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FAO/WHO Expert Consultation on the Safety Assessment of Foods Derived from Recombinant-DNA Animals

World Health Organization, Headquarters
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REPORT

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Executive Summary

A joint FAO/WHO Expert Consultation on the Safety Assessment of Foods Derived from Recombinant-DNA Animals was held at the Headquarters of the World Health Organization (WHO) in Geneva from 26 February to 2 March 2007. The objective was to provide scientific advice to FAO/WHO and their Member States on two sets of questions regarding: i) marker and reporter genes; and ii) non-heritable applications. The Codex *ad hoc* Intergovernmental Task Force on Foods Derived from Biotechnology had specifically requested advice on these questions. This Consultation built upon the conclusions and recommendations from the Joint FAO/WHO Expert Consultation on the Safety Assessment of Foods Derived from Genetically Modified Animals, including Fish (FAO/WHO 2004).

A variety of reporter and selectable marker genes are used extensively in plants and laboratory animals and are now being used in food animals. Few non-antibiotic resistance marker and reporter genes are currently used for producing recombinant-DNA animals intended for food and no studies are available on their food safety. It would be desirable to develop new selectable marker genes that do not confer antibiotic resistance.

Effective techniques for removal of specific DNA sequences, so called DNA excision systems, have been used primarily in laboratory animals and are beginning to be used in food animals. The continued validation and development of techniques for DNA excision is strongly encouraged. Recombinant-DNA animals intended for food use should be free of the introduced DNA excision genes in order to minimize the potential for unintended effects. There is a need for further research on the food safety of non-antibiotic resistance marker genes and of DNA excision systems.

Recombinant-DNA constructs introduced into animals can be designed to be heritable or non-heritable. Non-heritable constructs also may be used to improve production and animal health, or to protect against disease via administration of recombinant-DNA vaccines. Non-heritable constructs may become integrated into the genome of somatic cells.

Differences between recombinant-DNA constructs with respect to food safety are a function of whether the construct has been integrated into the genome or maintained episomally and not whether it is heritable or non-heritable. The primary qualitative difference between food consumption risks in recombinant DNA animals containing heritable and non-heritable constructs is whether excipients that facilitate delivery of non-heritable constructs remain present in recombinant- DNA animals.

Quantitative differences in animal health and food consumption risks are related to the increased potential of episomally maintained constructs to participate in horizontal gene transfer and possibly recombine to generate functional viral particles. Recent developments on non-viral episomal vectors provide a means to overcome many of the concerns associated with viral-based vector systems.

Further research is encouraged to understand whether food safety is affected by the use of viral sequences in the constructs and by potential effects of horizontal gene transfer. A guideline should be developed to address the identified animal health issues, including the safe use of virally derived vectors. A suitable venue for developing such a guideline would be the World Organisation for Animal Health (OIE).

There is an interrelationship between animal health and food or feed safety for recombinant-DNA animals. A particularly important issue is the urgent need to fully address the animal health and food safety issues raised by potential applications of recombinant-DNA vaccines, which are a type of non-heritable construct. Therefore, it is important that relevant bodies such as FAO, WHO, and OIE work together to adequately address interactions among these issues.

1. Introduction

A Joint FAO/WHO Expert Consultation on Safety Assessment of Foods Derived from Recombinant-DNA Animals was held at the Headquarters of the World Health Organization (WHO) in Geneva from 26 February to 2 March 2007. A total of 18 experts participated in the Consultation. The complete list of the participants is given in Annex 1.

Mrs. Susanne Weber-Mosdorf, Assistant Director-General, Cluster of Sustainable Development and Healthy Environment, WHO, opened the Consultation on behalf of the Director-General of WHO and the Food and Agriculture Organization of the United Nations (FAO).

In her opening remarks, it was recalled that WHO and FAO provide scientific advice and technical guidance to Member States as well as to Codex Alimentarius Commission with the aim of improving overall food safety and protecting human health as well as enhancing consumer confidence in the safety of the food supply.

It was expressed that, while recognizing that modern biotechnology could contribute directly and indirectly to enhancing human health and development, the use of the new technology might also introduce potential risks for human health and/or the environment. Therefore, there is a need for a common, evidence-based system to facilitate a coherent assessment of the safety of foods derived from modern biotechnology.

The Consultation elected Professor Anne R. Kapuscinski as Chairperson and appointed Dr Lisa Kelly as Rapporteur. The Consultation also decided to establish two in-session working groups: Working Group A focusing on issues of reporter and marker genes, and Working Group B addressing issues on non-heritable application.

