Guidelines for Application of Recombinant DNA Organisms in Agriculture, Forestry, Fisheries, The Food Industry and Other Related Industries

MINISTRY OF AGRICULTURE, FORESTRY AND FISHERIES
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CONTENTS

CHAPTER 1. GENERAL PROVISIONS

Section 1. Purpose

The purpose of these Guidelines is to establish basic requirements concerning the appropriate application of recombinant DNA (rDNA) organisms in agriculture, forestry, fisheries, and the food industry, as well as in other related industries regulated by the Ministry of Agriculture, Forestry and Fisheries, so as to assure the safe use of rDNA organisms and to achieve the soundoverall development of agro-industries.

Section 2. Definitions

1. "rDNA molecules" are defined as "vector" deoxyribonucleic acid (DNA) molecules combined with "donor DNA".

"rDNA techniques" are defined as methods by which to construct rDNA molecules by enzymes in vitro and to introduce the rDNA molecules into "host cells" in order to propagate "donor DNA" in the "host cell".

"rDNA organisms" are defined as (1) "host cells" into which rDNA molecules have been introduced (except living cells possessing the same genetic structure as that of naturally existing cells,), (2) cells or organisms derived via "rDNA techniques" from the living cells described in (1) above, (3) non-cellular organisms in which the rDNA molecules are introduced excluding those organisms used as "vectors".

2. "Host cells" refers to living cells into which rDNA molecules are introduced.
3. "Vectors" refers to DNA molecules used to transfer "donor DNA" to host cells by rDNA techniques.
4. "Donor DNA" refers to heterologous DNA segments to be combined with vectors. Heterologous DNA refers to DNA derived from organisms taxonomically different from their host cells.
5. "rDNA plants" refer to rDNA organisms (excluding rDNA organisms that are used as undifferentiated cells) whose host cells are plants (excluding microalgae and fungi, except those that form sporophores).

6. "rDNA microorganisms" refer to rDNA organisms whose host cells are microorganisms (including microalgae and fungi, except fungi that form sporophores). rDNA organisms that are used as undifferentiated cells, and whose host cells are animal or plant cells, are regarded as rDNA microorganisms.
7. "rDNA small laboratory animals" refer to rDNA organisms (excluding rDNA organisms used as undifferentiated cells) whose host cells are animals (restricted to mice, rats and other rodents) that are used as laboratory animals.
8. "rDNA live attenuated vaccines" refer to pharmaceuticals for animals that are "rDNA microorganisms" or non-cellular organisms into which rDNA molecules have been introduced and that are to be used directly for animals, but exclusively farming and companion animals, in order to prevent the spread of infectious diseases.
9. "Work area" is; defined as a location where rDNA organisms are handled directly.
10. "Work site" is defined as a site where the characteristics of rDNA organisms are produced or evaluated. A work site comprises work are as defined in 9 above and other sites where rDNA organisms are not handled directly.

CHAPTER 2. APPLICATION OF RECOMBINANT DNA ORGANISMS

Section 1. Fundamental principles

Any person or organization intending to produce rDNA organisms or to conduct sales of such organisms for use in agro-industries, or intending to produce related materials based on the use of rDNA organisms (excluding environmental applications that have previously complied with guidelines without specific measures for containment(referred to as hereinafter "open system"), shall conduct a total safety evaluation of the rDNA organisms on the basis of the characteristics of the hosts, the rDNA molecules, and the vectors involved. rDNA organisms shall be compared with their hosts on the basis of the evaluation criteria described in the following sections, and the use of the rDNA organisms must conform with the criteria described in the following sections.

Section 2. rDNA plants

1. General matters

1) When rDNA plants are propagated for purposes of obtaining breeding materials, they should be applied in a simulated model environment as described in Section 2-3. -1), and their initial safety must be confirmed.
2) rDNA plants whose safety has been confirmed in a simulated model environment referred to in 1) as above, can be applied in an open system as described in Section 2-3. -2).

2. Information required for a safety evaluation

1) Purposes of application of rDNA plants
2) Host cells or biological species to which the host cells belong
    (1) Taxonomic position
    (2) State of applications and distribution in the natural environment
    (3) Reproductive and propagative properties, and genetic characteristics
    (4) Weediness
    (5) Production of toxic substances
    (6) Other principal physiological characteristics
3) Donor DNA
    (1) Identified/unidentified
    (2) Structure and origin
    (3) Functions of the genes
4) Vectors
    (1) Names and origins
    (2) Characteristics
5) rDNA plants
    (1) Methods of preparing rDNA plants
        (i) Structure and construction methods of rDNA molecules
        (ii) Methods of introducing rDNA molecules into host cells
        (iii) Processes for cultivating a rDNA plants
    (2) Location of rDNA molecules in host cells, and stability of the expression
    (3) Differences between rDNA plants and host plants or the same biological species to which the host plants belong
        (i) Reproductive and propagative properties, as well as genetic characteristics
        (ii) Weediness
        (iii) Production of toxic substances
        (iv) Other physiological characteristics

6) Other information (obtained through the rDNA experiments or through cultivation of the rDNA plants, etc.)

3. Classification of applications

1) Application in a simulated model environment

This refers to the experimental application of rDNA plants in a specifically restricted area that is designed to simulate the environment of actual cultivation, but under such conditions so as to prevent the rDNA plants from either naturally propagating or influencing other plants in the outside area (e.g., via pollen).

2) Application in an open system

Applications of rDNA plants in an open system may be conducted following a confirmation of their safety in a simulated model environment.

4. Facilities and experimental equipment for rDNA plants

When rDNA plants are applied in a simulated model environment, the facilities and equipment used for rDNA plants should be installed in such a way as to fulfill the following conditions:

1) The work area should be clearly distinguished from other areas.

2) The work area should include an appropriate confined field that is designed to prevent the spread of rDNA plants taking into account of their reproductive and propagative properties, their physiological characteristics, the actual method of application in an open system, and their possible interactions with surrounding plants and/or animals outside the field.

5. Management of rDNA plants

When rDNA plants are applied in a simulated model environment, operations should be conducted in the field in conformance with the followings:

1) Cultivation of rDNA plants.

(1) Seeds and seedlings of rDNA plants should not be mixed with other seeds and seedlings.

(2) rDNA plants should be sown or transplanted in the work area in a manner designed to prevent their seeds and seedlings from spreading to the outside area.

(3) The propagation of other plants that have little relation to the application of rDNA plants should be minimized in the work area and in its vicinity.

(4) Appropriate measures should be taken to minimize the dispersion of pollen and seeds taking into account the nature of rDNA plants.

(5) When cultivating rDNA plants that are easily regenerated from their stems, leaves, tubers, rhizomes, roots, and so forth, the residual parts of rDNA plants should not remain in the work area, and appropriate measures should be taken to prevent their regeneration after usage in the simulated model environment.

