

Regulatory considerations for biotechnology-derived animals in Canada

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Summary

Regulatory initiatives relating to biotechnology-derived livestock have focused on animal health, environmental impact, and the general concept of the safety of the food and by-products derived from such animals. Existing regulatory frameworks have been stretched to accommodate these emerging concerns. Public concerns and the expectations of society mean that the regulatory infrastructure is subject to a high level of scrutiny and that regulations are expected to maintain a clear level of confidence, transparency and effectiveness. A sound regulatory regime should be 'neutral', neither 'facilitating' nor 'restricting' the approval of products or by-products derived from biotechnology-derived animals.

Keywords

Animal health – Biotechnology-derived livestock – Environmental impact – Food safety – Public acceptance – Regulation – Reproductive technologies – Transgenic technology.

Introduction

The principles of biotechnology have been unknowingly followed for years in the selection of animals to breed for particular phenotypic characteristics. The same biological mechanism used to produce a desired characteristic in an animal is now being exploited through modern genetic engineering, enabling new variants to be produced at a more rapid pace. Public unease about the applications of modern biotechnology is partly due to an ever-increasing number of genetically modified animals whose long-term fate and impact on the environment cannot be predicted with certainty. Although safety evaluations cannot be carried out in full, a rational approach to this problem is possible and could provide a sound basis for constructive discussion with the scientific community, members of the regulatory community and the general public. Regulatory policy for transgenic livestock is being developed concurrently with several rapidly advancing technologies for creating such animals (13). It is expected that this evolution of regulatory policy will determine whether the current collaboration between animal drug, veterinary biologic, environmental, public health and food safety agencies will continue to be appropriate. Regulatory policy

and industry practices associated with transgenic livestock, and with the welfare, safety and quality characteristics of these innovations, must be communicated effectively to gain consumer acceptance (12).

Before new biotechnology products enter the market, it is essential that such products be evaluated rigorously by the appropriate regulatory agencies to ensure their safety and efficacy. Concerns about the potential detrimental effects of the release of genetically modified organisms into the environment must be addressed by implementing science-based regulatory standards to ensure that all products are subjected to thorough safety assessments (14). On the basis of the 'precautionary principle', it is considered prudent for regulatory agencies to withhold approval if there is not sufficient information to eliminate the possibility of significant detrimental effects. The need to ensure the biosafety of genetically engineered micro-organisms and animals is undoubtedly one of the most critical challenges that the agricultural biotechnology industry and regulatory agencies face. Science-based regulatory agencies are now faced with the challenge of developing and implementing appropriate standards for assessing the safety of an increasingly broad range of novel biotechnology products (17).

The regulatory implications for reproductive technologies, including transgenic technology, were reviewed by Evans in 1999 (8). The present review will identify the key considerations for developing an effective regulatory framework, and touch on the concerns shared by industry and regulators in the development of policy to guide the use of transgenesis in livestock agriculture. The key message is, however, that for the development of regulatory instruments, the bodies promoting the use of regulatory controls, the authorities responsible for regulations and the stakeholders affected by regulations must all be fully involved in the process; acting together, these groups can provide the best possible balance of science, societal values, economic impact and general welfare. The main objective of this paper is to summarise the issues and challenges related to the regulation of animal health and production applications of agricultural biotechnology, with a focus on the relevance of the Canadian experience in developing and implementing regulatory standards for biotechnology-derived animals.

Biotechnology-derived animals

Background

Transgenic animals first emerged during the mid-1980s with reports of success in mice (15). The ability to alter the genome of an animal by introducing exogenous deoxyribonucleic acid (DNA) was a major technological advance in biotechnology and animal agriculture. Transgenic animals are produced by the introduction of a small fragment of DNA into pre-implantation embryos. Through this technique, scientists have been able to add, delete, silence or partially inactivate genes of interest. Genes can be inserted into animals in such a way that they are expressed in a particular tissue. Production of transgenic livestock provides a method of rapidly introducing 'novel' genes into poultry, cattle, swine, sheep, goats and fish. Transgenic animals are generally produced for four reasons:

- to improve animal health
- to increase productivity and improve product quality
- to mitigate the environmental impact of food animal production
- to produce bio-pharmaceuticals and industrial biochemical products.