For Working Group A, the Consultation appointed Professor Heiner Niemann as Moderator and Professor Kaare M. Nielsen as Rapporteur. For Working Group B, the Consultation appointed Dr Larisa Rudenko as Moderator and Professor Martin O. Makinde as Rapporteur.

All participants completed a Declaration of Interest as defined by FAO and WHO.

2. Background

The Codex Alimentarius Commission, at its 27th Session, re-established the Codex *ad hoc* Intergovernmental Task Force on Foods derived from Biotechnology (Codex Task Force) and entrusted it to elaborate standards, guidelines, or other principles, as appropriate, for foods derived from modern biotechnology.

The Codex Task Force, at its 6th Session held on 27 November - 1 December 2006, discussed the "Proposed Draft Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Animals" and agreed to ask FAO and WHO for scientific advice on two sets of questions[1]:

- ***Marker and Reporter Genes***

  - What developments have occurred in the development and use of reporter and selectable marker genes?

[1] ALINORM 07/30/34

- Are there non-antibiotic resistance marker or reporter genes that have been demonstrated to be safe to humans in food products, and if so, what are they?

- When removal of specific DNA sequences is desired, are reliable and safe techniques available to do this on a routine basis?

- ***Non-heritable Applications***[2]

- Are there relevant differences from a food safety perspective between animals with heritable and non-heritable traits, and if so, what are they?

- Are there specific food safety questions (e.g. with regard to types of vectors) that should be considered relative to the assessment of safety of food from animals containing heritable versus non heritable traits?

FAO and WHO, while recognizing the useful outcome of the Joint FAO/WHO Expert Consultation on the Safety Assessment of Foods Derived from Genetically Modified Animals including Fish (FAO/WHO 2004) decided to convene this Consultation to address in more depth those issues which are directly related to the work that has been undertaken by the Codex Task Force and to respond to the above specific questions.

The Terms of Reference of the Consultation covered the provision of answers to the above questions as well as addressing issues related to safety assessment of animal food derived from modern biotechnology, from a scientific perspective.

3. Scope

The Consultation focused on addressing the above questions raised by the Codex Task Force. In doing so, the Consultation considered known uses of marker and reporter genes and the difference between heritable and non-heritable applications in recombinant-DNA animals that can enter the human food supply. Discussions took into account scientific information regarding use of these techniques in terrestrial livestock, such as chickens and cattle, and aquatic farmed animals, such as finfish. Regarding non-heritable applications, the Consultation focused on presenting an approach for safety assessment of different applications, which is an early step of a risk assessment. The Consultation did not conduct a full risk assessment of any specific application.

This Consultation took note of the outcome of the previous Expert Consultation on GM animals (FAO/WHO 2004) and built upon its conclusions and recommendations. Thus, the overall approach to food safety assessment of recombinant-DNA animals involves a comparative safety assessment of the recombinant-DNA animal with its appropriate comparator, including a food intake assessment and integrated nutritional and toxicological evaluation, followed by a full risk characterization.

The Consultation noted that certain applications of marker and reporter genes and non-heritable genetic constructs may raise questions about effects on the health and welfare of the recombinant-DNA animal or on safety for animal health of feeds derived from recombinant-DNA animals (e.g. fish meal derived from a recombinant-DNA fish). Noting that these issues are beyond the scope of this Consultation, they should be addressed by relevant bodies such as OIE and FAO. The Consultation also noted but did not address the food safety of animals fed with feed derived from recombinant-DNA animals.

[2] *The term 'non-heritable applications' covers the direct introduction of nucleic acids into non-germ line tissue of animals that will enter the food supply.*

Definitions of important technical terms for purpose of this Consultation appear in the Glossary.

4. Marker and Reporter Genes

4.1 Introduction

The first recombinant-DNA farm animals were produced more than 20 years ago by microinjection of foreign DNA into pronuclei of zygotes. Despite significant shortcomings such as low efficiency, random integration and variable expression patterns, promising models for application have been developed in agriculture and biomedicine (Niemann *et al*., 2005). Among the various alternative methodologies that have been developed to overcome the limitations of the microinjection technology, somatic cell nuclear transfer (SCNT) holds the greatest potential as a tool for significant quantitative and qualitative improvement in the generation of recombinant-DNA farm animals. Specifically, this relates to prescreening of the transfected cells prior to use in SCNT and the possibility to achieve a targeted genetic modification *via* homologous recombination. Up to now, SCNT has been successful in eleven animal species (Niemann and Kues, 2001; Niemann *et al*., 2005). The technology has been steadily improved over the past decade and is now used for the generation of recombinant-DNA cattle, pigs, goats and sheep. However, the overall efficiency remains unsatisfactory and a proportion of the clones, particularly in cattle and sheep suffers from pathologies (e.g. large offspring syndrome) thought to be caused by failures in the epigenetic reprogramming of the transferred somatic nucleus. These pathologies are not observed in the offspring of cloned animals.

