCR studies for the presence of undeclared genetic modifications are shown in Table 1.

Table 1. PCR-primers for the detection of some marker and selective genes that are constituent parts of genetically engineered constructs

Target	Description	PCR-primers, 5' — 3'
Gene LacZ	Encodes the enzyme β-galactosidase; tt is used in the vector plasmid constructs	AGGGGGATGTGCT

	(pCR2.1, TOPO, pVIK 112, pBSKSII and others), includes the region of the poly-linker for the cloning of recombinant genes. In the presence of this enzyme, the colorless X-gal substrate turns into a colored product, the bacterial colonies expressing LacZ have a blue color.	
Gene gfp	Gene of the green fluorescent protein is used in a number of vector plasmids for genetic engineering manipulations (pKEN-gfpmut2, pRK415-gfp and others) as a luminous mark.	Gfp-F: TTGTTGATATTAGATGGCGATGTTA Gfp-R: TTTGGAAAGGGCAGATT GTGT
Gene amp	Encodes ampicillin resistance, used in a number of vector plasmids for antibiotic resistance selection (pBR322, pUC18, pBSKSII, pET, pCR2.1 TOPO and others)	Amp-F: GTGGGCTATATCGAACTGGA Amp-R: CCCGTCGTGTAGATA ACTACG
Gene Km	Encodes kanamycin resistance, used in a number of vector plasmids for selection by antibiotic resistance (pVIK112, pMC212 and others)	Km-F: CGGAAGGAATGTCTCCTGC Km-R: CGACATACTGTTCTTCCCCG
Gene tetO	Encodes tetracycline resistance, used in a number of vector plasmids for antibiotic resistance selection (pBR322 and others)	TetO-F: CGCCTGCAGAGGGCGGCACGGATCA TetO-R: CGCCTGCAGGGCCAACGTTCCAACC
Fl ori	Origin of plasmid replication (pBSKSII, pKEN-gfpmut2, pCR2.1 TOPO and others)	fl-F: TAAGCGCGGCGGGTGTGGT fl-R: TTGGGGTCGAGGTGCCGTAAA
Transpo Tn7L	The fragment provides a transposition of the recombinant genes from the plasmids to the genome (pRK415-gfp, etc.)	IncP- F: CAGAGCAGGATTCCCGTTGAG IncP- R: GGGCAGGATAGGTGAAGTAGG
R6K ori	Origin of plasmid replication (pVIK112, etc.)	R6K-F: GACAAA
Gene ermC	Encodes erythromycin resistance of plasmid vectors	ErmC-F: AGTAC AG AGGTGTA ATTTC G ErmC-R AATTCCTGCATGTTTTAAGG
Gene	Encodes chloramphenicol resistance of	Cat-F:

cat	plasmid vectors	ATGAACTTTAATAAAATTGATTT AGA Cat-R: AGCATTTTTCAGGTATAGGTG

If genetic modifications are found, further research is conducted to identify the specific genetic structure of the GMM.

Research period: not more than 90 days.

When receiving the results that contradict or do not correspond to the characteristics declared by the applicant/manufacturer, two repeated arbitration studies are conducted.

Annex 8. Draft 63060 - Procedure for Molecular Genetic Testing and Expert Assessment of the Biological Safety of GE animals

Draft

Attachment 3

Approved by the Order of the Ministry of Agriculture
of the Russian Federation
Dated _____ No ____

Procedure for molecular genetic testing and expert assessment of the biological safety of genetically-engineered-modified animals

I. Scope of application

1. This procedure establishes the process of molecular genetic testing and expert assessment of the biological safety of genetically-engineered-modified animals (hereinafter – GEMA) to be performed by the testing laboratories accredited with the national accreditation system.

II. General provisions

2. The primary objective of this procedure and instructions is provide assistance in ensuring an adequate level of protection in the area of safe transfer, processing and using live GEMA that have been created through the use of advanced biotechnology and that may affect the preservation and sustainable use of biological diversity and the human health.

3. The procedure is targeted at assessing risks and developing risk management measures to ensure GEMA safety for the environment and human health, as well as to prevent their uncontrollable spread.

4. The procedure shall establish on the territory of the Russian Federation the requirements for assessing the safety of GEMA necessary for their state registration exercised by the Federal Service for Veterinary and Phytosanitary Surveillance (Rosselkhoznadzor).

5. The procedure is mandatory for execution by Russian and foreign entities involved in scientific, research and administrative activities, irrespectively of their organizational and legal type, that operate on the territory of the Russian Federation in the areas of creating and using GEMA, as well as make transactions, including those in foreign trade, where GEMA are a subject matter.

6. The procedure is applied to all options of GEMA release into the environment, e.g. to those limited by

time and scale, as well as large-scale releases. However, the scope and type of the available data as well as the information required for assessing the risks associated with various types of intended releases into the environment will be different and defined on a case-by-case basis.

7. The procedure specifies general criteria for the assessment of risk arising from the first GEMA release into open system on the territory of the Russian Federation and/or GEMA imports for the purpose of breeding; the procedure also provides guidance for acquiring the reliable data necessary for assessing the biosafety and developing the risk management strategy.

8. The procedure suggests general approaches to the reduction of potential risks and the prevention of uncontrollable GEMA spread during their use/tests in open systems.

9. Duration of expert assessment:

application consideration – no more than 30 days;

complete expert assessment – no more than 200 days.

10. Justification of a refusal decision of the expert assessment

In case where the testing laboratories accredited with the national accreditation system make a refusal decision on the prospects of industrial use of the GEMA submitted for registration, the applicant shall be given a justified denial, specifying its reasons and ways of potential corrective actions.

11. Possibility of expert re-assessment after making a refusal decision

In case where the applicant has successfully eliminated the cause underlying the refusal decision made by the testing laboratories accredited with the national accreditation system, a re-assessment can be performed not earlier than 30 days from the date of receipt of such decision.