2) Waste disposal pertaining to rDNA plants

Wastes derived from rDNA plants should be disposed after appropriate inactivation designed to ensure their safety.

3) Storage of rDNA plants

(1) rDNA plants should be clearly labeled as "rDNA plants" on their containers, which should be safely stored in an appropriate facility set up in advance. A sign stating "rDNA Plants in Storage (for use in a simulated model environment) " should be posted in a clearly visible location at the storage facility.

(2) A catalogue of the stored materials, including the rDNA plants, should be prepared and maintained.

4) Transport

(1) rDNA plants that are to be transported outside the work area should be placed in a sealed container to prevent dispersion of the contents.

(2) Any containers to be used for transporting rDNA plants should be conspicuously labeled "Handle with Care" in red lettering on the surface.

5) Maintenance of facilities and equipment

Facilities and equipment used for handling rDNA plants should be given performance inspections immediately after their installation, and periodically thereafter.

6) Other requirements

(1) A sign stating "Application in a Simulated Model Environment (rDNA Plants)" should be posted at work areas where rDNA plants are being handled.

(2) The work areas should be kept clean.

(3) Working clothes should be worn in the work areas.

(4) Special attention should be taken that personnel in the work areas do not spread pollen, seeds, or other parts of rDNA plants outside the work area via attachment to their clothing, and so forth.

Section 3. rDNA microorganisms

1. General matters

1) Application of rDNA microorganisms to production processes

Production processes concerning rDNA microorganisms shall be classified into four categories as defined from (1) to (4) in Section 3-3.-1), according to the appropriate degree of safety required (e.g., pathogenic, non-pathogenic, etc.).

2) Application of rDNA microorganisms to an open system

(1) The propagation of rDNA microorganisms intended for utilization in an open system shall be conducted according to 1) as above.

(2) Following the safety evaluation in a laboratory, rDNA microorganisms should be applied in a simulated model environment as described in Section 3-3.-2) - (1), and their safety must be confirmed before being applied to an open system.

(3) rDNA microorganisms whose safety has been confirmed in a simulated model environment as described above, can be applied in an open system as described in Section 3-3. -2) - (2).

3) rDNA microorganisms to be used as rDNA live attenuated vaccines should be treated pursuant to the provisions of rDNA live attenuated vaccines described in Section 5.

2. Information required for safety evaluation

1) Purposes of the application of rDNA microorganisms

2) Host cells or the same biological species to which the host cells belong

(1) Taxonomic position

(2) Background information regarding utilization and distribution in natural environment

(3) Propagative properties and genetic characteristics

(4) Pathogenicity

(5) Production capability of toxic substances

(6) Other physiological characteristics

3) Donor DNA

(1) Identified/unidentified

(2) Structure and origin

(3) Functions off the genes

4) Vectors

(1) Names and origin

(2) Characteristics

5) rDNA microorganisms

(1) Methods of preparing rDNA microorganisms

(i) Structure and construction methods of rDNA molecules

(ii) Methods of introducing rDNA molecules into host cells

(iii) Developmental processes of rDNA microorganisms

(2) Location of rDNA molecules in host cells, and stability of the expression

(3) Differences between rDNA microorganisms and host cells or the same biological species to which the host cells belong

(i) Propagative properties and genetic characteristics

(ii) Pathogenicity

(iii) Production capability of toxic substances

(iv) Other physiological characteristics

(4) Survivability and methods of monitoring in natural environments

6) Other information (obtained through rDNA experimentation or obtained in the process of developing rDNA microorganisms, and so forth.)

3. Classification of usage

1) Application of rDNA microorganisms to the production process

(1) GILSP (Good Industrial Large-Scale Practice)

This refers to the application of rDNA microorganisms at the minimum level of physical containment that satisfies the following criteria.

(i) The: host cells must

1 Be non-pathogenic to humans;

2 Be uncontaminated by exogenous factors (viruses, etc.) that are pathogenic to humans; and

3 Have either an extended history of safe use or be subject to intrinsic environmental limitations that permit growth in an industrial setting but that permit only limited survival without adverse consequences in outside applications.

(ii) The; donor DNA and vectors must be

1 Well-characterized and free from known harmful gene sequences;

2 Size-limited as much as possible to only the DNA required to perform the intended function; and

3 Poorly mobilizable and unable to transfer any resistant markers to microorganisms not known to acquire them naturally.

(iii) rDNA microorganisms must

1 Be non-pathogenic to humans; and

2 Not have higher propagative abilities than their host cells.

(2) Category 1

This refers to the application of rDNA microorganisms at a certain level of physical containment, excluding rDNA microorganisms that fulfill GILSP conditions and that are not pathogenic for humans.

(3) Category 2

This refers to the application of rDNA microorganisms at a certain level of physical containment, including microorganisms that are capable of infecting humans but that have a minimal likelihood of being pathogenic for humans even when handled directly, and for which sufficient preventive measures and effective therapy exist for use in case of infection.

(4) Category 3

This refers to the application of rDNA microorganisms that do not meet Category-2 conditions, that are pathogenic for humans, and that require very careful handling. Such applications are to be conducted at a specified level of physical containment in case of infection,, and may be conducted only if hazards are relatively minimal, and if sufficient preventive measures and effective therapies exist.

In addition, rDNA microorganisms whose pathogenicity exceeds that specified in Category-3 applications shall be classified in a special category.

2) Application of rDNA microorganisms intended for an open system

(1) Application in a simulated model environment

This refers to the experimental application of rDNA microorganisms classified under GILSP or Category-1 applications in Section 3-3.-1). Applications are to be conducted in a specifically restricted area under conditions that minimize the spread of rDNA microorganisms to the outside area and that minimize gene transfers from the rDNA microorganisms to other organisms in the outside area.

(2) Application in an open system

This refers to the application in an open system of rDNA microorganisms whose safety has been confirmed in the simulated model environment.

4. Facilities and equipment used for rDNA microorganisms

Facilities and equipment used for applying rDNA microorganisms in GILSP, Category-1, Category-2, and Category-3 usages shall satisfy the conditions listed in the Appendix.

Facilities and equipment used for applying rDNA microorganisms in a simulated model environment should satisfy the following criteria:

1) There should be a work area clearly distinguished from other areas, and biohazard signs should be posted as necessary.

2) An appropriate confined field and management facilities should be established to utilize the rDNA microorganisms in the work area, taking into account their propagative properties, actual methods to limit their propagation, their physiological characteristics, and their application in an open system as well as the surrounding biota.