Several technologies are now available to introduce or remove genes with known function and products from recombinant-DNA farm animals will soon be ready to enter the food chain. With the advent of SCNT, it has become possible to employ molecular tools that allow precise modifications of the genome. These technologies include targeted chromosomal integration by site-specific DNA recombinases and even methods that are compatible with a temporally and spatially controlled transgene expression in farm animals used for food production. These molecular tools are well characterized through extensive studies in mice and other biological systems (Niemann and Kues, 2003). The first draft sequence of the genomes of some farm animals (e.g. dog, cattle, chicken, horse) are already available and others are expected to follow shortly.

The successful generation of recombinant-DNA animals using techniques such as SCNT is primarily dependent on the selection of transfected cells based on the use of appropriate marker and/or reporter genes. The prior Expert Consultation on GM animals (FAO/WHO 2004) recommended: “avoiding the use of unnecessary DNA sequences in the genetic construct, including marker genes.”

The purpose of this Consultation was to extend this assessment and to specifically address the following issues related to food production from recombinant-DNA animals:

- *Recent development and use of reporter and selectable marker genes in recombinant-DNA animal food production*
- *Availability of non-antibiotic resistance marker or reporter genes and their safe for humans in food products*
- *Reliability and safety of techniques used for the removal of specific DNA sequences*.

4.2 Main discussion

4.2.1 Recent developments and use of reporter and selectable marker genes in recombinant-DNA animals

Different methods are used to generate recombinant-DNA animals according to species. These methods are: (1) direct injection of DNA into embryo pronuclei or cytoplasm; (2) DNA transfer using transposons or lentiviral vectors; (3) DNA transfer by sperm incubated with DNA; (4) DNA transfer into pluripotent cells to generate chimeric transgenic animals; and (5) DNA transfer into somatic cells used to generate transgenic cloned animals. DNA transfer methods can include both random and targeted gene addition or replacement by homologous recombination. In methods 1, 2, and 3, the efficiency of integration may be sufficient to render the use of selectable marker genes unnecessary. However, selectable marker genes are essential for targeted gene addition or replacement or when cells to which the foreign DNA is transferred are abundant (methods 4 and 5).

Targeted gene transfer is a rare event, which often requires both positive and negative cell selection. Positive selection involves the elimination of cells in which the gene of interest was not integrated and is generally achieved by the use of antibiotic resistant marker genes. Negative selection involves the elimination of cells in which the gene of interest is not integrated specifically at the intended site and is facilitated by genes expressing cytotoxic substances.

For the purpose of this Consultation, the following definitions have been used:

a) A **marker gene** is used to determine if a piece of DNA has been successfully introduced into the animal cell. Marker genes are used either for selection and/or for screening.

b) A **selectable marker** is a gene introduced into animal cells that confers a trait suitable for artificial selection. It protects the cells from the effect of a selective agent that would normally kill them or prevent their growth. Among the positive selective agents, antibiotics are the most commonly used for animal cells. Common examples include puromycin, hygromycin, phleomycin for positive selection. Cytotoxic substances such as the gancyclovir derivatives generated by the herpes simplex thymidine kinase or the subunit A of cholera toxin are used for negative selection.

   Marker genes conferring resistance to antibiotics such as ampicillin and chloramphenicol may also be present in recombinant-DNA animals due to their frequent use in bacterial vector construction.

c) A marker gene used for screening or **reporter** purposes encodes a product that can readily be identified either qualitatively and/or quantitatively. Among the most prominent ones are fluorescent proteins such as the green fluorescent protein (GFP), beta-galactosidase (beta-gal), secreted alkaline phosphatase (AP), the luciferases and chloramphenicol acetyl transferase (CAT)[3].

[3] The CAT gene confers resistance to the antibiotic chloramphenicol in bacteria, but is used only for reporter purposes in animal cells

Another class of **reporter** genes relates to those genes that change the colour of the individual when used in a transgenic approach. Visible pigmentation in vertebrates results from the synthesis and distribution of melanin in skin and eyes. Tyrosinase is an enzyme in the pathway for melanin production in melanocytes. Mutation of the tyrosinase gene is a common cause of a similar phenotype in all vertebrates, known as albinism, due to a lack of melanin pigment. Therefore, the albino phenotype has been successfully corrected through the tyrosinase transgene, which can express active tyrosinase in transgenic mice and rabbits (Beermann *et al*., 1990; Aigner and Brem, 1993).

