

# Regulatory considerations in the development and application of biotechnology in Japan

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## Summary

In Japan, the development and application of living modified organisms (LMOs) are regulated under the Law Concerning the Conservation and Sustainable Use of Biological Diversity. Procedures are classified as Type 1 for the use of LMOs where no preventive measures against their dispersal into the environment are required, and Type 2 for the use of LMOs with preventive measures. During the period of development, risk assessment is the responsibility of the Ministry of Education, Culture, Science, Sports and Technology. The procedures for field use of LMOs, including recombinant vaccines for veterinary use and genetically modified animals, are described in detail. Control systems for xenotransplantation of the cells, tissues and organs of transgenic pigs are yet to be established.

## Keywords

Cartagena Protocol – Deoxyribonucleic acid – Genetically modified animal – Law Concerning the Conservation and Sustainable Use of Biological Diversity – Living modified organism – Pharmaceutical – Pig – Recombinant vaccine – Risk assessment – Xenotransplantation.

## Introduction

In 2004, the Law Concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms (LMOs) came into force (1). Several different ministries in Japan are involved in the application of this law, as follows:

- the Ministry of Finance
- the Ministry of Education, Culture, Science, Sports and Technology (MECSST)
- the Ministry of Health, Labour and Welfare (MHLW)
- the Ministry of Agriculture, Forestry and Fisheries (MAFF)
- the Ministry of Economy, Trade and Industry
- the Ministry of Environment.

The introduction of this new law led to the abrogation of the previous MECSST guidelines on experiments involving recombinant deoxyribonucleic acid (DNA) techniques for the development of LMOs (3), and of the guidelines for using recombinant DNA organisms for such developments

in agriculture, forestry, fisheries, the food industry and other related industries (2). Under the new law, experiments involving recombinant DNA techniques for the development of LMOs are regulated by the MECSST, while the application of LMOs for veterinary use is controlled by the MAFF.

The LMO Law aims to ensure the precise and smooth implementation of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity (hereafter referred to as the Cartagena Protocol) (8) by taking measures to regulate the use of LMOs.

The LMO Law divides the use of LMOs into two groups: Type 1 and Type 2. Type 1 procedures apply where LMOs are used without measures to prevent their dispersal into the environment. A person who creates or imports LMOs and makes Type 1 use of these LMOs must follow the regulations for such use and obtain the approval of the competent minister, as must any other person who wishes to make Type 1 use.

Type 2 procedures relate to the use of LMOs where preventive measures are to be taken against their dispersal

into the environment. Where the containment measures to be taken are stipulated by the ordinances of the competent ministries, a person who wishes to make Type 2 use of LMOs must take the measures prescribed. Where such measures are not stipulated, users must take containment measures that have been previously agreed by the competent minister.

When LMOs are at the development stage, both Type 1 and Type 2 measures are essentially similar to those described in the guidelines on experiments involving recombinant DNA techniques that were previously issued by the MECST. The field use of LMOs is regulated by the guidelines for Type 1 use. This paper will describe the procedures in effect that relate to LMOs, including recombinant vaccines for veterinary use and transgenic animals.

## Recombinant vaccines for veterinary use

The MAFF regulates the use of all veterinary medicinal products, including vaccines. Two different sets of regulations are relevant to the approval of recombinant vaccines for animals: the Cartagena Protocol and the Pharmaceutical Affairs Law of Japan. The former applies to all LMO products, and the latter to all veterinary medicinal products, whether or not they contain LMOs. The current system requires approval under both sets of regulations in the sequence outlined below.

A manufacturer developing a recombinant vaccine for animals first applies to the Minister of Agriculture, Forestry and Fisheries (hereafter referred to as 'the Minister') for approval of field or laboratory usage, on the basis of the Cartagena Protocol. The Food Safety and Consumer Affairs Bureau of the Plant Products Safety Division of the MAFF is responsible for processing these applications. Acting in the name of the Minister, the Division requests the opinion of the members of the Committee for Evaluation of Biological Diversity Effects (CEBDE), which is established within the MAFF.