5. Handling of rDNA microorganisms

In applying rDNA microorganisms to GILSP, Categoryl, Category2 and Category3 usages, or to a simulated model environment, rDNA microorganisms should be managed in compliance with the following:

1) Control of facilities and equipment for fermentation

(1) In GILSP or Categoryl applications, the leakage of rDNA microorganisms should be minimized in seeding the combination to the cultivation or fermentation equipment, in sampling, or in transferring recombinants between cultures. In Category2 or Category3 applications, appropriate measures should be taken to prevent leakage of rDNA microorganisms; if any such leakage occurs, disinfection should be undertaken in a speedy and approved mariner. In the case of application in a simulated model environment, measures should be taken as necessary to minimize the disposal of rDNA microorganisms from the work area.

(2) The leakage of aerosols from cultivation or fermentation equipment should be minimized in GILSP or Categoryl applications, and should be prevented in Category2 and Category3 applications.

(3) After completion of operations for employing rDNA microorganisms under GILSP or Categoryl applications or in a simulated model environment, the facilities and equipment used should be washed and disinfected; facilities used for Category2 or Category3 applications should be sterilized by fully approved methods.

2) Disposal of wastes

Wastes should be disposed after inactivating them as required to attain the prescribed safety level. They should be inactivated by approved methods in Categoryl applications (including certain applications of rDNA microorganisms in a simulated model environment), and should sterilized by fully approved methods in Category2 or Category3 applications.

3) Storage

(1) Containers used to store materials, including rDNA microorganisms, should be clearly labeled as containing "rDNA microorganisms" and should be safely stored in specific storage facilities set up in advance, and especially so in the case of Category2 and Category3 applications. Signs describing applications as required levels, i.e. ", rDNA

Microorganisms in storage (GILSP applications), "rDNA Microorganisms in storage (Category) applications)", "rDNA Microorganisms in storage (Category2 applications)", "rDNA Microorganisms in storage (Category3 applications)", or "rDNA Microorganisms in storage (Application in a simulated model environment)" should be posted in conspicuous places in each storage facility.

(2) A catalogue of the stored materials harboring rDNA microorganisms should be prepared and maintained.

4) Transportation of rDNA microorganisms

(1) If rDNA microorganisms are transported outside the work area, such materials should be placed in a container of sufficient strength, and the container should be sealed to prevent leakage of the contents. In Category2 or Category3 applications, especially precautions should be taken so that the leakage of the contents does not occur hermetically, even if the container is damaged.

(2) The container to be used to transport rDNA microorganisms should be clearly labeled on a conspicuous part of its surface with "Handle with Care" in red lettering.

5) Control and maintenance of facilities and equipment

(1) The facilities and equipment used for handling rDNA microorganisms should be given performance inspections immediately after their installation and periodically thereafter.

Sterilization equipment used in Category2 or Category3 applications should be inspected every six: months.

(2) Whenever part of equipment used for such purpose as packing and sealing is/are modified or replaced, the airtightness and other functioning of the equipment should be examined.

(3) The airtightness of fermentation or cultivation equipment and related equipment used in Category2 or Category3 applications should be confirmed by fully approved methods during their operation.

(4) All facilities and equipment used in Category2 and Category3 applications should be identified by serial number, and their use should be strictly controlled.

(5) Sterilization equipment should be disinfected by approved methods on the occasion of replacement, periodic inspection, and modification of the apparatus itself.

6) Other requirements

(1) Signs indicating "GILSP Applications," "Category1 Applications," "Category2 Applications," "Category3 Applications," or "Applications in a Simulated Model Environment" should be posted at each work area during work periods.

(2) Work areas should be kept clean; and, especially when utilizing rDNA microorganisms in industrial manufacturing processes, insects and rodents should be exterminated.

(3) Personnel) should wear designated work clothing in the work area. Particular clothing should be used exclusively in the case of Category2 or Category3 applications. In addition, in Category3 applications, personnel should completely change clothing and should take a shower when entering and leaving the work area.

Section 4. rDNA small laboratory animals

1. General matters

rDNA small laboratory animals whose safety has been confirmed in a laboratory or a breeding room should be utilized as described in Sections 4-3 and 4-4. However, in certain cases, the use of rDNA small laboratory animals should be exceptionally restricted.

The persons: responsible for the use of rDNA small laboratory animals must obey the laws and ordinances relating to the utilization of laboratory animals; e.g., "the Law concerning the Protection and Management of Animals" (Law No. 105, 1973), "the Ordinance concerning the Breeding and Custody of Laboratory Animals" (Notice No. 6, Prime Minister's Office, 1980), and so forth.

2. Evaluation factors
1) Type of rDNA, small laboratory animals employed
2) Host animals or the same biological species to which the hosts belong
(1) Taxonomic position
(2) Background information regarding utilization by humans and distribution in the natural environments
(3) Reproductive and propagative properties, as well as genetic characteristics
(4) Survival and reproductive abilities in natural environments
(5) Other physiological characteristics
3) Donor DNA
(1) Identified/unidentified
(2) Structure and origin
(3) Functions of genes
4) Vectors
(1) Names and origin
(2) Characteristics
5) rDNA laboratory small animals
(1) Methods of preparing rDNA small laboratory animals
(i) Structure and construction methods of rDNA molecules
(ii) Methods of introducing rDNA molecules into host cells
(iii) Developmental processes of rDNA small laboratory animals
(2) Location of rDNA molecules in host cells, and stability of the expression
(3) Differences between rDNA small laboratory animals and host animals or the same biological species to which the recombinant animals are derived.
(i) Reproductive and propagative properties, and genetic characteristics
(ii) Survival and reproductive abilities in natural environments
(iii) Production of infectious viruses
(4) Other physiological characteristics
6) Other information (obtained through rDNA experiments or through the breeding of rDNA small laboratory animals, etc.)

3. Facilities and equipment used for rDNA small laboratory animals
Facilities and equipment used for the employment rDNA small laboratory animals should be installed to satisfy the following criteria.
1) Facilities and apparatus for rDNA small laboratory animals should be installed in a work area clearly distinguished from other areas. A work area may be formatted as the whole area for facilities in which rDNA small laboratory animals are bred (hereinafter referred to as "breeding facilities").
2) Appropriate equipment should be set up to prevent the escape of rDNA small laboratory animals through doorways, ventilation ducts, drainage locations, and windows in the breeding facilities and the work area, taking into consideration the animals, reproductive and propagative properties and their physiological characteristics.
3) Cages and other equipment used for the breeding and maintenance of rDNA small laboratory animals (hereinafter referred to as "breeding container") should not be easily openable by force, vibration, or the movement of the animals.