III. Assessment of the risk associated with the GEMA release into the environment

12. Risk assessment has the following objectives:

a) to identify potential hazards posed by a GEMA to the environment (e.g. human health), arising from the GEMA release into the environment due to the genetically engineered modification performed in the animal and to safeguard the preservation and sustainable use of biological diversity;

b) to conduct comprehensive assessment of the likelihood of a hazard occurrence and scenarios if the hazard occurs;

Risk assessment is a scientific process of estimating the potential of a hazard to give rise to an adverse outcome. This estimation is based on a combination of the likelihood of the hazard occurring and the consequences if the hazard occurs.

c) to prepare a report on the potential risk and recommendations concerning the biosafety status of the GEMA.

13. The following guidelines should be employed to assess the risk associated with the GEMA use (release) in open systems:

a) risk assessment should be carried out in a scientifically based and transparent manner, taking into consideration the recommendations of experts and the guidelines developed by the relevant international organizations;

b) a lack of scientific knowledge or scientific consensus should not be interpreted as a reference to a

certain level of the existing risk or missing risk, or the risk acceptability;

c) risks associated with live modified organisms or products containing such organisms should be considered in context of risks caused by non-modified recipients (analogues) or parent organisms in the probabilistic potential receiving environment;

d) the assessment of risks should be performed on a case-by-case basis. The necessary information may vary with respect to its contents and level of details in every particular case, depending on the relevant live modified organism, its potential use and the probabilistic potential receiving environment;

e) the risk assessment must describe value of all completed studies and identify all areas of uncertainty;

f) the risks from the integration of GEMA created through the use of biotechnology are usually similar to the risks arising from the use of non-modified organisms or organisms modified by other methods;

g) for assessing the GEMA risks, a differentiated approach to various species should be used. As a rule, the following four major groups of animals are distinguished: mammals, birds, fishes and insects;

h) the risk should be re-assessed continuously and revised as updates become available;

i) a scientifically based and lifelong monitoring of GEMA is required – a system of regular observations of the spread, population number, physical and behavioral condition of the GEMA, as well as the structure, quality and the area of their habitat.

14. GEMA biosafety assessment includes the following three major stages:

a) to detect and characterize hazards and to estimate the risk arising from the GEMA release into the environment;

b) to develop risk management plans and methods, including monitoring;

c) to make decision on the GEMA biosafety.

15. The GEMA designing entity is responsible for identifying hazards and assessing risks. The risk analysis should also include a potential beneficial impact (e.g. non-target) of the GEMA on the receiving environment and human health.

16. Risks associated with live GEMA containing detectable novel combinations of reproducible genetic material should be considered in the context of risks caused by non-genetically modified recipients or parental organisms in the probabilistic potential receiving environment;

17. There are multiple common factors to be taken into consideration for identifying hazards arising from a transgene entry into the environment.

18. A transgene entry into the environment occurs through:

the vertical transfer of a genetically engineered construct;

the expansion to new territories (same as through the invasion of new or exotic species);

the horizontal transfer of genetically engineered construct with the use of microbiological mediators or through the combination of these factors.

19. Risks arising from the GEMA use and release into the environment:

potential changes in the natural ecosystems and impact on biological diversity from the GEMA use (release);

potential impact of the products of expression of a built-in gene on non-target organisms;

potential recombination between animal viruses and viral sequences of GEMA transgenes;

horizontal transfer of genes from a GEMA to the environmental microorganisms and its potential effects;

vertical transfer of a genetically engineered construct to the live environmental organisms.

20. Initial GEMA classification by risk level is based on the potential hazard of a transgene contained in the GEMA and a vector used for the transfer:

the GEMA created with virus-free vectors and posing no obvious hazards to humans or the environment (vectors – bacterial plasmids; genes coding structural cell proteins);

the GEMA created with virus vectors, posing no obvious hazards to humans or the environment (vectors – elements of viral genomes; defective viruses; genes that do not encode physiologically active proteins);

the GEMA created with virus or virus-free vectors and posing a potential hazard to humans and the environment (vectors – bacterial plasmids or defective viruses; genes that encode hormones, growth factors and other physiologically active proteins);

the GEMA created with virus or virus-free vectors and carrying genes with a predictable threat to humans and the environment (genes that encode prions, onco-proteins, products of viral genes, etc.).

21. Risk assessment includes the following:

to identify potential adverse effects of GEMA on the receiving environment, including human health;

to evaluate the probability of these adverse effects;

to identify and evaluate beneficial effects of GEMA on the receiving environment, including human health;

to assess the cumulative outcome from the GEMA release into open system and/or its industrial applications.

22. To assess GEMA risks for the environment the following data will be needed:

molecular characteristics of vector, transgene and its expression in the GEMA;

investigation of GEMA genotypic and phenotypic changes, including its reproductive function, behavior, health and welfare;

investigation of the transgene stability through the GEMA lifespan and its multiple generations;

projection of potential non-intended transgene effects on the GEMA;

assessment of the potential for horizontal gene transfers;

assessment of potential ecological effects caused by the GEMA; description of habitat areas and effects on biodiversity;

direct and indirect studies of toxicity and allergenicity of foodstuffs and feeds derived from the GEMA;

demonstration of the efficacy and the evaluation of potential environmental effects, as well as the appropriate measures to alleviate the consequences that could reduce or alter the identified impacts toward the desirable direction;

evaluation of the environmental risks through the regular assessment of ecotoxicity and biodegradability of every ingredient;

describing differences between the GEMA and conventional organisms of the same species as regards the contents of proteins, fats, carbohydrates, amino acids, vitamins, trace elements, etc.

23. The risk assessment associated with the GEMA use should be based on the review of known research and empirical data, as well as the experimental results pertaining to GEMA characteristics with consideration of the following:

recipient organism;

donor organism;

vector;

novel trait(s);

objective and geographic location of the proposed release;

scope of the release;

potential receiving environment.