The CEBDE has separate subcommittees for plants, animals and micro-organisms. However, rather than working through these subcommittees, the Committee sends applications relating to recombinant vaccines to the Council on Drugs and Food Sanitation and follows the Council's procedure: first, the application is discussed at the Investigative Committee for Veterinary Drugs (ICVD) Applying Recombinant DNA Technology, established under the Subcommittee on the Technology of Organism

Origin (STOO) on the basis of the Cartagena Protocol; then, when necessary, the application is discussed at the STOO itself. Since the members of the STOO and the ICVD are also members of the CEBDE, in practice both the STOO and the ICVD are used as forums for discussion.

The Plant Products Safety Division sends the result of the discussion to the Minister in the names of the members of the CEBDE and informs the applicant of the approval of field or of laboratory usage, in accordance with the Cartagena Protocol.

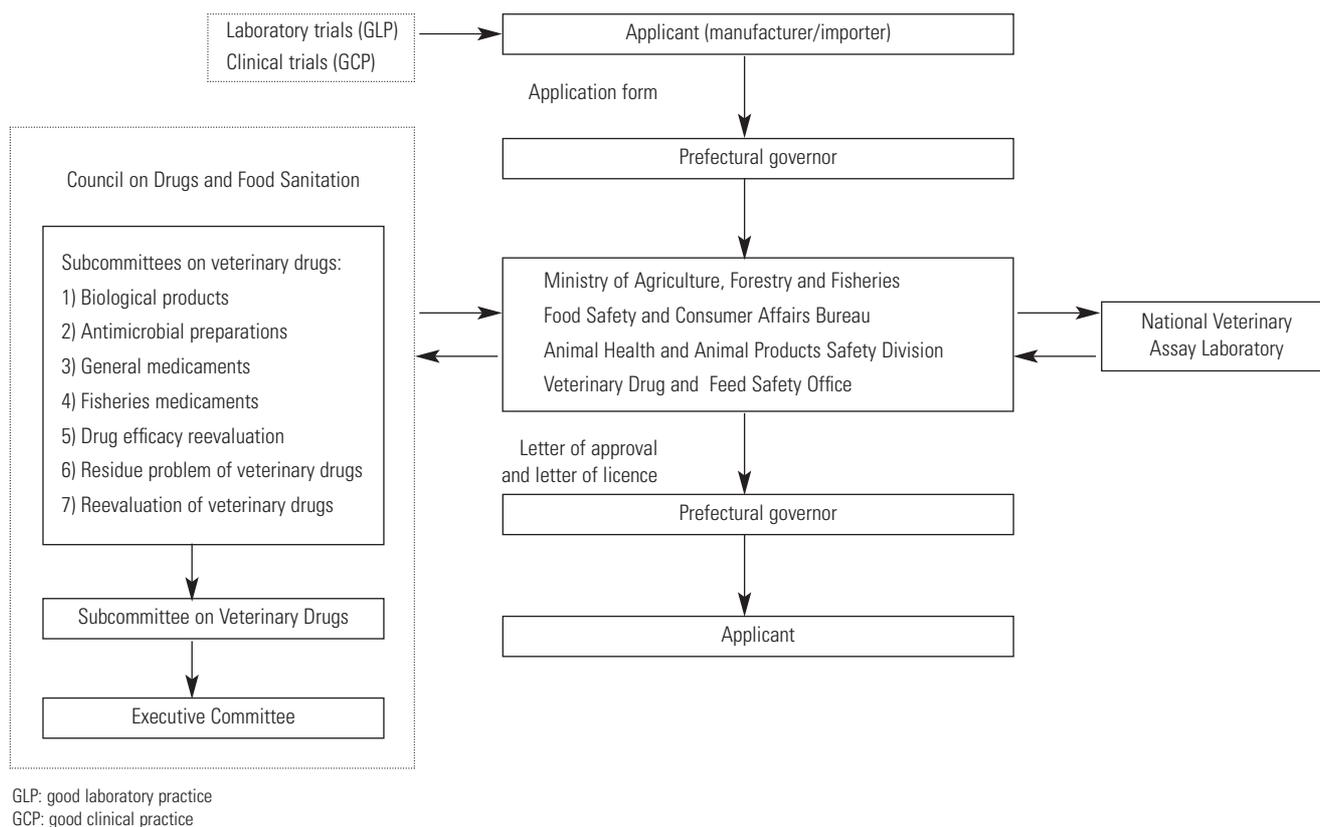
Subsequent to these procedures, manufacturers must also gain approval for field use under the Pharmaceutical Affairs Law. To do this, the vaccine manufacturer must produce a pilot batch, implement clinical tests and collect all the data required to make an application for approval. The approval procedures are the same as those required for ordinary veterinary drugs as shown in Fig. 1.