Another approach to identify and/or discriminate animal cells with an introduced DNA or recombinant protein is the use of unique identifiable DNA or protein sequences (epitope or poly-histidine tag sequences).

Marker genes are being increasingly used in recombinant-DNA animals used for food. Recent applications are as follows:

- Recombinant-DNA cattle produced with altered beta and kappa casein composition in their milk after selecting appropriate recombinant-DNA donor cells (Brophy *et al*., 2003). A similar strategy was employed for the production of recombinant-DNA pigs showing alterations in their fatty acid pattern with a significant shift towards more poly-unsaturated fatty acids (Lai *et al*., 2006). Moreover, cows with a knockout of the prion gene have been produced.

- Gene constructs harbouring a selectable gene, either neomycin or puromycin resistance genes, and a marker gene (GFP gene) were transfected into chicken primordial germ cells (PGC). The selected cells were injected into early chicken embryos giving rise to fluorescent animals (van de Lavoir *et al*., 2006). This first successful approach of producing recombinant-DNA chicken paves the way towards agricultural applications in poultry.

- The salmon melanin concentrating hormone (MCH) gene was used as a reporter gene for the generation of recombinant-DNA medaka that exhibits white body color by enhanced MCH expression (Kinoshita *et al.,* 2001). MCH is a cyclic heptadecapeptide that is produced in pituitary, concentrates melanin granules in the melanophores and lightens the body color of fish. Similarly, the tyrosinase marker gene has been successfully used in the production of recombinant-DNA fish (Hyodo-Taguchi *et al*., 1997; Inagaki *et al*., 1998).

4.2.2 Availability of non-antibiotic resistance marker or reporter genes and their safety for humans in food products

4.2.2.1 Availability

The 2004 Expert Consultation on GM animals recommended avoiding the use of unnecessary DNA sequences in the gene construct, including marker genes (FAO/WHO, 2004). However, due to the proven utility and consistent performance of antibiotic resistance genes, limited efforts have been put into the development of alternative marker genes that allow identification and positive selection of transfected cells. Today a range of screenable marker genes (reporter genes) is available that allows for the identification, but not the positive selection of animal cells with introduced DNA.

Among the most prominent are fluorescent proteins such as the green fluorescent protein (GFP), beta-galactosidase (beta-gal), secreted alkaline phosphatase (AP), the luciferases and chloramphenicol acetyl transferase (CAT). The GFP gene encodes a protein that fluoresces under light of specific wavelengths in vivo or in vitro without causing damage to the cell. Luciferases are commonly used and require substrates. These may be applied either to cell extracts or to intact cells or even to tissues. In contrast, detection of beta-galactosidase activity generally requires fixation and disruption of the tissue to be analysed.

4.2.2.2 Safety considerations

One of the important considerations in the safety assessment of food derived from recombinant-DNA animals is the safety of any newly expressed proteins, including those expressed by marker genes remaining in the organism. For non-antibiotic resistance marker genes, the assessment would generally focus on the safety of the expressed protein, which must be determined on a case-by-case basis. Depending on the knowledge of the expressed protein, this assessment may range from a limited evaluation of the available data on the biochemical function of the protein and its expression in the recombinant-DNA animal, to, in the case of less well documented proteins, extensive toxicity testing including animal studies (FAO/WHO, 2004). This information is then used as part of the overall comparative safety assessment to reach a conclusion about the safety of the food derived from the recombinant-DNA animal.

A standard food safety assessment of a newly expressed recombinant protein includes:

- biochemical composition and function of the protein;
- expression of the protein in the recombinant-DNA animal (site of expression, expression levels, integrity of expressed protein);
- stability of the protein to heat, processing and digestion;
- toxicological evaluation; and
- allergenicity.

Depending on the outcome of the assessment, additional studies may be required (e.g. immunological studies).

In the case of the GFP family of reporter genes, some studies are available, including those from recombinant-DNA plants, from which some information with respect to food safety may be extrapolated. A limited amount of data on GFP toxicity has been published. Some experiments with transfected animal cells (plasmids expressing the GFP gene) suggest that GFP has some cytotoxicity (Liv *et al.,* 1999). Lines of animals expressing the GFP gene have been obtained in most of the animal species in which transgenesis is possible. They all survived with no observed adverse effects. The observed in situ cytotoxicity of GFP does not necessarily imply that this protein is toxic when administered orally. Rats fed for 26 days with pure GFP or canola expressing the GFP gene did not show any significant difference with control animals for growth and several other parameters. GFP was rapidly degraded in the presence of pepsin and almost completely digested by rats. No significant amino acid sequence similarities to known allergens were found in the GFP sequence although the Consultation noted that the comparison was done to only approximately half of the known allergens. The conclusion of this study was that the GFP gene is a relevant substitute to replace antibiotic resistant selectable genes (Richards *et al*., 2003). The Consultation agreed, however, that further studies on the biochemical function and expression of GFP in animal tissues would be necessary to draw firm conclusions with respect to its safety in recombinant-DNA food animals.