24. The following key criteria are taken into account for assessing the risk:

for the recipient organism:

mode of reproduction and distribution;

invasive potential;

capability to produce viable fertile offspring with the wild-living related species;

for the donor organism:

pathogenicity;

toxicity and allergenicity of the products of expression of the target gene;

for the vector:

molecular biological characteristics;

safety characteristics;

for the recombinant DNA:

DNA fragment or full-sized gene replica;

promotor and other regulatory sequences;

selective antibiotic-resistance marker genes or reporter genes;

for the expressed protein:

toxicity and allergenicity (known or potential) for humans and organisms-inhabitants of the potential receiving environment.

for the GEMA:

conformity to the declared genetically engineered construct (insert);

toxicity and allergenicity;

ability to transfer transgene to other organisms;

ability to produce viable fertile offspring with the related and non-related animal species;

invasive potential;

type of interaction between the GEMA and target organisms;

capability for impact and type of impact of the GEMA on non-target organisms;

ability to transfer transgene to the potential receiving environment (qualitative assessment of vertical and horizontal gene transfer);

potential for the recombination between animal viruses and viral nucleotide sequences of the GEMA transgenes;;

potential trophic chains.

for the potential receiving environment:

geographic location of the planned GEMA release site;

specifics which may cause adverse effects of the GEMA;

remoteness from the places of residence;

the capability to have mating with the animals living closely to the site of release

the ability of animal organisms, other than the GEMA, to receive genes from the genetically modified animals in the potential receiving environment as a result of the release;

data on the natural ecosystems (including their organisms-inhabitants) which can enter in interaction with the GEMA or get exposed to their impact, e.g. indirectly.

25. Long-term risk evaluation and decision making in case of the GEMA release into the environment require that a specific scenario is developed on a case-by-case basis. However, there are multiple common factors to be taken into consideration for identifying hazards arising from transgene entry into the environment.

26. The risks associated with live modified organisms and their metabolic products, i.e. processed materials (feces, urine, saliva, blood, cells, etc.) derived from a live modified organism should be considered in comparison with the risks caused by non-genetically modified organisms in the potential receiving environment (a principle of composition equivalence). The principle is based on the GEMA comparison with the original conventional analogue. The comparison is made for contents of the major food groups (proteins, fats and carbohydrates), micronutrients, minor non-food biologically active components, anti-alimentary and toxic substances, allergens, typical for a particular organism species or determined by the properties of transferred genes.

27. The objective of the comparative approach is to identify changes between the GEMA and compared organism(s) that may cause an adverse impact. The selection of organisms for comparison may have a significant influence on the relevance and interpretation of the results obtained in the process of risk assessment, as well as on the conclusions based on such results. To this end, it is necessary to select one or more organisms from those identified for comparison, taking into consideration their potential for providing relevant information required for the risk assessment objectives. For considering variations resulting from the interaction with the environment, it is advisable to perform the evaluation of GEMA

and compared organism at the same time and in the same location under the same environmental conditions.

28. The iterative method can be also employed for the risk assessment procedure, where certain stages can be revised as updates are becoming available or circumstances that may reverse the conclusions, are changing.

29. After review of the submitted data, the Rosselkhoznadzor will assess the risk from the GEMA use (release) in open system. For this purpose, it will compile a report on the presence of risk for every particular GEMA, for particular conditions of its release or use in open systems and forward this report to the Russia's Ministry of Agriculture. The report will serve as a basis for making decision on the GEMA biosafety.

IV. Method used for the biosafety assessment

30. To analyze a potential transgene (construct) spread in open systems (e.g. during the monitoring) the following two major approaches or their combinations are recommended:

a) protein-specific methods based on the detection of protein expressed by incorporated DNA:

- *ELISA (Enzyme-linked Immunosorbent Assay)* is used to measure the required protein in a sample;
- *Rapid immunochromatographic test kits on paper-based platform - LFS (Lateral Flow Strip)* technology is immunochromatography in thin layer using protein-specific antibodies based on the difference in mobility between the immobilized and conjugated antibodies. It is can be recommended as a primary "field" method for GEMA monitoring/screening;
- *immunoblot* is used to characterize the transgene's protein product nativity and identity to the original gene;
- b) methods characterizing the genome region of foreign DNA integration, positions and levels of transgene expression:
- *PCR (polymerase chain reaction*);
- *nucleic acid hybridization (Southern/Northern Blot)* in the analysis of DNA/RNA sequence.

31. To determine functional activity of a transgene product, the test kits with cultures of postnatal diploid human fibroblasts are used.

32. For analyzing functional status of the GEMA cells *in vitro*, it is advisable to derive primary cell cultures from this organism.

33. For investigating immunological status of GEMA, immunoglobulins in the blood serum should be determined by gamma-globulin, using the precipitation method.

34. For assessing potential allergenicity typical for the cell metabolic products of the GEMA released into the environment, it is possible to use an experimental model of systemic anaphylaxis which develops in the laboratory animals (rats) after their intraperitoneal sensitization followed by intravenous injection of the challenging dose of homologous protein antigen.

35. The assessment of potential allergenicity of novel expressed proteins gives consideration to the following: protein size (length of the known allergens is usually not below 10-40 kD); protein resistance to digestion and processing (most of the known allergens are digested slowly in the gastro-intestinal tract and are resistant during processing); similarity of the protein structure to that of the known

allergens. If a gene is transferred from the donor organism which is an allergen, it should be proven that the derived product does not contain allergens.

36. To investigate carcinogenic and mutagenic effects of the GEMA metabolic products on the operational personnel and the environment, several methods are used:

chromosome aberration test in the mammalian bone marrow cells;

carcinogenicity tests in mice or rats.

37. To assess embryotoxicity and teratogenicity, the GEMA tissue homogenate is fed to pregnant rats. Similar groups of the control animals are fed with homogenate of the same tissue of animals of the same species. The following criteria characterize damaging effects of the transgene products during pregnancy:

а) embryo deaths characterizing embryotoxic properties;

б) congenital abnormalities (malformations) characterizing teratogenic effect.

38. Chronic toxicity of the GEMA should be studied in at least two animal species. The groups of small laboratory animals should include not less than 10 animals, and the groups of large animals – not less than four animals. The main route of administration of the GEMA tissue homogenate is through gavage for 7 days a week.

39. The transgene impact on the GEMA reproductive function should be investigated by mating these animals and, in parallel, in animals of the same species containing no trasgene. Then indicators of the two animal groups are compared. The suppression of reproductive function can be caused by multiple factors: gametogenesis disorder; impairment of the endocrine function of genital glands, hypophysis and adrenal glands; affection of the hypothalamus and some of the cerebral centers, etc. The reproductive function impairment can develop either due to the occurrence of insertion mutation resulting from gene integration in the genome, or the expression of transgene integrated in the genome. In case of the GEMA entry into open system, it cannot be ruled out that the transgene will be transferred to the genome of the local animal population as a result of random mating and thus modify the local population genetic pool. Continuous monitoring of the GEMA is required for the rapid detection, prevention and elimination of adverse processes and events in order to preserve biological diversity, ensure steady-state condition of animals and their scientifically based use.