4. Handling of rDNA small laboratory animals
1) The breeding of rDNA small laboratory animals should be conducted in accordance with the following.
(1) Breeding of rDNA small laboratory animals rDNA small laboratory animals should be bred in breeding containers clearly distinguished from other containers used for breeding non-rDNA animals. In addition, it is desirable that rDNA small laboratory animals be individually distinguished; however, if individual distinction is difficult, rDNA small laboratory animals may be managed according to breeding group.
(2) Disposal of wastes relating to rDNA small laboratory animals Wastes relating to rDNA small laboratory animals (including the carcasses of rDNA small laboratory animals) should be disposed of after sterilization or incineration, as necessary.
(3) Transportation of rDNA small laboratory animals
(i) In transporting rDNA small laboratory animals outside the work area, containers possessing sufficient strength and structure to prevent escape the animals should be used.
(ii) The words "Handle with Care" in red lettering should be clearly displayed on all containers that contain rDNA small laboratory animals.
(4) Maintenance and management of facilities and equipment

Facilities and equipment used for handling rDNA small laboratory animals should be performance-tested at the time of installation and periodically thereafter, and their original performance level should be maintained.

(5) Other requirements

(i) A sign stating "Application of rDNA small laboratory animals" should be posted at each work area being used for the handling of rDNA small laboratory animals.

(ii) The work areas should be kept clean.

(iii) Working clothes should be worn in the work areas.

2) Transfer of rDNA small laboratory animals

If rDNA small laboratory animals are to be transferred to other facilities and to other personnel, the person responsible must notify the receiving personnel of the following:

(1) That the animals being transferred are rDNA organisms; and

(2) That experiments employing rDNA small laboratory animals are subject to regulations specified in the "Guidelines for Recombinant DNA Experiment" (Decision of the Prime Minister on August 27, 1979) and the "Guidelines for Recombinant DNA Experiments in Universities and Other Research Institutions" (Notice No. 4 of the Ministry of Education, Science and Culture, 1991).

Section 5. rDNA live attenuated vaccines

1. General matters

1) Production of rDNA live attenuated vaccines rDNA live attenuated vaccines should be produced pursuant to the appropriate safety requirement defined in Section 5-3. -1)

2) Use of rDNA live attenuated vaccines in the open air (1) Propagation of rDNA live attenuated vaccines to be used in the open air should be conducted according to 1) above.

(2) After the safety evaluation in a laboratory or a breeding room, rDNA live attenuated vaccines should be tested, if necessary, under a model environment as described in Section 5-3. -2)-(l.) on their safety with respect to their use in the open air. Animals, inoculated with the live vaccines, should be handled in the manner described in Sections 5-4 and -5.

2. Information required for safety evaluation

1) purposes of the application of rDNA live attenuated vaccines

2) Host cells or non-cellular organisms into which the rDNA molecules have been introduced, and the species to which the host cells or the organisms belong

(1) Taxonomic position

(2) Background information regarding the utilization and the distribution in the environments

(3)Propagative properties and genetic characteristics

(4) Pathogenicity

(5) Capability of producing toxic substances

(6) Other main physiological characteristics

3) Donor DNA

(1) Identified/unidentified

(2) Structure and origin

(3) Functions of genes

(4) History of industrial use

4) Vectors

(1) Names and origin

(2) Characteristics

5) Original lines of rDNA live attenuated vaccines

(1) Methods of preparing the original lines of rDNA live attenuated vaccines

(i) Structure and construction methods of rDNA molecules

(ii) Methods of introducing rDNA molecules into host cells or non-cellular organisms

(iii) Breeding history of the original lines of rDNA live attenuated vaccines

(2) Location of rDNA molecules in host cells or non-cellular organisms, and stability of the expression

(3) Differences between the original lines of rDNA live attenuated vaccines on the one hand and host: cells or non-cellular organisms into which the rDNA molecules have been introduced, or the species to which the host cells or the non-cellular organisms belong on the other.

(i) Propagative properties and genetic characteristics

(ii) Other main physiological characteristics

(4) Survivability and methods of monitoring in the environment

6) Animals inoculated with rDNA live attenuated vaccines or the species to which the animals belong

(1) Taxonomic position

(2) Distribution in the environment and history of contact with human beings

(3) Other main physiological characteristics

7) rDNA live attenuated vaccines (1) Biological properties toward animals to be inoculated

(i) Pathogenicity

(ii) Capability of producing toxic substances

(iii) Possibility of reproduction in the animal body, affinity for internal organs and sustained infection

(iv) Discharge from the inoculated animal and possibility of infection to other animals kept together

(v) Influences of high-dose inoculation

(vi) Methods of distinction of inoculated animals from non-inoculated or naturally infected ones

(vii) Other main physiological characteristics

(2) Influences on the environment

(i) possibility of outflow to the environment by ways other than excretion (such as outflow caused by rDNA live attenuated vaccines added to feed or water

(ii) Survivability in the environment

(iii) Influences on non-inoculated animals to which the rDNA live attenuated vaccines are applicable

(iv) Influences on the microflora

(v) Influences on human beings

3. Classification of usage

1) Application of rDNA live attenuated vaccines to the production process rDNA live attenuated vaccines should be produced in the same way as described in Section 3-3. -1) except for the regulations concerning Category2 and Category3.

2) Application of rDNA live attenuated vaccines intended for use in the open air

(1) Application in the determinant model environment

This refers to the experimental applications conducted in a specifically restricted area, simulated for the environment in which animals inoculated with live attenuated vaccines are reared. And the area must be designed in such way to minimize the spread of rDNA live attenuated vaccines beyond the border and to minimize the gene transfer from the vaccines to other organisms outside the area.

(2) Application in the open air

This refers to the application in the open air of rDNA live attenuated vaccines whose safety has been confirmed in a determinant model environnment.

4. Facilities and. equipment used for rDNA live attenuated vaccines

Facilities and. equipment used for applying rDNA live attenuated vaccines to the production process should be regulated pursuant to the provisions of Section 3-4. except for the description concerning the applications of Category2, Category3 and the simulated model environment.

Facilities and. equipment used for applying rDNA live attenuated vaccines in adeterminant model environment should satisfy the following criteria:

1) There should be a work area clearly distinguished from the other areas

2) An appropriate livestock houses and stock farms should be established to utilize the rDNA live attenuated vaccines in the work area, taking into account their propagative properties, actual methods to limit their propagation, their physiological characteristics, and their application in the open air as well as the surrounding biota.

5. Handling of r:DNA live attenuated vaccines

rDNA live attenuated vaccines applied to the production process should be handled pursuant to the provisions of Section 3-5. except for the description concerning the applications in Category2, Category3 and simulated model environment.

In applying rDNA live attenuated vaccines to a determinant model environment, rDNA vaccines and the animals inoculated with those vaccines should be handled in compliance with the following:

1) Control of rDNA live attenuated vaccines

Necessary, measures should be taken to minimize the dispersal of rDNA live attenuated vaccines from the work area, depending on the form of application.