The Consultation was also unable to draw any firm conclusions in relation to the food safety of beta-galactosidase, alkaline phosphatase, CAT and luciferase expressed in recombinant-DNA food animals due to a lack of available studies. There is a clear need for data on the safety of such proteins if these marker genes are to be used in the development of recombinant-DNA animals used for food.

In the case of peptide sequences that are incorporated into gene constructs to serve as unique identifiers / tags for the recombinant protein, the Consultation noted that studies on the safety of such sequences need to be undertaken on the entire fusion protein, not just the isolated peptide sequence.

4.2.3 Reliability and safety of techniques used for the removal of specific DNA sequences

Specific recombination systems are well known from various bacterial and fungal systems in which enzymes including Cre, flippase or R act on specific target sequences, such as lox, FRT or RS, respectively. These systems have been adapted to other biological systems and already play a role in the production of recombinant-DNA plants, and are now beginning to be used in animal cells for the purpose of generating recombinant-DNA animals for food production. These recombination systems are generally comprised of three major elements: two pairs of short DNA sequences (the site-specific recombination sequences) and a specific enzyme, i.e. the site-specific recombinase. Recombinase-mediated DNA rearrangements include site-specific excision, integration, inversion and interchromosomal recombination, thus allowing a broad range of applications. Thus functional systems for the removal of unnecessary DNA sequences are available and can be applied in animal cells.

Information has been published on the function of the Cre-lox recombinase system. Data on the function of other site-specific recombinases is too limited to allow assessment of efficiency and safety. A concern that has been raised for the use of such systems is off-target effects where high levels of recombinase expression may result in genome rearrangements at cryptic target sites. The most important off-target effect associated with the use of the Cre-lox is the tendency of the recombinase to induce recombination between cryptic lox sites in mammalian genomes. High levels of recombinase activity were associated with chromosomal aberrations (Loonstra *et al*., 2001; Schmidt *et al*., 2000). Furthermore, the faulty excision of very large pieces of DNA may be associated with another set of unwanted side effects. These unintended effects may also be associated with other site-specific recombination systems.

The combination of tissue specific promoter elements with the Cre DNA recombinase enables restriction of a gene knockout to a certain cell type or tissue and should thus play a role in the future production of specific food commodities. Indeed, recent research shows that gene excision can be more precisely controlled by using inducible promoters (e.g. tetracycline inducible system) for the Cre recombinase gene and by using inducible active forms of Cre recombinase (e.g. inducible by 4-hydroxy tamoxifen).

The following example indicates the capacity of the Cre-lox system to eliminate selectable marker genes in recombinant-DNA animals. The two alleles of the PRNP and immunoglobulin genes (Mu) were knocked out in somatic cells that were then used in SCNT to generate recombinant-DNA cows that lacked the expression of the target genes. Two positive selective marker genes flanked by lox sequences and a negative selection gene were used to obtain the cells for SCNT. Polymerase chain reaction tests confirmed that the marker genes were efficiently eliminated by the site-specific recombination system (Kuroiwa *et al.,* 2004). No health compromising effects attributable to the recombination system could be identified in the cows subsequently examined (Richt *et al.,* 2007).

5. Non-heritable Applications

5.1 Introduction

In recent years there have been major developments in the production of recombinant-DNA food animals using heritable constructs (HCs) and non-heritable constructs (NHCs).

Recombinant-DNA animals with heritable constructs are produced by the introduction of recombinant-DNA constructs into early embryos, gametes, and somatic cells used for somatic cell nuclear transfer (cloning). In contrast, non-heritable recombinant-DNA food animals are produced by the direct introduction of nucleic acids into somatic cells of animals. Since these two classes of constructs use different components and technologies, they may pose different types of hazards and may therefore require different food safety evaluation strategies.

As mentioned in the scope of this report, the Consultation considered whether food animals bearing heritable and non-heritable constructs pose different food safety risks and if they do, what those changes might be.