40. The DNA marking methods should be used for genetic marking of a GEMA. In addition to the presence of transgene, it is important to have genome passport of the GEMA. Such approach enables to identify more accurately the cases of uncontrolled animal mating. Genome analysis is also used for assessing potential genome rearrangements of the GEMA. It is advisable to conduct monitoring of the GEMA genome stability which implies the analysis of hyper-variable sequences in the recipient organism, in the GEMA immediately after its creation, and in the GEMA at different stages of ontogenesis.

41. In addition to the information on characteristics of the GEMA parent stock, it is necessary to review and assess specific data on the potential interaction between the transgenes and other genetic elements (e.g. promoters and other regulating elements), proteins, metabolites or other modified traits, as well as endogenous genes and their products in the packaged GEMA. To this end, emphasis should be made on the transgenes belonging to the same biochemical pathways or physiological processes.

V. Biosafety tests

42. Biosafety testing comprises the GEMA controllable release under the conditions of limited and secured experimental sites, using special measures for limiting risks.

43. The objective of biosafety testing is to receive experimental data and information necessary to make a scientifically-based comprehensive evaluation of the risk arising from the GEMA use (release) in open systems or for acquiring additional data in accordance with p. 12 of the Rules on the State Registration of Genetically-Engineered-Modified Organisms Intended for Release into the Environment as well as Products Derived from the Use of Such Organisms or Containing Such Organisms" approved by Resolution of the Government of the Russian Federation # 839 dated September 23, 2013 "On the State Registration of Genetically-Engineered-Modified Organisms Intended for Release into the Environment as well as Products Derived from the Use of Such Organisms or Containing Such Organisms" at the time of submission an application for the GEMA state registration.

44. Tests are performed at the sites with natural and climatic conditions similar to those at the site proposed for the GEMA release into the environment.

45. Tests are performed within a contract concluded between the GEMA owner (and/or the applicant) and the GMO Safety Testing Center. The testing program shall be forwarded for notification to the Expert Advisory Board.

Test results are documented in the report to be submitted to the Expert Advisory Board. The level of details of the information described in the report depends on the level of potential risks and can be determined on a case-by-case basis by the applicant (owner) and/or the Expert Advisory Board.

The general principles of risk minimization during the GEMA safety testing should be in agreement with the earlier detected risk degrees.

46. Entities conducting biosafety tests should consider the following minimal requirements:

- requirements for the prevention of undesirable transgene transfer during the GEMA tests;
- requirements for the location of experimental site;
- requirements for the prevention of unauthorized access to the experimental site;
- requirements for the safe storage and transportation of GEMA specimens;
- requirements for the GEMA use;
- requirements for the experimental site monitoring measures;
- requirements for the personnel performing test.

VI. Recommendations for risk reduction

47. One of the key problems is to combine the risk assessment (determining the probability of the occurrence of potential hazard) with the risk management system – a decision-making and risk monitoring system, where socioeconomic and cultural factors may play a crucial role in determining the risk/benefit ratio and, correspondingly, the risk acceptability (i.e. the genetically-engineered-modified organism *per se*). Though these two components of the biosafety definition are mutually informative and complimentary, every effort should be made to split them in order to end up with the opportunity to make a non-biased and scientifically based decision on the biosafety of a particular organism.

48. Where a GEMA is released into the environment, the type of applicable risk reduction measures should be consistent with the degree of identified risks.

Designers of every novel GEMA should develop in-house risk reduction measures, taking into consideration the specifics of a particular genetically modified organism and to provide the relevant information.

49. Nowadays, the international criteria theoretically allow minimizing harmful consequences associated with various GEMA. While developing GEMA, a designer is driven by the consideration of benefits of the introduced modifications. First, it is related to the increase in fertility of organisms, improvement of their productive characteristics, resistance to different diseases and the ability to produce substances useful for humans, etc. Along with the assigned phenotypic traits, the GEMA may possess some unexpected properties harmful for humans and/or animal populations of the same species in case of their uncontrollable mating (synthesis of components causing allergic reactions, the emergence of hazardous compounds with mutagenic, carcinogenic or toxic effects). Adverse events may also include the development of such properties in the GEMA as the impairment of reproduction process, malignant neoplasm, excessive aggressiveness, viral infection, etc.

50. General recommendations on the reduction of acceptable risk at the initial stage comprise housing GEMA in the conditions isolated from humans and other animals of the same species:

the GEMA care takers should use protection equipment in compliance with the requirements established depending on the transgene origin and GEMA specific characteristics;

the use of GEMA products is highlighted in the special Guidelines (Biomedical assessment of food products derived from genetically modified sources);

The GEMA should be housed, used, transported, euthanized and disposed in the conditions of operating physical barriers that prevent contacts of such animals with the public and the environment outside the containment system. At this stage, quarantine rules for housing various GEMA species (drosophila, fishes, chickens, mice, rats, rabbits, sheep, goats, pigs, cows) should be applied which correspond to the current rules of quarantine conditions for housing non-genetically modified animals.

51. General precautions include but not limited to:

provision of the relevant awareness and training of the GEMA handling individuals;

the application of control procedure to be used for taking appropriate measures in case of unexpected consequences during or after the release;

the implementation of control over the spread of the released GEMA and their offspring into the environment by the genes flowing out from the released GEMA;

the provision of authorized access to the release site;

the restraint of relocation using such physical tools as fences, filters, islands, water barriers, nurseries, etc.;

the application of reproductive isolation by using sterile GEMA;

the isolation of GEMA from wild-living animals of the same species;

the regulation of resistance or distribution of such reproductive structures as larvae or eggs.

52. At the stage of creation and risk assessment, the GEMA should be separated strictly from the environment. Handling and use of different GEMA classes (mammals, birds, fishes, insects) are described in the specific rules approved by the Russia's Ministry of Agriculture for housing non-genetically modified animals.

53. Physical, chemical and biological types of protection are required.

For example, physical protection in case of mammals will include electrical fence, double barrier, and internal trench surrounded by buffer zones.