2) Disposal of wasters

Wasters should be disposed after being inactivated as required to attain the prescribed safety level.

3) Storage

(1) Containers used to store materials, including rDNA live attenuated vaccines, should be clearly labeled as comtaining "rDNA live attenuated vaccines" and should be safely stored in specific storage facilities set up in advance. Storage facilities in use should be indicated clearly with the indication "rDNA live attenuated vaccines in storage (on a application in a determinant model environment.) " The indication should be put on the facility at a recognizable place.

(2) A catalogue of the stored materials harboring rDNA live attenuated vaccines should be prepared and. maintained.

4) Transportation of rDNA live attenuated vaccines

(1) If rDNA live attenuated vaccines are moved from the work area, they should be placed in a container of sufficient strength, and the container should be closed well to prevent leakage of the contents.

(2) The container to be used to transport .rDNA live attenuated vaccines should be clearly labeled and the label should be posted at a recognizable place of the surface, indicating "Handle with Care" in red.

5) Control and maintenance of facilities and equipments

Facilities and equipment used for handling rDNA live attenuated vaccines should undergo performance inspections immediately after their installation and periodically thereafter.

6) Rearing of animals inoculated with rDNA live attenuated vaccines

In the area where animals inoculated with rDNA live attenuated vaccines are reared and around that area, the number of animals that have nothing to do with the application of rDNA live attenuated vaccines, should be minimized. In addition, it is desirable that animals inoculated with rDNA live attenuated vaccines be individually distinguished; in cases when individual distinction is difficult, the animals may be managed in breeding groups.

7) Disposal of wastes relating to animals inoculated with rDNA live attenuated vaccines

Wastes relating to animals inoculated with rDNA live attenuated vaccines (including the carcasses and the products of those animals) should be disposed of, if necessary, after sterilization or incineration.

8) Transportation of animals inoculated with rDNA live attenuated vaccines

In transportation of animals inoculated with rDNA live attenuated vaccines outside the work area, appropriate measures for the category of the animals should be taken to prevent the animals from escaping.

9) Maintenance and management of facilities and equipment used for breeding animals inoculated with rDNA live attenuated vaccines

Facilities and equipment used for handling animals inoculated with rDNA live attenuated vaccines should undergo performance inspections immediately after their installation and periodically thereafter.

10) Other requirements

(1) The indication stating "Application in a determinant model environment (on application to a of determinant model environment) " should be placed at each work area during the work period.

(2) Work areas should be kept clean.

(3) Personnel should wear working clothes prepared exclusively for the work in the work area.

(4) Due attention should be paid so as not to carry rDNA live attenuated vaccines, attached, for example, on the clothes of the personnel, resulting in their diffusion outside the work area.

CHAPTER 3. MANAGEMENT SYSTEMS

Section 1. Establishment of management systems by person responsible

The person responsible (This term applies to a person who is entrusted with the operation of applications in a simulated model environment) should establish management systems in compliance with the following, so as to ensure the safe application of rDNA organisms.

1. Appoint an operations administrator and a safety operations manager for each work site or institute where work will be conducted, and appoint replacements for the above operations administrator end safety operations manager to execute their duties in case of their absence due to illness or other reasons.

2. Establish a safe operations committee and appoint the members of that committee. The committee's members shall be required to periodically review the safety of the applications of rDNA organisms.

3. Oversee the operations administrator in the execution of the duties prescribed in Section 2 below.

4. Oversee the safety operations manager in the execution of the duties prescribed in Section 3-2 below.

Section 2. Operations administrator

The operations administrator should fully understand the following guidelines and should perform the duties detailed below.

1. Observe these guidelines in planning and executing operations, and appropriately manage and supervise each entre operation in close cooperation with the safety operations manager.

2. Prior to initiating operations, educate and train operations personnel regarding safety.

3. Post in a clearly visible location within the work area and storage facilities necessary information related to the handling of rDNA organisms.

4. Require, as appropriate according to the level of operations, that persons entering a work area be accompanied by operations personnel, and ensure that such persons obey the directions of the operations personnel upon entering a work area.

5. Make, and maintain for five years from the date of completion of an operation, records of the following items:

1) Reference names of each rDNA organisms and container number;

2) Storage and history of rDNA organisms employed;

3) The biological properties of rDNA organisms employed and the dates of their inspection;

4) Name and address, and the purpose of application, of organizations from which rDNA organisms were transferred (in the case of rDNA small laboratory animals, the date, number of animals, and the; places of transfer also are required);

5) Results of health examinations of personnel;

6) Records of reviews made by the Safe Operations Committee (including documents used as a basis for confirming that operating standards conform to the Guidelines); and

7) Records of the periodic inspections of facilities and equipment.

Section 3. Safety operations manager

1. The safety operations manager who is to assist the operations administrator should be appointed from among personnel who are knowledgeable about and acquainted with rDNA technology, so as to ensure safety in the application of rDNA organisms.

2. The safety operations manager should fully understand these guidelines and should properly discharge the following duties:

1) Ensure that operations are carried out in accordance with these Guidelines.

2) Advise or report information to the administrator as necessary.

3) Conduct any operations necessary to secure safety.

Section 4. Operation personnel

Operations personnel should properly discharge the following duties.

1. Understand. the operations fully and maintain safety conducting them.

2. Give appropriate instructions to ensure full safety when any persons enter the work area without operations personnel.

Section 5. Safe operations committee

1. There should be formed a Safe Operations Committee consisting of members from appropriate fields, considering that members will need to process highly specialized knowledge of recombinant technology and organisms, as well as have overall general knowledge.

2. The committee should investigate and consider the following matters and advise the responsible person at his (her) request.

1) The suitability of handling methods for rDNA organisms;

2) The suitability of education, training, and health care regarding the safety of personnel in conducting operations;

3) Treatments necessary in case of accidents, and improvements of operating procedures that are advisable to present accidents;

4) Other factors necessary to secure of operating safety; and

5) The suitability of production and sale methods concerning rDNA small laboratory animals, in the context of "the Law concerning the Production and Management of Animals" and other laws and ordinances concerning the applications of rDNA small laboratory animals).

3. The committee may request reports from the operations administrator or the safety operations manager as necessary.

Section 6. Education and training of personnel

The operations administrator should ensure that the operations personnel fully understand these Guidelines and. should ensure that such personnel receive appropriate education and training with regard to the following matters prior to initiating applications involving rDNAorganisms.

1. Knowledge of safety levels of rDNA organisms being handled;

2. Knowledge of the proper handling of rDNA organisms according to their safety evaluation;

3. Knowledge of the proper use of facilities and equipment required for handling rDNA organisms;

4. Knowledge of the safety of operations to be conducted; and

5. Knowledge of measures to be taken in case of accidents.

Section 7. Health care

1. The person responsible should ensure that health examinations of operations personnel are conducted prior to their initiation of operations and at intervals of no longer than one year thereafter.