There is a great deal that is not yet understood, including how genes that are knocked out are compensated for by other pathways and how epigenetic phenomena affect gene expression in the whole animal. Technologies in this field are becoming more and more sophisticated but much remains to be learnt in order to optimise these procedures in a responsible manner.

Applications

There are numerous potential applications of transgenic methodology for developing new or altered strains of agriculturally important livestock (Table I). Practical applications of this technology in livestock production include:

- improved milk production
- increased growth rates
- increased disease resistance
- increased prolificacy
- altered cell and tissue characteristics for biomedical research.

In agriculture, the use of transgenic animals is being investigated as a potential way of developing breeding stock to enhance production traits (such as growth rate and feed efficiency), mitigate environmental impacts or increase disease resistance. Potential human health applications of this technology include producing biopharmaceuticals, and generating organs, tissues and cells for xenotransplantation.

There have also been reports of the production of transgenic cows with altered casein, and others that have an increased resistance to mastitis, with the secondary benefit of reducing dependence on anti-microbial agents. Growth hormone genes have been inserted into sheep to produce faster-growing lambs, with increased feed efficiency for a net increase in productivity. Transgenic pigs have been produced by inserting in their genome a phytase gene that is expressed in salivary glands (9). This allows such swine to digest phosphorus more efficiently, which reduces the excretion of phosphorus into manure.

Production of biopharmaceuticals (animal 'pharming') has been achieved by generating transgenic animals that express novel proteins in secretions such as milk or seminal plasma. A range of polyclonal and monoclonal human antibodies, industrial biochemicals such as spider silk, and other proteins for treatment of human diseases have been generated through animal 'pharming'. The latest achievement has been the 'double-knockout pig' (16), which may eventually lead to the production of organs and cells such as pancreatic islet cells to treat diabetes, or kidneys for transplantation into humans.

It is important to note, however, that the production capability of genetically selected and/or genetically modified animals will be realised only when their true genetic potential is attained through appropriate environmental and management measures (2).

Table I
List of transgenic animals and the stated purposes for which they are being developed

Type of animal	Modification	Intended end use
Cattle	Produce pharmaceutical proteins	Pharmaceutical production
	Increase disease resistance	Human consumption
	Increase milk and meat production	Human consumption
	Produce organs which are less likely to be rejected	Xenotransplantation
Chickens	Produce pharmaceutical proteins in eggs	Pharmaceutical production
Goats	Produce pharmaceutical proteins	Pharmaceutical production
	Produce industrial proteins	Production of new materials
Monkeys	Develop rhesus monkeys as research models for human diseases	Research
Pigs	Boost lean meat production	Human consumption
	Produce pharmaceutical proteins	Pharmaceutical production
	Create a research model for human diseases	Research
	Decrease phosphorous content in pig manure to improve feed efficiency and environmental protection	Human consumption
	Produce organs which are less likely to be rejected	Xenotransplantation
Sheep	Produce pharmaceutical proteins	Pharmaceutical production
	Increase milk and wool production	Human consumption, production of fibre
Fish	Increase growth rates and food conversion efficiency	Human consumption
	Increase disease resistance or cold tolerance	Human consumption, ornamentals and companion animals
	Alter the colour of a fish under certain conditions	Ornamentals and companion animals, environmental detectors of pollutants
	Engineer fish with sterile or deleterious genes	Biological control of non-indigenous species
	Produce pharmaceutical proteins	Pharmaceutical production

Criteria for developing biotechnology-derived animals

Whether transgenic technology is adopted for use in agriculture will largely depend on the perceived benefits and risks for humans, as well as the potential impact on the overall health and welfare of farm animals. Some of the more pertinent criteria that must be considered in the light of potential safety concerns are discussed below.