For birds and insects, a mesh should be installed over the site where tests are carried out and GEMA are housed. For fishes, special electrical and mechanical filters are required to prevent the release of GEMA into the outer water reservoirs.

Chemical protection might include disinfection and pesticides.

To ensure biological protection, mature males and females should be kept separately; animals could be castrated, or animals of the same sex could be used in experiments to avoid reproduction; cycle seasonality could be considered for this purpose, etc.

For example, two strategies are available for creating genetically modified mosquitoes: self-limiting and self-propagating strategies. The first strategy is targeted at ensuring control of mosquito vectors by suppressing their population growth or decreasing their competence.

For this purpose, genetically-engineered-modified mosquitoes are created that are unable to produce viable offspring. For example, it is achieved by interrupting development at the larval stage. Other self-limiting strategies target metabolic processes of the mosquito vectors and aim at lowering their fitness and thus reducing their populations. Self-propagating strategies promote the spread and persistence of the transgene through populations of the same mosquito species. As opposed to the self-limiting strategy, the genetically-engineered-modified mosquitoes produced through self-propagating strategies are intended to be heritable and to spread through the target population and, thus, to persist in the ecosystem at least in the medium term. The objective of the self-propagating strategies is, hence, population replacement of the non-modified mosquitoes with the population genetically-engineered-modified mosquitoes with a lower capacity for disease transmission.

54. Disposal and destruction of dead GEMA is carried out in compliance with the Veterinary and sanitary rules for collection, disposal and destruction of biological waste approved by the Chief Veterinary Officer of the Russian Federation on December 04, 1995, No. 13-7-2/469.

55. Any scientist involved in GEMA research should have appropriate education, qualification and training required for the completion of task in a full scope.

Appendix No. 1.
to the Guidelines for Risk Assessment and Management of the Use (Release)
of Genetically-Engineered-Modified Animals
in Open Systems

Materials to be submitted by the applicant to the Rosselkhoznadzor together with an application for the state registration of GEMA

1. General information (the applicant, authors, contact phone numbers and addresses).

2. Genetically-engineered-modified animal – name, identifier of the transformation event.

3. Information on the DNA-donor organisms and the recipient animal:

3.1. donor organism:

3.1.1. name (trivial and in the Latin language), taxonomic status;

3.1.2. information on genes – carriers of potential:

− pathogenicity;

− toxicity;

− allergenicity;

3.1.3. potential gene exchanges with other organisms;

3.2. recipient animals:

3.2.1. name in the Latin language, synonyms;

3.2.2. trivial name, synonyms;

3.2.3. taxonomy of the genus;

3.2.4. related species;

3.2.5. morphological description;

3.2.6. genetics of recipient animal;

3.2.7. capacity for hybridization

- hybridization with wild-living related species, including those in the regions proposed for release;

- hybridization with farm animals, including those in the regions proposed for release;

3.2.8. agricultural applications of recipient organism;

4. Molecular characteristics of vectors and cloning methods:

4.1. description of DNA nucleotide sequences and methods employed for the DNA integration into the animal genome, including:

− structural conformity of the integrated gene(s) to the natural gene;

− number of inserts (number of genetic loci)

− number of copies (number of copies of heterologous DNA in every genetic locus);

− exogenous DNA integration site;

− insert integrity;

− selective marker and/or reporter genes;

− data on molecular structure of the insert required for its identification in the integrated DNA structure, including, if necessary, specific primers.

5. Genetically-engineered-modified animal:

5.1. differences of a genetically-engineered-modified animal from the recipient animals (molecular biological and morphological);

5.2. transfer of the built-in properties and their stability, depending on the number of reproduction cycles;

5.3. transgene expression (including the distribution of expressed protein contents in GEMA).

5.4. expressed protein characteristics:

5.4.1 equivalence to the naturally occurring protein (including metabolic and immune characteristics);

5.4.2. toxicity (for humans and organisms – natural inhabitants of the potential receiving environment);

5.4.3. allergenicity;

5.4.4. capacity for degradation (degradation mechanism and period, toxicity of degradation products).

6. Conditions of the release of transgenic animal in open system (or industrial applications) and description of the potential receiving environment:

6.1. objective of the release (or industrial applications);

6.2. locations of the release (or industrial applications);

6.3. date of the release (or industrial applications) of the GEMA;

6.4. GEMA quantity;

6.5. methods of the removal of transgenic material from the potential receiving environment, as and when required;

6.6. description of the release site, including the surrounding area:

6.6.1. geographic location;

6.6.2. distance to the residential space;

6.6.3. local flora and fauna;

6.6.4. target and non-target ecosystems.

7. Data on the GEMA interaction with the environment:

7.1. The GEMA properties influencing their survival and uncontrollable reproduction;

7.2. The GEMP interaction with the environment;

7.3. probability of transgene transfer to other organisms;

7.4. the GEMA interaction with target and non-target organisms:

7.4.1. direct or indirect GEMA effects through the food chain;

7.4.2. possibility of the recombination of the natural animal viruses intrinsic for the potential receiving environment with the nucleotide sequence of GEMA transgene (if any);

7.4.3. potential GEMA impact on ecology of the receiving environment.

8. Potential threat reduction strategy and plan.

9. Risk assessment summary.

10. Monitoring и emergency response plan.

The scope and level of details of provided information depend on the level of potential risk and might be determined on a case-by-case basis by the Expert Advisory Council.

Annex 9. Draft 63139 - Procedure for Molecular Genetic Testing and Expert Assessment of the Biological Safety of GE Microorganisms for

Agricultural Applications

Appendix No. 1
Approved by Order of the Ministry of Agriculture of Russia
Dated __ No.___

Procedure for molecular genetic testing and expert assessment of the biological safety of genetically-engineered-modified microorganisms for agricultural applications

I. Scope of application

1. This procedure establishes the process of molecular genetic testing and expert assessment of the biological safety of genetically-engineered modified microorganisms for agricultural applications to be performed by the testing laboratories accredited within the national accreditation system.