Based on an evaluation of the available scientific evidence on current methods used to generate recombinant-DNA food animals with heritable and non-heritable constructs, as well as an analysis of the methods presented in the Proposed Draft Guideline for the Conduct of Food Safety Assessment of Food Derived from Recombinant-DNA Animals, being elaborated by Codex Task Force, the Consultation attempted to identify whether different food safety assessments would be required for recombinant-DNA animals produced by HC and NHCs.

5.2 Background

Although similar molecular tools and genetic elements can be used in heritable and non-heritable genetic applications, there are some important differences.

In general, recombinant-DNA animals with HCs are produced using one of three broadly characterized methods: microinjection into early embryos, somatic cell nuclear transfer of transgenic donor cells, or introduction of recombinant-DNA into gametes (usually sperm) (Smith, 2002; Lavitrano, et al. 2003; Sorrell *et al*., 2005; Wheeler, 2007). Animals with HCs are often produced to enhance production characteristics such as growth, modify nutritional requirements, improve carcass composition, milk and wool production, increased feed conversion efficiency, wound healing, therapy, and for developing resistance to animal diseases including those which can induce food-borne diseases in humans (reviewed by Kopp *et al*., 2004; Sun *et al*., 2006; Kochhar and Evans, 2007; Wheeler, 2007).

Recombinant-DNA animals with NHCs can be produced by physical and chemical means (e.g. microinjection, electroporation, gene gun (biolistic) methods, liposomes). Most of these methods require the presence of excipients (materials that serve as vehicles) associated with delivery. For example, chemical means such as liposome-mediated transfection by definition involves the presence of liposomes; gene guns deliver recombinant-DNA constructs on beads, often made of gold. These applications of recombinant-DNA animals with NHCs are similar to those with HCs, and include production characteristics, therapy, and developing resistance to animal diseases Draghia-Akli *et al*., 1997; Southwood *et al*., 2004; Thacker *et al*., 2006; Richt *et al*., 2007)

Biological methods include the use of viral sequences associated with packaging, cell entry, and nuclear targeting functions. These are most commonly derived from retro-, lenti-, adeno-, and adeno associated-, or herpes viruses (see Table 1). In addition, there are recent developments of non-viral vectors for introducing recombinant-DNA into food animals (Manzini *et al*., 2006).

Recombinant-DNA vaccines can be considered to comprise a class of NHCs. The genetic material of recombinant-DNA vaccines could be made up of plasmids, virus-based vectors, or DNA fragments encoding antigenic peptides derived from the pathogen of interest (Pachuk *et al*., 2000). The general intent of the vaccine is to induce an antigen-specific cellular or humoral immune response in the animal (Jechlinger, 2006). Although effective delivery of recombinant-DNA vaccines is still under development, promising methods include using transdermal aerosols, electroporation, and liposomes. For example, intramuscular injection has been shown to be a relatively inefficient method of administering recombinant-DNA vaccines to cattle (Hurk *et al*., 1998), while intradermal high pressure jet administration appears to be more efficient (Carter and Kerr, 2003) Jecklinger *et al*. (2006) have shown that recombinant-DNA vaccines can persist episomally in the tissues of treated animals for some time.

5.3 Main discussion

Although the questions refer to heritable and non-heritable "traits", for the purpose of this discussion it is more appropriate to refer to "constructs" that are heritable or non-heritable. The reason for this is that "trait", as defined by FAO, refers to phenotype or one of the many characteristics that define an organism, whereas this analysis is based on consideration of the actual recombinant-DNA genes themselves. Heritable constructs (HCs) are defined as constructs that are stably integrated into the genome and transmitted from generation to generation, whereas non-heritable constructs (NHCs) may be integrated into the genome of somatic cells but are not expected to be vertically transmitted.

When recombinant-DNA constructs are introduced into animals, multiple effects can occur. These are often characterized as "intended" and "unintended" or "direct" and "indirect". Intended and unintended effects categorize outcomes based on the objective of the modification. Intended effects include changes in the recombinant-DNA animal brought about deliberately by introduction of the recombinant-DNA construct and its predicted gene product(s) (e.g., increased growth rate, resistance to infection). These may or may not pose direct or indirect effects on food safety. Unintended effects can occur as a result of multiple changes in the recombinant-DNA animal resulting from the interaction of the recombinant-DNA construct or its gene product(s) with the physiology of the animal. These may or may not pose direct or indirect effects on food safety.