Benefits to human health, animal health and the environment

A key objective is to enhance the quality of life for humans without compromising (and preferably while improving) the health and welfare of domestic livestock. The assessable benefits include:

- reduced antibiotic use
- reduced pathogen loads
- enhanced metabolic efficiencies
- increased disease resistance.

The costs and benefits of transgenic animal development may need to be assessed in the context of intended use. For example, the development of transgenic livestock intended

to become founders of lines used for pharmaceutical production may be justified because it has the potential to be of great benefit to society. Another example is the development of transgenic animals that have reduced pollutants in their excretion and which, therefore, benefit the environment (as mentioned above, pigs have been developed with phytase genes in their salivary glands to reduce phosphorus output). The goals of preserving human health and the environment are well served by such biotechnologies that reduce environmental pollution.

Safe and ethical propagation

The criteria for selecting desirable traits to be propagated through transgenic technology are largely based on the demand for specific commercially valuable characteristics. Good science alone cannot ensure public acceptance of transgenic livestock; if the implications of this technology are poorly communicated to the public the likelihood that they will accept such animals is severely reduced. When developing and regulating the commercial applications of biotechnology, the animal biotechnology industry and regulatory agencies must address society's expectations about the safety of this technology and must consider their ethical concerns regarding the conservation of genetic material, the maintenance of diversity and the sustainability of agriculture.

Animal welfare considerations

Some aspects of gene transfer also have the potential to cause unpredicted harm, such as infectious disease hazards or impaired reproduction. For example, some transgenic technologies could potentially lead to the activation and recombination of endogenous retroviruses, leading to increased virulence. Moreover, using nuclear transfer techniques to propagate genetic modifications may increase risks to the reproductive health and welfare of both surrogate dams and transgenic offspring, due to foetal oversize and perinatal mortalities (11, 18). Modern producer practices, societal values and expectations, and regulatory initiatives will weigh heavily against the development of any animals, transgenic or not, that would result in significantly increased risk of abortions and stillbirths, dystocia, neonatal losses, physiological or anatomic dysfunctions, or other impairments.

Source of transgenes

Transgenic animals may be generated by the introduction of genes obtained from a number of sources, including:

- animals of the same species
- animals of a different species
- microbes
- human cells
- *in vitro* nucleic acid synthesis.

Transgenes originating within the same food animal species or transgenes of synthetic origin may be preferable, since they can alleviate potential concerns about the incorporation of genetic material from other species into food animals. Concern about the origin of transgenes in food-producing animals is clearly an issue of human perception and acceptance rather than a safety issue, as humans have ingested DNA from many sources for millions of years.

Preserving the integrity of species

Stringent controls will be required to assure the maintenance of biological diversity and the genetic integrity of species. Unaltered germplines may prove to be an invaluable 'gene bank' in the event that novel infectious diseases or heritable genetic defects are inadvertently introduced into modified sub-populations as a consequence of genetic alterations. Regulatory policies should respect the deeply held moral convictions of democratic societies, as they can profoundly influence public acceptance of human-controlled gene transfer among animals.

Regulatory considerations

The regulation of products derived from biotechnology can be based on the principles used for products produced

using conventional animals. Regulations and standards for determining the responsible use of animal biotechnology in food and agriculture are based on principles that take into account criteria such as benefits and risks, the scientific basis of biotechnology and effects on the environment; they should also consider animal welfare and social acceptability (12). Transgenic animals may be most readily accepted if the end result of the genetic manipulation is to provide a better quality of life for humans or animals, or to provide 'environmentally friendly' alternatives to 'factory farms'.