Under the Pharmaceutical Affairs Law, the manufacturer first submits an application form to a prefectural governor; this form is addressed to the Minister. The prefectural governor sends the application to the MAFF, where the Minister requests the opinions of the Council on Drugs and Food Sanitation. The application is discussed by the Subcommittee on Veterinary Drugs and the Council sends the resulting opinion to the Minister. Once the decision has been approved at Ministerial level, it is sent to the applicant through the prefectural governor.

After approval, recombinant vaccines for veterinary use must undergo national assay by the National Veterinary Assay Laboratory of the MAFF. The assay is performed on each lot/batch of products before distribution, and a pharmaceutical inspector will monitor the disposal of rejected products.

## Genetically modified animals

The MAFF controls the field use of genetically modified animals. The initial steps are essentially similar to those described for recombinant vaccines. Acting in the Minister's name, the Plant Product Division requests the opinion of the subcommittee on animals in the CEBDE. Risk assessment is discussed at the Agriculture, Forestry and Fisheries Research Council (AFFRC) of the MAFF. The AFFRC working group on genetically modified animals has considered a range of factors (discussed below) for the risk assessment and risk management of genetically modified animals in industry where the host animal is neither insect nor aquatic (the group did not consider procedures for companion animals).



**Fig. 1**  
**Procedures for approval of veterinary drugs**

### Procedures of risk assessment

Annex III of the Cartagena Protocol, lists the various steps involved in risk assessment and includes several ‘points to consider’, such as information relating to the intended use of the LMO, and information about the location into which the LMO will be released. It is appropriate to follow these steps for risk assessment for genetically modified animals. (In the following sections, ‘LMO’ refers to genetically modified animals.)

### Basic approach to identifying environmental effects

The Cartagena Protocol does not prescribe a basic approach for identifying environmental effects. However, some international initiatives have already started to classify environmental effects associated with LMOs for the purpose of risk assessment. In Japan, current environmental risk assessment follows such initiatives, using knowledge about the host that has already been gained through previous experience, on a case-by-case basis, and gradually moving from the use of simulated field environments to the use of open field environments.

There are no internationally recognised guidelines for the risk assessment of genetically modified animals, although

safety measures for the biotechnological scale-up of crop plants are widely used for recombinant plants. An OECD report published in 1993 identifies six safety issues that may have negative environmental effects (6):

- gene transfer
- weediness
- trait effects
- genetic and phenotypic variability
- biological vector effects and genetic material from pathogens
- human safety.

The OECD has also produced publications on biotechnology and micro-organisms, including reports on designing small-scale field research with genetically modified micro-organisms (5) and on biotechnology and the scaling-up of micro-organisms as biofertilizers (7). On the basis of these reports, it is considered that the following characteristics of micro-organisms need to be taken into account:

- a) exposure considerations:
- survival, persistence and dispersal
  - gene transfer

b) scale-dependent considerations:

– scale of use should be taken into consideration as the probability of harmful effects may depend on the quantities used

c) potential adverse effects:

– trait effects:

- functional-genes
- selected marker-genes
- non-selected marker-genes
- regulatory genes

– target effects

– non-target effects:

- effects on indigenous microbial populations
- increased growth of non-target plants (i.e. weediness)
- potential pathogenicity and other harmful effects (on plants, animals, humans and mineral cycling)

d) effects of contamination

e) effects derived from mixing different micro-organisms.

Although these factors were originally considered in terms of plants or micro-organisms, they are in principle applicable to animals. On the basis of the characteristics of LMOs, the environmental effects mentioned earlier can be categorised into three general groups:

a) effects of LMOs

b) effects of products made with LMOs

c) effects of the dissemination of introduced genes.

Table I gives some examples of the potential adverse effects of each group.