Direct effects causing food safety concerns can be thought of as adverse outcomes resulting from the human consumption of edible products from recombinant-DNA animals containing the recombinant-DNA construct or its gene products. Adverse indirect effects may arise from human consumption of edible products from the recombinant-DNA animal that contain hazards due to the construct or gene product perturbing the food animal's physiology. Examples include those affecting the synthesis of an anticipated nutrient, or alteration of the concentration of a metal-sequestering protein that may pose no risk to the recombinant-DNA animal, but which may pose a human food consumption risk. Alternatively, these indirect effects may adversely affect the recombinant-DNA animal, but be neutral with respect to human food consumption risks (e.g. stimulating local irritation in a non-edible tissue that, by virtue of lack of exposure to humans via food consumption, poses no risk to humans).

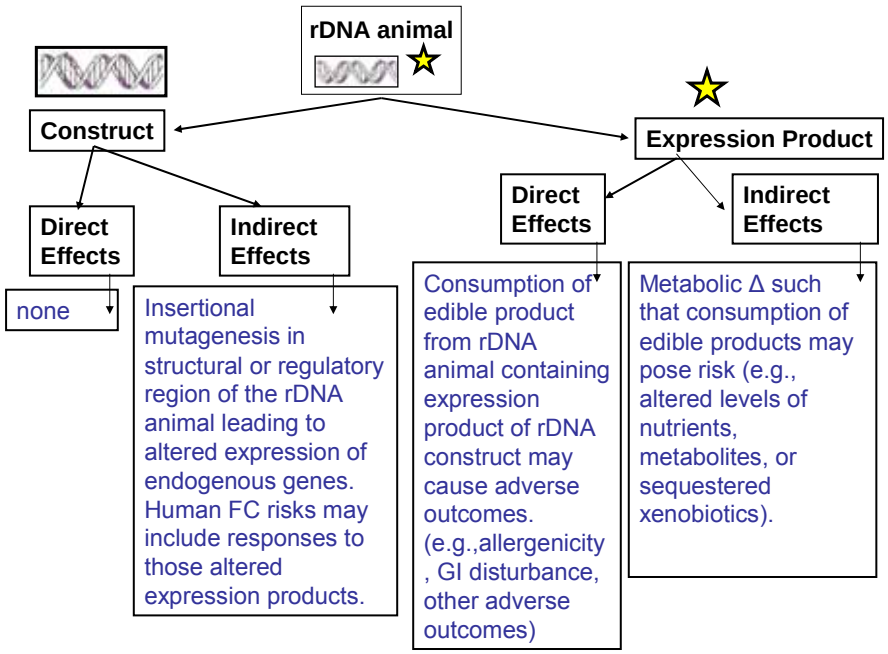

Figure 1. Conceptual Overview of Considerations for Food Safety Evaluation for recombinant-DNA(rDNA) Animals Containing Heritable Constructs *(FC, GI, Δ denote food consumption, gastrointestinal, changed, respectively).*

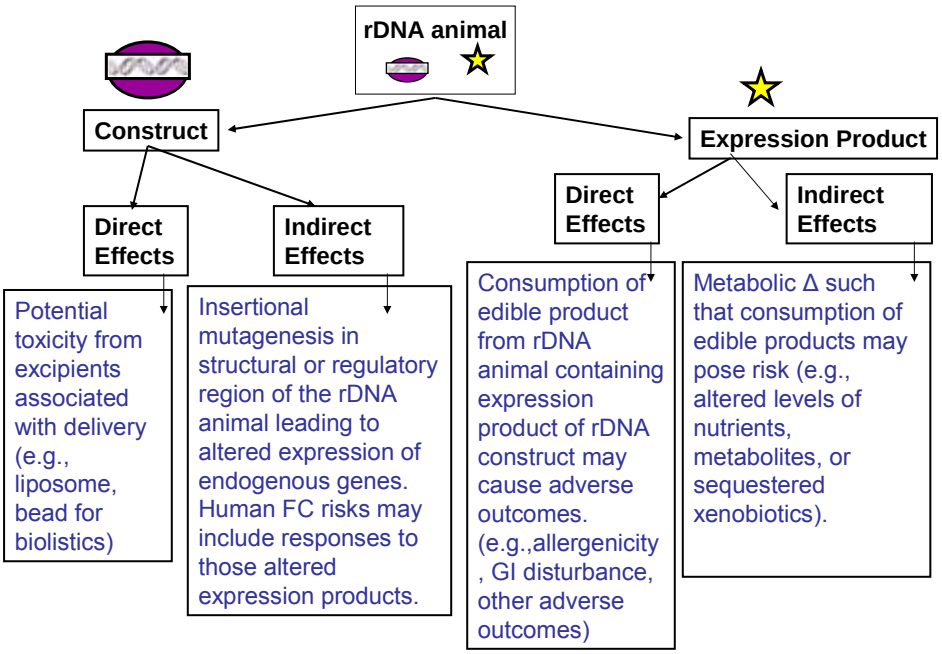

Figure 2. Conceptual Overview of Considerations for Food Safety Evaluation for recombinant-DNA (rDNA) Animals Containing Non-Heritable Constructs *(FC, GI, Δ denote food consumption, gastrointestinal, changed, respectively).*