Producers of transgenic food animals may encounter considerable resistance among some sectors of the general public, as well as among producers of conventional or 'organic' livestock who are concerned that the presence of transgenic animals in the general livestock population may result in inadvertent mixing with the conventional or 'organic' products. Consequently, regulatory initiatives must be founded on principles that encompass careful evaluation and monitoring of the products of animal biotechnology to assure appropriate segregation, identification and tracking of transgenic animals. These principles include:

- establishing high standards for safeguarding human health and animal health and welfare
- developing clear technical standards and assessment guidelines
- providing a sound scientific basis for evaluating associated risk
- consulting and involving stakeholders and the general public in the development of regulations
- building upon existing regulations and technical standards
- maintaining genetic diversity and conserving the environment.

Regulation of biotechnology-derived animals in Canada

The responsibility for regulating biotechnology-derived animals is shared between Environment Canada (EC) and Health Canada (HC), with support from the Canadian Food Inspection Agency (CFIA). The EC and HC are both responsible for environmental safety assessments, while HC also assesses the food safety of products and by-products obtained from biotechnology-derived animals. The CFIA is responsible for feed safety assessments and also for risk assessment from the animal-health perspective. This approach is based on principles that are an integral part of the foresight processes that formed the basis for a federal regulatory framework as early as 1993.

Table II
Possible causes of unintended effects in transgenic animals

Event	Mechanism	Comments
Gene disruption	Insertional mutagenesis through the random insertion of the transgene into the host's genome	It has been estimated that between 5% and 10% of established transgenic mice lines carry such mutations (35). The severity of any effect on the host will largely depend on the function of the particular host gene. Such mutations tend to be recessive and so do not become evident until individuals are produced which are homozygous at the site of insertion (36)
Activation of host gene	Action of inserted promoter and/or enhancer elements on host genes adjacent to or some distance from the transgene integration site	A whole range of phenotypic consequences are said to be possible, including interference with normal development and cancer induction later in life (e.g. gene activation is the mechanism of cancer induction in animals infected by a variety of retroviruses) (36)
Ectopic expression	The novel protein is expressed in tissues where, or at a time when, the promoter is not expected to be active	Often referred to as 'leaky expression'. May be due to the action of a neighbouring enhancer element or could result from basal-level transcription at the site of integration (36)
Pleiotropy	In addition to, or instead of, the intended effect, expression of the transgene results in multiple, often seemingly unrelated, phenotypic effects	Can have both positive and negative impacts on the host animal. For example, expression of the rainbow trout growth hormone gene in carp had a positive influence on survival from fingerling size upwards when they were subjected to a series of stressors and pathogens (6), whereas growth hormone expression in transgenic Coho salmon has resulted in severe morphological abnormalities to the head and jaw due to overgrowth of cartilage (12)

Federal regulatory framework for biotechnology

The principles that formed the basis of the original framework continue to provide direction for all biotechnology regulatory initiatives in Canada, as mentioned in discussions at the CFIA consultation in 1999 (1). The framework allows the benefits of biotechnology products and processes to be achieved in a way that protects health, safety and the environment. The following key principles form the backbone of the framework:

- a) to maintain Canada's high standards for protecting the health of workers, public and the environment
- b) to use existing legislation and regulatory institutions to clarify responsibilities
- c) to continue to develop clear guidelines for evaluating products of biotechnology, in line with national priorities and international standards
- d) to provide a sound scientific basis on which to assess risk and evaluate products
- e) to ensure the development and enforcement of Canadian biotechnology regulations in an open and consultative manner

f) to contribute to the prosperity and well-being of Canadians by fostering a favourable climate for investment, development innovation and adoption of sustainable Canadian biotechnology products and processes.

A key aspect of the Government of Canada's regulatory approach is that it is product-based rather than process-based. In other words, a product derived from a transgenic animal (milk, meat or a protein) would be regulated, not the process which introduced a transgene or a novel trait in the animal. This approach allows the regulatory agencies to take into consideration the substantial history of information related to foods that have long been safely consumed by humans, so as to help identify potential safety and nutritional issues.

Regulatory approach

The regulatory approach is based not only on characteristics of the product, but also on adherence to the established performance standards and on the use of science-based risk assessment methodology to protect the health of people, livestock and the environment.