## Assessing the environmental effects of living modified organisms

Previous Japanese guidelines on safety assessments for genetically modified animals related to small laboratory animals used under controlled conditions. Differences between genetically modified animals and host animals (survival ability in the natural environment, productivity of infectious viruses, reproduction, etc.) are assessed on the basis of the characteristics of the recipient animal and the functions of the introduced genes. In future, however, a wider range of factors must be taken into account in risk assessments, as genetically modified animals may be used in uncontrolled spaces such as barns or pastureland.

The AFFRC's working group has discussed items assessed under current safety guidelines for genetically modified animals, as well as items assessed for recombinant crop plants and items listed in Annex III of the Cartagena Protocol. The elements they considered are summarised below.

### Type of recombinant animals employed

#### *Host animals or animals of the same biological species as the host*

a) taxonomic position

b) background information about use by humans and distribution in the natural environment

c) reproductive and propagative properties, as well as genetic characteristics

d) survival and reproductive abilities in the natural environment

e) other major physiological characteristics.

#### *Donor deoxyribonucleic acid*

a) structure and origin

b) functions of genes.

**Table I**

### Examples of possible adverse effects associated with the use of micro-organisms and recombinant animals

Effects of LMOs	Effects of products made with LMOs	Effects of the dissemination of introduced genes
LMOs may escape and multiply in the surrounding environment, and eventually disturb the original ecological system by competing with native types.	Milk containing antibacterial proteins may spill and affect soil microbes.	Natural, non-modified organisms that are related to the LMOs may acquire the introduced LMO traits as a result of hybridisation with escaped LMOs.
	Infectious viruses may be produced from a vector virus that was used to develop LMOs, and affect wild relatives.	Excreta or body fluid may contain fragments of the introduced gene that may confer pathogenicity or toxin-producing ability on wild micro-organisms in barns or pastures.

### *Vectors*

- a) name and origin
- b) characteristics.

### *Genetically modified animals*

a) methods of preparing and distinguishing genetically modified animals:

- structure and construction methods of recombinant molecules
- methods of introducing recombinant molecules into recipient cells
- developmental and maintenance processes of genetically modified animals
- methods of detecting and identifying recombinant molecules

b) location of recombinant molecules in recipient cells; stability of expression

c) differences between genetically modified animals and host animals or animals of the same biological species from which the genetically modified animals are derived:

- reproductive and propagative properties; genetic characteristics
- survival and reproductive abilities in the natural environment
- production of infectious viruses
- other major physiological characteristics.

### **Methods of handling genetically modified animals**

Risk assessment should identify the differences between genetically modified animals and recipient animals by collecting existing knowledge as well as implementing experiments, and then reveal the possibility of the adverse effects mentioned in Table I. The assessment should also take into account the intended use of the genetically modified animals.

## **Approaches to risk management**

### **Risk assessment as a basis of risk management**

Possible environmental effects associated with the use of genetically modified animals are summarised below. Risk assessment evaluates the likelihood of such adverse effects by identifying the differences between a genetically modified animal and its recipient, on the basis of information about the recipient, introduced genes and vectors. The kind and scale of risk management

to be implemented depend on the magnitude of the assumed effect.

Possible adverse effects associated with the use of genetically modified animals are in principle the same as those associated with plants or micro-organisms, and examples of these are given in Table I.

When using genetically modified animals it is critical to ensure that genetic material is not released into the environment. Special conditions may need to be imposed to prevent, for example, contamination of soil micro-organisms by milk spillage or escape of genetic material in body fluids or excreta (see Table I).

### **Risk management of living modified organisms used in enclosed facilities**

There are currently guidelines for small genetically modified laboratory animals, and these guidelines should be followed in the management of LMOs that are intended to be used in indoor facilities.

### **Risk management of modified organisms in barns or pastureland**

Adequate measures to ensure the prevention of any escape, taking into account the nature of the animals concerned, are required for LMOs intended to be used in barns or pastureland. Without such measures, there is always a risk of LMOs escaping and disturbing the ecological system by competing with native species (Table I).

Appropriate measures to prevent escape must be chosen after assessing the characteristics of the LMO under consideration. In order to ensure safe use of genetically modified animals, it is important to confirm the effectiveness of such measures.

Potential adverse effects such as disruption of the environment and ecological system, or hybridisation of non-modified native species with escaped LMOs (Table I) may occur if LMOs escape into the surrounding environment. Such effects will not occur if the measures for escape prevention are sufficiently effective. In particular, the threat of hybridisation is premised on the existence of wild relatives that are hybridisable with the LMO. However, it is uncommon for livestock animals in Japan to have hybridisable wild relatives in the surrounding environment.