2. The guidelines shall cover microorganisms containing genetic material modified with the use of genetic engineering technologies, except those cases where the genetic engineering manipulations result in the creation of microorganisms that can be derived from the known naturally occurring processes taking place *in vivo*:

DNA transfer between microorganisms of the same taxonomic species or taxonomically related species;

the decrease or increase in gene activities due to various mutations and, consequently, the reduction or activation of synthesis of the substances that are present in a particular microorganism;

genetic modifications generated through the cell conjugation; DNA transformation; and, protoplast transduction or fusion that may effectively occur in nature;

mutagenesis with the use of any mutagens and genetic constructs not associated with the insertion of foreign DNA;

and, the transfer of foreign DNA containing only the characterized non-coding regulatory regions (including operators, promotors, terminators and origins of replication) into a recipient microorganism.

II. General provisions

3. Responsibility of applicants for the provided information on genetically-engineered-modified microorganisms (hereinafter – GMM) under the conditions of designed use is established in compliance with the legislation of the Russian Federation.

4. Definition of the scope of necessary expert assessment

At the stage of preliminary review of a package of documents required for the state registration of GMM with the Federal Service for Veterinary and Phytosanitary Surveillance (Rosselkhoznadzor) (application for the state registration of a genetically-engineered modified (GMM) strain), the appropriateness of the strain assignment to GMM category and the fulfillment of mandatory requirements applied to GMM submitted for registration are verified, and the necessary scope of expert assessment is defined.

The necessary scope of expert assessment is established depending on the implementation of the recommendations on creating GMM and on the declared conditions of its use (the scope of expert assessment is defined in accordance with Appendix #3 to the Procedure).

6. The following four options are available for performing the expert assessment:

notifying process – in case where the applicant has fulfilled all the requirements and recommendations for the GMM creation and for its use under the standard conditions in the containment systems

(implying no release of the used microorganisms into the environment);

expedited process for containment systems – in case of insignificant deviations from the recommendations for the GMM creation or from the standard conditions of its use;

expedited process for open systems (based on the use of microorganisms implying their release into the environment) – only for testing in the open systems;

complete process – in case of using GMM in the open systems or in case of significant deviations from the recommendations for the GMM creation or from the standard conditions of its use;

III. Process of GMM expert assessment and registration

7. The following institutions are involved in the process of GMM expert assessment and registration:

BioResource (in the area of microbial bioresources) Centers (BRC) – state research organizations (or divisions within the organizational structure of a state research organization) specializing in the implementation of targeted nation-wide important operations in the area of microbial bioresources (genetic resources), including the establishing, maintaining and assurance of regulated availability of the national collection of microbial bioresources throughout the territory of the Russian Federation;

testing laboratories accredited within the national accreditation system which, in turn, identify organizations for conducting additional and experimental inspections, if necessary, to perform the expert assessment of GMM properties and specify requirements for them.

8. BRC functions

BRCs perform a preliminary expert assessment composed of the following stages (a detailed description of the expert assessment stages and registration see in Appendix No. 4 to the Procedure):

to accept the package of documents from an entity (physical or legal entity, irrespective of the type of property) authorized by the GMM owner to submit an application and to perform on his/her behalf all actions for the GMM state registration (hereinafter – the Applicant). The list of documents is presented in Appendix No.5 to the Procedure);

to prepare a report on the assignment to GMM category;

to prepare a report on the fulfillment of the mandatory requirements during the construction of GMM designed for industrial applications in the contained or open systems;

to deposit GMM or to convey GMM for deposition to the authorized collection;

to prepare a GMM strain certificate;

to develop recommendations on the required scope of expert assessment.

9. Functions of the testing laboratories accredited within the national accreditation system:

to verify the required scope of expert assessment determined by BRC;

to conduct expert assessment;

to prepare an expert assessment report and to submit a notification on the possibility of registration to the registration agency with the attached results of expert assessment and complete data on the registered GMM;

to develop recommendations for the GMM designing entities;

to develop recommendations on the conditions of GMM industrial use;

to analyze the data of monitoring conducted by the registration agencies and potential consequences in case of emergency situations.

IV. Process of expert assessment

10. BRC identifies:

whether a strain submitted for registration belongs to the GMM category;

compliance of a GMM strain submitted for registration with the mandatory requirements;

the authorized collection of microorganisms where the strain submitted for registration should be deposited (deposition may take place in the BRC);

an option (scope) of the expert assessment necessary for registration of the submitted strain.

11. The Applicant shall transfer for deposition the GMM strain requiring registration to the BRC or, upon BRC's referral, to the collection and provide a package of documents based on the option of expert assessment, except the case where the notifying registration process is applicable.

12. Testing laboratories accredited in the national accreditation system, shall, within the established period of time, depending on the option of expert assessment, prepare an expert report on the possibility of GMM use for industrial purposes. In case of refusal decision, it should be supported by arguments, and the documents should be returned to the Applicant; in case of positive decision – the notification with attached necessary documents are conveyed to the registration agency.

13. Forwarding request for additional information during expert assessment

In case where an expedited expert assessment is performed, the testing laboratories accredited in the national accreditation system may request additional data on the GMM or ways of its applications to find out significant deviations from the recommended biosafety standards.

In case where a complete procedure of expert assessment is performed, the testing laboratories accredited in the national accreditation system may request additional data on the GMM or ways of its applications that are essential for determining the biosafety of GMM use.

In case where during the expert assessment process, some disagreements are found in the submitted documents or it turns out that the provided information is insufficient, the procedure of expert assessment of GMM strain is suspended; the testing laboratories accredited in the national accreditation system shall, within 3 days, notify in writing the Applicant about such suspension and provide a list of necessary changes for eliminating drawbacks in the documentation. The duration of suspension cannot exceed 90 days. If the identified disagreements cannot be fixed by the Applicant within the specified time period, the application is canceled.

14. Duration of the expert assessment procedure:

preliminary application review – no more than 30 days;

notifying process – no more than 15 days;

expedited expert assessment process for containment systems – no more than 45 days;

expedited expert assessment process for testing in open systems – no more than 60 days;

complete expert assessment process – no more than 90 days.

15. Justification of the refusal decision of expert assessment.

In case where the testing laboratories accredited in the national accreditation system make a refusal decision on industrial applications of the GMM strain submitted for registration, the Applicant shall be given a justified refusal specifying its reasons and the ways of potential corrective actions.