All new substances, including living organisms, are governed by the Canadian Environmental Protection Act, 1999 (CEPA). This act is co-administered by EC and HC. The term biotechnology is defined in CEPA as 'the application of science and engineering to the direct or indirect use of living organisms or parts or products of living organisms in their natural or modified forms' (7). This legal definition casts a very wide net. By extension of the definition, all livestock manipulated through science and engineering are considered to be biotechnology-derived animals. Transgenic animals and clones of animals would fall under the category of biotechnology-derived animals. The classification of a product as 'new' is determined by whether or not the substance appears on the domestic substances list (DSL) maintained by the CEPA registry. Any substance not on the DSL is considered to be new.

Developers must notify EC and HC whenever a new substance is manufactured or imported; 'new' animals reported to these authorities are then evaluated for any risk to the environment and human health prior to import or manufacturing. The risk of products of biotechnology is further assessed on the basis of the use for which the products are intended. A case in point is a transgenic animal modified to better metabolise phosphate, and hence reduce the phosphate load in the environment. The intent of developers of such an animal could be to introduce the milk of these animals into the human food chain, and to render the rest of the animal into animal feed. Risk would have to be separately assessed for these distinct uses.

Risk assessment for such animals may in future be dealt with in a similar way to plants with novel traits (PNTs). In Canada, regulators from the Plant Biosafety Office (PBO) and the CFIA have a policy of 'no split approvals' for PNTs. In accordance with this policy, developers are requested to submit three notification packages simultaneously to three Government departments and agencies. Specifically, one notification package must be submitted to the PBO and the CFIA for release of the PNT into the environment; a second goes to HC for a safety assessment of the product as a novel food; and a third to the CFIA for an examination of the safety of the PNT as a novel feed. Currently, EC, HC and the CFIA are in discussion to determine whether the current approach of 'no split approvals' can be extended to biotechnology-derived animals. For a transgenic animal intended for multiple uses, under a no-split-approval policy as described above, the three notifications would be directed to EC and HC for release into the environment, to HC for approval as a novel food and to the CFIA for animal health and novel feed assessments. Until a decision has been taken about this policy, a two-tiered approach will apply.

Under this two-tiered approach, notifiers will first apply to EC and HC for approval to release the biotechnology-derived animal into the environment. Once the application is granted, the notifiers must apply to the appropriate

department, depending on the intended use of the product derived from the animal. For example, an application for approval of milk to be introduced into the human food chain must be made to HC, while the CFIA is responsible for approval of feed for consumption by livestock. Notifiers are encouraged to discuss and meet with regulatory officials prior to submitting the notification packages for assessment.

Health Canada's Novel Foods Regulations (10) uses 'substantial equivalence' as a guide to the safety evaluation of three main aspects:

- molecular characterisation
- nutritional similarities
- toxicological assessment, including allergenicity data.

The product is evaluated for 'substantial equivalence' to its conventional counterpart, and if the conditions laid down for food safety are met, then the product may be released for use. However, if any unintended effects are observed during the post-approval phase, the developers are required to report the findings and the product may be subject to re-evaluation.

Officials of the CFIA are responsible for administering both the Health of Animals Act (HAA) and Regulations (5, 6) and the Feeds Act and Regulations (3, 4). The HAA is 'an act respecting diseases and toxic substances that may affect animals or that may be transmitted by animals to persons, and respecting the protection of animals'. The CFIA officials examine animal-health concerns such as the potential transmission of animal pathogens during the import of conventional livestock and their germplasm. In addition, Agency officials in the Animal Biotechnology Unit and the Biohazard Safety and Containment Units have proposed a model for establishing a mechanism to contain animal pathogens that may be transmitted or borne by transgenic animals bred via retroviral vectors. Following peer review, this model will provide a basis for developing guidelines for the containment of transgenic animals developed through retroviral vectors.