Nonetheless, it is technically very difficult to monitor the possible effects of LMOs. Therefore, unless the possibility of escape by, for example, spillage of products or body fluids containing LMOs can be completely eliminated, such LMOs should not at present be employed in open spaces such as barns or pastureland.

## Xenotransplantation

Clinical trials of xenotransplantation using tissues or cells derived from genetically modified animals should be conducted under the 'Guidelines on issues of infectious diseases in public health associated with clinical trials of xenotransplantation' of the MHLW (4).

Xenotransplantation is defined as a procedure that involves the transplantation, implantation or infusion into a human recipient of either live cells, tissues or organs from a non-human animal source; or a procedure that involves the transplantation, implantation or infusion into a human recipient of body fluids, cells, tissues or organs of humans that have had *ex vivo* contact with live nonhuman animal cells, tissues or organs.

Donor animals should be free from known zoonotic infectious agents and other normal flora or commensals in animals that may cause disease in humans, especially in immunosuppressed patients, by xenotransplantation. As it

is technically difficult to guarantee that all tissues or organs for xenotransplantation are free of such agents, the microbiological conditions of the source animals are important. In terms of the microbiological conditions described in the guidelines, the pig is considered to be a plausible animal.

Experiments on the production of transgenic or knockout pigs for xenotransplantation are conducted under the supervision of the MECSSST. The commercial use of transgenic pigs is monitored by the MAFF. The control system for the commercial use of cells, tissues or organs derived from transgenic animals for clinical trial remains to be determined.

## Aspects réglementaires du développement et de l'application des biotechnologies au Japon

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### Résumé

Au Japon, la production et l'utilisation des organismes vivants modifiés (OVM) sont régis par la Loi relative à la conservation et à l'utilisation durable de la diversité biologique. Les procédures relèvent du Type 1 pour l'utilisation des OVM ne nécessitant pas de mesures préventives contre leur dispersion dans l'environnement, et du Type 2 pour l'utilisation des OVM devant s'accompagner de mesures préventives. Pendant la période de développement, l'évaluation du risque incombe au ministère de l'Éducation, de la Culture, de la Science, des Sports et de la Technologie. Les règles à suivre pour l'utilisation pratique des OVM, y compris les vaccins recombinants à usage vétérinaire et les animaux génétiquement modifiés, sont décrites en détail. Des systèmes de contrôle relatifs à la xénotransplantation de cellules, de tissus et d'organes de porcs transgéniques doivent encore être institués.

### Mots-clés

Acide désoxyribonucléique – Animal génétiquement modifié – Appréciation du risque – Loi relative à la conservation et à l'utilisation durable de la diversité biologique – Organisme vivant modifié – Pharmaceutique – Porc – Protocole de Carthagène – Vaccin recombinant – Xénotransplantation.

# Examen de la reglamentación sobre la producción y la aplicación de la biotecnología en Japón

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## Resumen

En Japón, la producción y la utilización de organismos vivos modificados (OVM) están reglamentadas por la Ley sobre la Protección y la Utilización Duradera de la Diversidad Biológica. La reglamentación comprende dos categorías de normas: las que se aplican a las utilidades de OVM para las que no es preciso tomar medidas contra su dispersión en el medio ambiente (Categoría 1), y las aplicables cuando esas medidas son necesarias (Categoría 2). La evaluación de riesgos durante el período de producción recae bajo la responsabilidad del Ministerio de Educación, Cultura, Ciencia, Deporte y Tecnología. En este artículo se describen detalladamente las normas sobre la utilización de OVM sobre el terreno, incluidas las relativas a las vacunas recombinantes de uso veterinario y los animales modificados genéticamente. El país todavía no cuenta con mecanismos de control de los xenotransplantes de células, tejidos y órganos de cerdos transgénicos

## Palabras clave

Ácido desoxirribonucleico – Animal modificado genéticamente – Cerdo – Evaluación del riesgo – Ley sobre la Preservación y la Utilización Duradera de la Diversidad Biológica – Organismo vivo modificado – Producto farmacéutico – Protocolo de Cartagena – Vacuna recombinante – Xenotransplante.



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