16. A possibility of additional expert assessment after refusal decision.

In case where the Applicant has eliminated the cause underlying the refusal decision made by the testing laboratories accredited in the national accreditation system, the re-assessment can be made not earlier than 30 days from the date of receipt of such decision.

V. Monitoring of consequences of the GMM industrial use

Monitoring is a system of control of the potential GMM spread in open system and its interaction with the environment. It also includes the evaluation and projection of potential changes in the condition of the open system exposed to GMM. Monitoring is one of the potential tools for receiving information on the necessity to implement the appropriate measures, such as to revise the risk management strategies; take emergency response measures; to conduct an additional risk assessment or re-assessment underlying rationale of made decision.

17. Monitoring is conducted by the federal executive body responsible for GMM registration and by other federal executive bodies authorized by the Government of the Russian Federation.

18. Monitoring periodicity

Monitoring performance is based on the results of regular (scheduled and extraordinary) inspections envisaged in the operating sanitary rules and standards. Experts conducting review of the monitoring results shall assess all incoming data, including public appeals.

19. An opportunity to appeal to the expert assessment agency with information on adverse effects caused by GMM use

Any individual or legal entity has the right to address the testing laboratories accredited in the national accreditation system with an application on the adverse GMM effects on public health and the environment.

20. Review of the monitoring results and the appeals associated with the effects of industrial GMM applications

The monitoring results are reviewed by the testing laboratories accredited in the national accreditation system.

Based on the monitoring results of industrial GMM applications, the revision of the requirements for biosafety assurance may take place.

Appeals of individuals or legal entities are considered in the following two stages:

first, a preliminary review of the appeal and its relevancy is carried out (in case where justifications of the adverse effects on public health and the environment are not available, no further review of the appeal takes place).

second, the causes of negative impact are identified and the biosafety assurance measures are adjusted to the point that the registration of a particular GMM is cancelled and its industrial use is discontinued.

21. Procedure of revision of the expert assessment report on the monitoring results

Based on the monitoring results, the testing laboratories accredited in the national accreditation system may revise the expert assessment results in view of additional information received with respect to the biosafety of GMM industrial use.

Appendix #1

to the procedure for molecular genetic testing and expert assessment of the biological safety of genetically-engineered-modified organisms for agricultural applications

Requirements for inserted genetic material

For the state registration of a GMM strain all the requirements posed to inserted genetic material must be fulfilled.

1. Limited size. The inserted genetic material should be composed only of the following components:

1.1. Structural gene(s),

1.2. Regulatory sequence allowing the expression of solely the structural gene(s),

1.3. Associated nucleotide sequences necessary for the "relocation" of genetic material, including linkers, homopolymers, adaptors, transposons, and restriction enzyme sites,

1.4. Nucleotide sequences for transferring a vector,

1.5. Nucleotide sequences for maintaining a vector.

2. The genetic material characteristics should contain the following information:

2.1. nucleotide sequence of the inserted genetic material;

2.2. functions of all products whose synthesis is controlled or governed (coded) by the inserted genetic material;

2.3. functions of sequences involved in the regulation of expression of the inserted genetic material;

2.4. the absence of associated nucleotide sequences, except those enlisted in item 1.3.

3. The absence of particular sequences. Such characterization of inserted sequence means that neither a portion, nor the whole piece of the sequence coding toxins will enter the inserted sequence and, thus, the recipient. (The list of hazardous toxins is under development*). The enlisted toxins have a high degree of toxicity and a polypeptide structure. Other types of toxins (modified amino acids, heterocyclic compounds, complex polysaccharides, glycoproteins, peptides) are not included in the list for two reasons. The first reason is associated with their mild toxicity; the second – with the multi-gene regulation of activity of these toxins. For the synthesis of such toxins it is necessary to transfer a large number of expressed genetic sequences to a recipient microorganism; and there is little likelihood of such scenario if the above requirements for the limited size of inserted genetic material are satisfied.

4. Low ability to mobilization. This requirement is necessary for ensuring a low rate of the transfer of genetic material to other microorganisms from the environment. Such transfer may occur through conjugation, transduction or transformation; during this process the genetic material may enter multiple microorganism populations, and, as a result, it will be not possible to predict their properties. The recommended frequency of DNA transfer is below 10^{-8}.

The application of the above criteria to inserted genetic material enables to minimize the risk of entering a non-characterized genetic material into the recipient and the risk of unpredictable consequences of the genetic modification.

The applicant should provide description of completed gene-engineering manipulations, and full nucleotide sequence of the inserted genetic material specifying all elements and their functions.

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*Currently, a detailed list is not available in the Russian Federation. It can be built on the list of toxins from Decree of the President of the Russian Federation dated 20.08.2007 No. 1083 “On approval of the list of microorganisms, toxins, equipment and technologies subject to export control” with addition of the toxins enlisted under TSCA, §725.421 (http://www2.epa.gov/laws-regulations/summary-toxic-substances-control-act).

Appendix # 2

to the procedure for molecular genetic testing and expert assessment of the biological safety of genetically-engineered-modified organisms for agricultural applications

Requirements for recipient strains

All recipient strains are divided into three groups:

1. A recipient strain is included in the list of recommended recipients (Table 1, column 1, Appendix #3 to the Procedure).

2. A recipient strain is not included in the list of recommended recipients, but the strain is well-characterized and has a long-term history of the safe industrial applications (Table 1, column 2, Appendix #3 to the Procedure).

3. A recipient strain is not included in the list of recommended recipients, and data on the biosafety of its industrial applications are not available (Table 1, column 3, Appendix #3 to the Procedure).

For including in the list of recommended recipients, a candidate strain should meet the following criteria:

The first criterion requires that a microorganism should be clearly identified and categorized. Information about genotypic and phenotypic characteristics of the microorganism should be available.

The second criterion requires availability of the information on relations between the studied microorganisms and the closely related microorganisms that have potential adverse effects on human health and the environment.

The third criterion implies availability of a long-term history of the safe microorganism application for commercial purposes.

The fourth criterion requires that the research has not demonstrated potential harmful impact of the microorganism on human health or the environment.

The fifth criterion implies a low survival rate of the microorganism in the environment.