The Feeds Section of the CFIA is responsible for administering the Feeds Act and Regulations and for approving feeds. In Canada, a feed is considered 'novel' if it comprises an organism or organisms, or parts or products thereof, that have a novel trait or have not been previously used in Canada. The Feeds Act and Feeds Regulations (3, 4) require that all single-ingredient feeds be evaluated prior to their use in livestock feeds. This applies to both imported and domestically manufactured products. The relevant standards and labelling requirements are specified in the legislation. Novel feed products being developed or tested in feeding trials are subject to regulatory requirements. Data submissions for a

novel feed should include a description of the organism and the genetic modification, the intended use, and the environmental fate, including a determination of whether the gene products or their metabolic products will reach the human food chain. The importation of a novel feed requires a safety assessment of the feed before entry into Canada is authorised.

Conclusions

Genetically modified livestock in agriculture are already an experimental reality, and may soon become a commercial reality. The challenge is to see whether the existing regulatory model will work and whether current marketing practices will be appropriate for this challenge. The acid test of regulatory policy and industry practices will be their capacity to build consumer confidence. At the same time, scientists engaged in the development of transgenic livestock intended to supply food must recognise that regulators and society see transgenic technology as a considerable shift from traditional animal breeding practices. Livestock breeding is – and will continue to be – a balancing act of multiple trait selection, and it is naïve to

believe that transgenes will become so important as to monopolise the selection process.

Regulatory requirements for transgenic livestock are not yet definitive, but clearly have the potential to affect important areas such as trade certification, animal identification, product identity and traceability. Based on the principle of responsible stewardship, the government should build public confidence by developing clear and appropriate regulatory pathways for the oversight of transgenic livestock technology. With the technology clock ticking, regulators, producers and consumers have a narrow window of opportunity to develop a consensus about this technology and work together in a responsible, transparent manner to propose a science-based regulatory system. ■

Canada : aspects réglementaires concernant les animaux issus des biotechnologies

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Résumé

Les initiatives réglementaires relatives aux animaux d'élevage issus des biotechnologies visent la santé animale, l'impact environnemental et le principe général de sécurité des aliments et des produits dérivés. Les cadres réglementaires existants ont été élargis pour tenir compte de ces nouvelles préoccupations. Les craintes du public et les attentes sociales poussent les infrastructures réglementaires à un niveau de contrôle et de responsabilisation tel que les dispositions en vigueur devraient permettre d'assurer un degré de confiance, de transparence et d'efficacité acceptables. Tout régime réglementaire doit rester « neutre », c'est-à-dire ne pas « faciliter » ni « restreindre » la procédure d'agrément des produits et des sous-produits tirés des animaux issus des biotechnologies.

Mots-clés

Acceptation du public – Animal d'élevage issu de la biotechnologie – Impact environnemental – Réglementation – Santé animale – Sécurité alimentaire – Technologie de la reproduction – Technologie transgénique.

La dimensión reglamentaria de la obtención de animales por medios biotecnológicos en Canadá

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Resumen

En Canadá, las iniciativas para reglamentar la obtención de bovinos por medios biotecnológicos se han centrado en la sanidad animal, los posibles efectos ambientales y el concepto general de inocuidad de los alimentos y subproductos procedentes de esa clase de animales. Lo que se ha hecho para dar cabida a estas nuevas preocupaciones es dar una interpretación flexible a los dispositivos reglamentarios vigentes. Las preocupaciones y expectativas de la sociedad llevan a endurecer los controles y la exigencia de responsabilidad dentro de dichos dispositivos hasta niveles que en principio basten para mantener un grado adecuado de fiabilidad, transparencia y eficacia. Un ordenamiento reglamentario bien concebido debe ser 'neutral', esto es, ni 'facilitar' ni 'dificultar' la aprobación de productos o subproductos procedentes de animales obtenidos por medios biotecnológicos.

Palabras clave

Aceptación pública – Bovino obtenido por biotecnología – Efecto ambiental – Inocuidad de los alimentos – Regulación – Sanidad animal – Técnica de transgénesis – Tecnología reproductiva.



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