2. The person responsible should examine the measures to prevent and treat any infection or illness that could possibly arise from the engaging of personnel in Category2 or Category3 applications.

3. The person responsible should require operations personnel to undergo immediate health examinations and take other appropriate measures if it is the possible that personnel have been infected in conducting of Category2 or Category3 applications.

Blood-sera samples should be collected for operations personnel who will be engaged in Category 3 applications prior to the initiation of operations and should be stored for two years after such personnel cease work on the operations.

CHAPTER 4. APPROVAL AND REPORTS

1. In order to ensure the safe application of rDNA organisms, the person responsible may request the Minister of Agriculture, Forestry and Fisheries to approve the safety criteria regarding facilities, equipment, and procedures utilized, to ensure compliance with these Guidelines.

2. The person responsible should collect information relating to rDNA organisms and their applications, and should report immediately to the Ministry of Agriculture, Forestry and Fisheries any new information that might influence the safety evaluation of rDNA organisms.

CHAPTER 5. OTHER MATTERS

1. The person responsible should make efforts to accumulate sufficient knowledge to ensure the safety of rDNA microorganisms intended to be applied in an open system.

2. rDNA organisms whose hosts are animal cells (excluding rDNA organisms that have not progressed sufficiently to be differentiated as individuals) shall be kept in a specifically controlled environment for a specified length of time. If the person responsible requests the Ministry of Agriculture, Forestry and Fisheries to approve a safety evaluation of rDNA organisms whose hosts are animal cells, the approval shall be made on a case-by-case basis.

The species, characteristics, and purposes of applying rDNA animals shall be taken into consideration, and. the regulations concerning their usage shall be applied appropriately.

3. At present, provisions relating to rDNA microorganisms in these Guidelines shall be applied to non-cellular organisms (containing rDNA molecules) that are directly injected into plants. However, plants into which non-cellular organisms are injected shall not be classified as rDNA organisms.

4. At present, a person responsible who intends to conduct sales of rDNA plants that might be able to propagate naturally in Japan, or who intends to produce foods, drinks, oils, or fats using such rDNA plants as materials, should evaluate the initial safety of such plants as described in Chapter 2-Section 2-1-1) .

5. Regarding matters not specified in these Guidelines, in certain applications the director general of the relevant bureau may establish necessary additionally provisions in order to implement of these guidelines.

APPENDIX

	GILS P	Category 1	Category 2	Category 3
1 Extent of sealing of facilities and apparatus				
(1) Handling of rDNA microorganisms in exhaust gases	Minimization* of leakage	Minimization of leakage	Prevention of leakage	Prevention of leakage
(2) Performance of adjusting valves	Minimization* of leakage	Minimization of leakage	Prevention of leakage	Prevention of leakage
2 Conditions of work area				
(1) Biohazard sign	Not necessary	Optional	Necessary	Necessary
(2) Air lock on doorway	Not necessary	Not necessary	Not necessary	Necessary
(3) Decontamination and washing facilities for operation personnel	Optional	Necessary	Necessary	Necessary
(4) Shower facilites	Not necessary	Not necessary	Optional	Necessary

(5) Disposal facilities for waste water from decontamina -tion, washing and shower facilities	Not necessary	Not necessary	Optional	Necessary
(6) Ventilation	Optional	Optional	Optional	Necessary
(7) Maintenance of air pressure negative to atmosphere in work area	Not necessary	Not necessary	Optional	Necessary
(8) Application of HEPA filters to ventilation facilities	Not necessary	Not necessary	Optional	Necessary
(9) Design of work area to pre-vent contents from spreading outside the area in case spillage occurs	Not necessary	Not necessary	Optional	Necessary
(10) Design for sealing of work area to enable sterilization by fumigation	Not necessary	Not necessary	Optional	Necessary

*: It means that the extent of leakage will be reduced to a permissible level, according to the safety of the specific rDNA microoganisms involved.

Guidelines for Safety Assessment of Feed Produced by the Recombinant DNA Techniques

1. Objectives

The objectives of the Guidelines are to establish basic requirements for safety assessment of manufacturing, import and sales of feed produced by recombinant DNA techniques and thereby to ensure the safety of the feed.

2. Definitions of terminology

(1) Recombinant

The term is defined as Living cells where recombined DNA (Deoxyribonucleic acid, body of gene) is inserted (except for cases whose genetic constituent is equivalent to living cells existing in natural environment), and living organisms derived from living cells containing recombined DNA. A recombined DNA (hereinafter referred to as "recombinant DNA") is produced by techniques that DNA proliferative in living cells and donor DNA (derived from livings whose taxonomic classification is not identical to those of transferred living cells) are enzymatically recombined in vitro , and transferred to living cells in order to propagate donor DNA (hereinafter referred to as "recombinant DNA techniques").

(2) Host

The term is defined as living cells into which recombinant DNA is transferred.

(3) Vector

The term is defined as DNA molecules which are used to transfer donor DNA to host cells by recombinant DNA techniques.

(4) Donor DNA

The term is defined as heterologous DNA segments to be combined with vectors.

(5) Product

The term is defined as substances produced by recombinant DNA techniques.

3. Scope of Application

The Guidelines are applicable to the products that are regarded as equivalent to the existing products used as feed.

In addition, the Guidelines are applicable only if the products or their raw material are made or composed by recombinants whose hosts are spermatophytes presently used as feed.

Whether or not the products are regarded as equivalent to the existing products is assessed based upon the following information (1) to (4):

(1) Information on the genetic material

(2) Information on broad breeding history as domestic animals

(3) Information on components of products

(4) Information on difference in usage between the hosts and the recombinants

4. Production methods of recombinants

It is fundamental principle to use hosts, vectors, donor DNAs and recombinants that fall into the criteria specified in Attachment, when feed derived from recombinant is manufactured, imported, or sold.

5. Safety assessment

Whether or not feed derived from recombinants has safety regarded as equivalent to that of conventional feed made from spermatophytes (hereinafter called "existing feed") used as hosts should be assessed in accordance with the safety assessment items specified below, and to ensure the safety the evaluation of properties of recombinants based upon characteristics of hosts, vectors, and donor DNAs as well as the comparison between recombinants and hosts should be made.