Only a concurrent implementation of all the criteria allows making a conclusion on the safety of microorganism and its inclusion in the list of recommended recipients. Microorganisms shall be added to the list of recommended recipients e by the authorized state expert agency upon completion of thorough studies, taking international experience into consideration.

List of recommended recipients**

Acetobacter aceti

Aspergillus niger

Aspergillus oryzae

Bacillus licheniformis

Bacillus subtilis

Clostridium acetobutylicum

Escherichia coli K-12

Penicillium roqueforti

Saccharomyces cerevisiae,

Saccharomyces uvarum.

Trichoderma reesei QM6a

Bacillus amyloliquefaciens subsp.amyloliquefaciens

The Applicant should provide data on the taxonomic identification of a recipient strain; their reliability will be verified by the authorized BRC at a preliminary stage of the expert assessment procedure.

** Currently, a detailed list is not available in the Russian Federation. In the United States the list of approved recipients is provided under TSCA, §725.420 (http://www2.epa.gov/laws-regulations/summary-toxic-substances-control-act)

Appendix # 3

to the procedure for molecular genetic testing and expert assessment of the biological safety of genetically-engineered-modified organisms for agricultural applications

Establishing the necessary scope of expert assessment

Depending on the employed recipient strain and proposed conditions (standards) of the production with the use of GMM, multiple options of expert assessment procedure are available for making a registration decision. The selection of options depends on whether the GMM strain designers have satisfied the safety criteria for GMM strains in accordance with the international practice.

Table (see below) is given in this Appendix for expediting a preliminary process of expert assessment, depending on the recipient microorganism and the biosafety assurance standard.

Table 1 – Conformity of the expert assessment to the recipient strain and production process

Group of recipient strain[1]			Production conditions								Procedure of expert assessment
			Open system	Containment system	Standard conditions of GMM use[2]						
1	2	3			1	2	3	4	5	6	
+				+		+					Registration is not required[3]

+				+				+			1
	+			+				+			2
+				+					+		2
+				+						+	4
		+		+							4
+			+		+						3
+			+				+				4

1 – The groups of recipient strains are given in Appendix #2.
2 - The standard conditions of GMM use are given in Appendix #1 to the Procedure.
3 – Testing is the containment systems is an exception, and no expert assessment is required. In this case the activity is governed by the operating sanitary rules and the standards for microorganisms (non-GMM).

Appendix # 4

to the procedure for molecular genetic testing and expert assessment of the biological safety of genetically-engineered-modified organisms for agricultural applications

Description of the stages of expert assessment and registration process

Preliminary expert assessment (conducted by the BRC)

Stage 1

The applicant shall provide data on the following:

- the strain submitted for registration (including data on its origin and taxonomic identification) and proposed areas of its applications;
- the inserted genetic material;
- the scale and conditions of proposed industrial production.

Stage 2

In accordance with this procedure, it shall be determined whether the strain belongs to the GMM category.

Stage 3

Based on the review of provided information, a report is developed:

- on compliance with the requirements applied to the inserted genetic material;
- on the implementation of recommendations to the used recipient strain (in accordance with Appendix #2 to the Procedure);
- on compliance of the proposed conditions of its use to the recommended standards.

Stage 4

The BRC will identify collection where the registered GMM strain should be deposited. The Applicant shall deposit the registered GMM strain based on the collection rules.

Stage 5

The BRC will issue a certificate of registered GMM strain.

Stage 6

An option (scope) of expert assessment required for the strain registration is determined.

Expert assessment (performed) by the testing laboratories accredited in the national accreditation system

Stage 7

The Applicant shall submit documents, required for performing a particular option of expert assessment, to the testing laboratory accredited in the national accreditation system. Upon receipt of the package of documents on the GMM strain submitted for registration by the expert review agency, the necessary scope of expert assessment determined by the BRC is confirmed.

Stage 8

The testing laboratory accredited in the national accreditation system will perform expert assessment of data provided by the Applicant on the GMM strain submitted for registration and conditions of its proposed use.

Stage 9

The testing laboratory accredited in the national accreditation system will, within the established period of time, issue an expert report on the prospects of industrial applications of the GMM and, in case of a positive decision, will forward the package of documents to the registration agency. In case of a refusal decision, the expert review agency will provide motivations for its conclusion and return the documents to the Applicant. In case of a positive decision, the expert review organization may impose some restrictions on the intended use of the GMM (e.g. by limiting production output or recommending additional measures of physical protection).

Registration (performed by Rosselkhoznadzor)

Stage 10

Verification of the data about the Applicant (designer, proposed producer)

Stage 11

Registration of the GMM strain for potential industrial applications under the declared conditions or justified refusal.

Stage12

Issuance of the certificate of state registration and entry into the Consolidated Register of GMO.

Appendix # 5

to the procedure for molecular genetic testing and expert assessment of the biological safety of genetically-engineered-modified organisms for agricultural applications

Form for providing information on the GMM and the conditions of its use

An application on the state registration of a GMM strain is filled out in the Russian language and

contains the following data:

1. Full name and location of the Applicant and producer (designer) of the modified organism;

2. Identification tax payer number (INN) of the Applicant

3. Name of the modified organism and its taxonomic status;

4. Characteristics of the GMM required for the description of inserted genetic modification and new properties of the GMM;

(characteristics of the modified organism necessary for the description of inserted genetic modification and new properties of the GMM are provided in accordance with Appendix #1 to the Procedure);

5. Data on the place of origin of recipient organism;

6. Assessment of the risk associated with the use of modified organism (vs. an original organism) and recommendations on the alleviation of acceptable risk;

7. Registration number of the certificate of state registration of modified organism(s) used for the development of a modified organism submitted for registration (in case where the modified organism submitted for registration was developed on the basis of another modified organism);

8. Information on the registration of the modified organism abroad (if available);

9. Type of proposed intended use;

10. Conditions of proposed use of the GMM;

11. Economic value of using the GMM;

12. Information on the availability of a positive decision of the state environmental management agency (if necessary for using the GMM in open systems) for intended use envisaging the release into the environment.

The registration authority will request data required for state registration, concerning the performance and results of the state assessment of ecological impact held by the Federal Service for Surveillance in the Sphere of Environmental Management, pursuant to the procedure established in the legislation of the Russian Federation. The above conclusion can be provided by the Applicant upon its own initiative.