(1) Host

a. Taxonomic position (Crop name and scientific name (variety and line)
b. Distribution in natural environment
c. Present usage as feed or food and food additives in Japan and other countries
d. Productivity of harmful substance in host and ancestral or related species to the hosts
e. Foreign factors (virus etc.) (host should not be contaminated with pathogenic exogenous factors.)
f. Reproductive and fertile properties and genetic characteristics
f-1 Survival, reproductive and fertile abilities under experimental conditions simulating ordinary or natural environments
f-2 Reproductive and fertile patterns and period, and crossability
f-3 Restrictive conditions for survival, reproductive and fertility
f-4 other genetic properties and origin
g. Constituents and nutrients
h. Parasiticity, fixing conditions and other physiologically important properties.

(2) Vector

a. Name and origin
b. Properties
b-1 Number of DNA sequences
b-2 Cleavage map by restriction enzymes
b-3 Presence of nucleic acid sequence to code for a known toxin
b-4 Transmissionality
b-5 Host dependency
b-6 Drug resistance

(3) Donor DNA

a. Origin
b. Properties
b-1 Structure (Presence or absence of exogenous open reading frames, and possibilities of their transcription and expression are required to be attached.)
b-2 Function
b-3 Identified/unidentified
b-4 Number of DNA sequences
b-5 Cleavage map by restriction enzymes
b-6 Presence of nucleic acid sequence to code for a known toxin
b-7 Expression site and timing, and amounts of expression
b-8 Number of copies of the donor DNA and its stability
c. Antibacterial-resistant Marker
c-1 Structure and function
c- Mechanism of the manifestation of the resistance, the method of use, and related metabolites
c-3 Antibacterial substances associated with resistance, and their usage for domestic animals
c-4 Inactivation methods of antibacterial-resistant markers and related metabolites
c-5 Estimated amount of antibacterial substances inactivated after addition to feed or oral administration and the possibility that inactivation may cause problems

(4) Recombinant

a. Recombination method and the structure of recombinant DNA
b. Introduction method of target gene into hosts
c. Expression stability and location of target gene

d. New acquired properties
e. Inactivation methods of the recombinant
f. Constituents and nutrients
g. Difference between recombinant and host
g-1 productivity of harmful substances
g-2 Reproductive and fertile abilities, and genetic characteristics
g-2-1 Survival, reproductive and fertile abilities under experimental conditions simulating ordinary or natural environments
g-2-2 Reproductive and fertile patterns and period, and crossability
g-2-3 Restrictive conditions for survival, reproductive and fertile abilities
g-3 Other physiologically important properties
h. Approval and application status in other countries.

(5) Application of products for feed

(6) Toxic studies
a. If safety equivalent to existing feed is not confirmed on basis of (1) to (5), necessary toxic studies among the followings should be conducted.
In principle, tests should be performed according to testing methods described in Circular by Director of Livestock Industry Bureau published in March 16, 1992, named "Assessment methods of Feed Additives."
a-1 Single dosage toxicity study
a-2 Repeated dosage toxicity study (short-term)
a-3 Repeated dosage toxicity study (chronic)
a-4 Breeding and Fertility study
a-5 Carcinogenicity study
a-6 Mutagenicity study
a-7 Teratogenicity study
a-8 Other necessary tests
b. Breeding studies with use of domestic animals
If the safety of the product equivalent to that of existing feed is not ensured according to studies described in 6.a, breeding studies with use of target domestic animals should be conducted.
In principle; tests should be performed according to testing methods described in Circular named "Assessment methods of Feed Additives."

6. Principle for usage of products
(1) If the products for feed are produced by recombinant DNA techniques or feed is composed by the products made from recombinants, manufacturers, importers or distributors (hereinafter referred to as "business agents") of them are required to conduct safety assessment of the products in accordance with the Guidelines to confirm the safety for feed.
(2) The business agents may request the Minister of Agriculture, Forestry and Fisheries to confirm the compliance of the products to the Guidelines to ensure the safety for feed.

7. Miscellaneous provision
(1) Living cells and living organisms derived from the living cells described below are deemed to recombinant defined by 2 (1) for a while, and these should be manipulated under the Guidelines Living cells and living organisms where inserted is recombinant DNA that DNA proliferative in certain living cells (whose origin is limited to spermatophytes, and so forth) is recombined with other DNA derived from an organism of the taxonomically identical variety to the host, and living cells and living organisms derived from the living cells to which recombinant DNA is inserted, having equivalent sequences to living cells existing in nature.
(2) Other articles required for the application of the Guidelines than ones prescribed by the Guidelines will be defined separately by Director-General of Livestock Industry Bureau or Director-General of Fisheries Agency.

Attachment

Host	Vector/Donor DNA	Recombinant
l.Non-pathogenic. 2. No adventitious	1 Well characterized and free from known harmful sequences. 21imited in size as	l.Non-pathogenic.

agents (.virus etc.) .	much as possible to the DNA required to perform the intended functions; Should not increase the stability of the construct in the environment (unless that it requirement of the intended function). 3.Should be poorly mobilizable. 4.Should not transfer any drug-resistance genes to microorganisms not known to acquire them naturally.	

Note: The above attachment is made based on the OECD recommendation "Safety Considerations for Industry, Agriculture and Environmental Application of Organisms Delivered by Recombinant DNA Techniques " published in 1986.

Guidelines for Safety Assessment of Feed Additives Produced by the Recombinant DNA Techniques

1. Objectives

The objectives of the Guidelines are to :establish basic requirements for safety assessment of manufacturing, import and sales of feed additives produced by recombinant DNA techniques and thereby to ensure the safety of the feed additives.

2. Definitions of terminology

(1) Recombinant

The term is defined as Living cells where recombined DNA (Deoxyribonucleic acid, body of gene) is inserted (except for cases whose genetic constituent is equivalent to living cells existing in natural environment), and living organisms derived from living cells containing recombined DNA.

A recombined DNA (hereinafter referred to as "recombinant DNA ") is produced by techniques that DNA proliferative in living cells and doner DNA (derived from livings whose taxonomic classification is not identical to those of transferred living cells) are enzymatically recombined in vitro, and transferred to living cells in order to propagate doner DNA (hereinafter referred to as "recombinant DNA techniques").

(2) Host

The term is defined as living cells into which recombinant DNA is transferred.

(3) Vector

The term is defined as DNA molecules which are used to transfer doner DNA to host cells by recombinant DNA techniques.

(4) Doner DNA

The term is defined as heterologous DNA segments to be combined with vectors.

(5) Product

The term is defined as substances produced by recombinant DNA techniques.

3. Scope of Application

The Guidelines are applicable to the products that are regarded as equivalent to the existing products used as feed additives.

In addition, the Guidelines are applicable only if the products or their raw material are made or composed by recombinants whose hosts are spermatophytes or only if the products derived from microorganisms do not contain recombinants.

Whether or not the products are regarded as equivalent to the existing products is assessed based upon the following information (1) to (4):

(1) Information on the genetic material

(2) Information on broad breeding history as domestic animals

(3) Information on components of products

(4) Information on difference in usage between the hosts and the recombinants

4. Production methods of recombinants

It is fundamental principle to use host, vector, doner DNAs and recombinant that fall into the criteria specified in Attachment, when feed additives derived from recombinant are manufactured, imported, or sold.

5. Safety assessment

Whether or not feed additives derived from recombinant (hereinafter called "target feed additives") have safety regarded as equivalent to conventional ones should be assessed in accordance with the safety assessment items specified below, and to ensure the safety the evaluation of properties of recombinants based upon characteristics of hosts, vectors, and doner DNAs as well as the comparison between recombinants and hosts should be made.

(1) Host

a. Taxonomic position (Crop name and scientific name (variety and line) for spermatophytes, and scientific name and strain for microorganism)

b. Distribution in natural environment

c. Present usage as feed or food and food additives in Japan and other countries

d. Productivity of harmful substance in host and ancestral or related species to the hosts

e. Pathogenicity (non-pathogenic) of in host and ancestral or related species to the hosts only if host is microorganism.

f. Foreign factors (virus etc.) (host should not be contaminated with pathogenic exogenous factors.)

g. Reproductive and fertile properties (proliferation property in case host is microorganism) and genetic characteristics

g-1 Survival, reproductive and fertile abilities under experimental conditions simulating ordinary or natural environments (proliferation ability in case host is microorganism)

g-2 Reproductive and fertile patterns (proliferation pattern in case host is microorganism) and period, and crossability

g-3 Restrictive conditions for survival, reproductive and fertile abilities (proliferation ability in case host is microorganism)

g-4 other genetic properties and origin. Parasiticity, fixing conditions and other physiologically important properties

(2) Vector

a. Name and origin

b. Properties

b-1 Number of DNA sequences

b-2 Cleavage map by restriction enzymes

b-3 Presence of nucleic acid sequence to code for a known toxin

b-4 Transmissionality

b-5 Host dependency

b-6 Drug resistance

(3) Doner DNA,

a. Origin

b. Properties

b-1 Structure (Presence or absence of exogenous open reading frames, and, possibilities of their transcription and expression are required to be attached.)

b-2 Function

b-3 Identified/unidentified

b-4 Number of DNA sequences

b-5 Cleavage map by restriction enzymes

b-6 Presence of nucleic acid sequence to code for a known toxin

b-7 Expression site and timing, and amounts of expression

b-8 Number of copies of the doner DNA and its stability

c. Antibacterial-resistant Marker

c-1 Structure and function

c-2 Mechanism of the manifestation of the resistance, the method of use, and related metabolites

c-3 Antibacterial substances associated with resistance, and their usage for domestic animals

c-4 Inactivation methods of antibacterial-resistant markers

c-5 Estimated amount of antibacterial substances inactivated after addition to feed additives or oral administration and the possibility that inactivation may cause problems

(4) Recombinant

a. Recombination method and the structure of recombinant DNA

b. Introduction method of target gene into hosts.

c. Expression stability and location of target gene

d. New acquired properties

e. Inactivation methods of the recombinant

f. Difference between recombinant and host

f-1 Productivity of harmful substances

f-2 Pathogenicity (non-pathogenic) only if host is microorganism.

f-3 Reproductive and fertile abilities, and genetic characteristics (proliferation ability in case host is microorganism)

f-3-1 Survival, reproductive and fertile abilities under experimental conditions simulating ordinary or natural environments (proliferation ability in case host is microorganism)

f-3-2 Reproductive and fertile patterns and period, and crossability (proliferation pattern in case host is microorganism)

f-3-3 Restrictive conditions for survival, reproductive and fertile abilities (proliferation ability in case host is microorganism)

f-4 Other physiologically important properties

(5) Application of products for feed additives

(6) Target feed additives

a. The product derived from microorganism is required to submit the information as below:

a-1 Evidence demonstrating absence of contamination by recombinants.

a-2 Levels of impurities in products derived from manufacturing and their safety evaluations.

a-3 Purification procedures of products and effectiveness of purification procedures.

b. Approval and application status in other countries.

(7) Toxic studies

a. If safety equivalent to existing feed additives is not confirmed on basis of (1) to (6) , necessary toxic studies among the followings should be conducted.

In principle, tests should be performed according to testing methods described in Circular by Director of Livestock Industry Bureau published in March 16, 1992, named "Assessment methods of Feed Additives."

a-1 Single dosage toxicity study

a-2 Repeated dosage toxicity study (short-term)

a-3 Repeated dosage toxicity study (chronic)

a-4 Breeding and Fertility study

a-5 Carcinogenicity study

a-6 Mutagenicity study

a-7 Teratogenicity study

a-8 Other necessary tests

b. Breeding studies with use of domestic animals

If the safety of the product equivalent to that of existing feed additives are not ensured according to studies described in a. , breeding studies with use of target domestic animals should be conducted.

In principle, tests should be performed according to testing methods described in Circular named "Assessment methods of Feed Additives".

6. Principle for usage of products

(1) Manufactures, importers or distributors (hereinafter referred to as "business agents") of feed additives produced by recombinant DNA techniques are requested to conduct safety assessment of the products in accordance with the Guidelines to confirm the safety for feed additives.

(2) The business agents may request the Minister of Agriculture, Forestry and Fisheries of confirmation regarding the compliance of the products to the Guidelines to ensure the safety for feed additives.

7. Miscellaneous provision

(1) Living cells and living organisms derived from the living cells described below are deemed to recombinant defined by 2 (1) for a while, and these should be manipulated under the Guidelines: Living cells and living organisms where inserted is recombinant DNA that DNA proliferative in certain living cells (whose origin is limited to spermatophytes or microorganisms, and so forth) is recombined with other DNA derived from an organism of the taxonomically identical variety to the host, and living cells and living organisms derived from the living cells to which recombinant DNA is inserted, having equivalent sequences to living cells existing in nature.

(2) Other articles required for the application of the Guidelines than ones prescribed by the Guidelines will be defined separately by Director of Livestock Industry Bureau or Director of Fisheries Agency.

Attachment

Most	Vector/Donor DNA	v Recombinant
1. Non-pathogenic. 2. No adventitious agents (virus etc.) ,	1. Well characterized and free from known harmful sequences. 2. Limited in size as much as possible to the DNA required to perform the intended functions; Should not increase the stability of the construct in the environment (unless that it requirement of the intended function) . 3. Should be poorly mobilizable. 4. Should not transfer any drug-resistance genes to microorganisms not known to acquire them naturally.	1. Non- pathogenic. .

Note: The above attachment is made based on the DECD recommendation "Safety Considerations for Industry, Agriculture and Environmental Application of Organisms Delivered by Recombinant DNA Techniques" published in 1986.