Figures 1 and 2 are summaries of the thought processes employed in developing the methodology for identifying hazards and risks that may occur in the consumption of recombinant-DNA food animals with HCs and NHCs, respectively. They do not comprise a full hazard identification or a comprehensive risk assessment. Their layout separates the effects that arise from the recombinant-DNA construct itself and from its expression products, and to identify them as direct or indirect effects.

In order to determine whether HCs and NHCs pose different human food consumption risks, it is important to characterize the types of constructs that are used, their fate and persistence in the resulting recombinant-DNA animal, their expression profiles and the potential hazards they may pose to the recombinant-DNA animals themselves. Risks to human health do not arise until the consumption of the actual food from these recombinant-DNA animals. Table 1 provides a summary of the major types of HCs and NHCs currently used in such applications. It must be noted, however, that this analysis of NHCs is based in large part on the experiences derived from human gene therapy and, although directly extrapolated to food animals, requires empirical demonstration. In the identification of potential hazards for humans *via* food consumption, Table 1 takes into account the methods outlined in Figures 1 and 2.

One of the food consumption risks associated with consumption of recombinant-DNA animals with NHCs is referred to as “excipient effects”. Excipient effects refer to the direct and indirect toxicity that may occur as the result of consuming tissues with these materials. The amount of exposure can range from zero to the total administered to the recombinant-DNA animal, depending on the tissue consumed and the distribution and fate of the recombinant-DNA construct and its excipients in the recombinant-DNA animal.

As shown in Table 1, it is important to note that many NHCs are integrated into the chromosomes of somatic cells. Examples include those that are derived from retroviruses or transposons (reviewed by Kay *et al*., 2001). NHCs that remain episomal include those that are adenovirus or herpes virus derived (reviewed by Thomas *et al*., 2003). Adeno-associated virus derived recombinant-DNA constructs are generally taken to be episomal, although there is some evidence of integration (Recchia *et al*., 1999).

Although most of this information stems from human gene therapy, it is important to note that development of this technology has been accompanied by a concerted international effort to ensure that these virally-derived vectors are “as safe as possible”, as summarized by the American Society for Gene Therapy (http://www.asgt.org/) and the European Society for Gene and Cell Therapy (http://www.esgt.org/). This includes efforts to limit the degree to which homologous recombination can occur with endogenous viruses that could lead to: (1) insertion into regions of the genome that could affect growth and development; (2) recombination leading to reconstitution of active and infectious viral particles; or (3) instability of the integrated construct. An additional concern that has been the subject of significant research and development has been elimination of the expression of viral proteins that could lead to inflammatory responses. It is anticipated that similar efforts will be undertaken for vectors used in recombinant-DNA food animals (see Section 7: Recommendations).

A complication was noted with the definition of recombinant-DNA animal as it appeared in the previous Expert Consultation (FAO/WHO, 2004) which implied that recombinant-DNA animals only referred to animals containing heritable constructs. Animals are being produced for food and other purposes using NHCs. Differences between the nature and extent of food consumption risks in recombinant-DNA animals produced using heritable and non-heritable constructs exist principally when excipients are used to introduce NHCs into recipient animals, and when recombinant-DNA constructs are intended to remain episomal. Recent scientific evidence indicates that recombinant-DNA vaccines can persist episomally in the tissues of treated animals for some time (Jecklinger *et al*., 2006).

Table 2 gives an overview of the differences between recombinant-DNA animals with HCs and NHCs as they relate to food safety assessment. The purpose of this table is to identify the key construct characteristics that differ between these animals that have implications for differences in the way that food safety assessments should be performed. It is important to emphasize that the purpose of this table is not to identify potential food consumption risks that may arise in recombinant-DNA animals, but rather to identify whether a different food consumption safety assessment would be required for recombinant-DNA animals with these two classes of constructs.

Based on these differences identified between recombinant-DNA animals with HCs and NHCs, the conclusions and recommendations on non-heritable applications are given in Sections 6 and 7 respectively.

Table 1: Characteristics of Methods of Generating recombinant-DNA (rDNA) Animals and their Potential Food Consumption Hazards (*adapted from Thomas et al. 2003*)

Type of Construct	Integrated/ Episomal	Primary delivery	Transmissibility***	Fate/Persistence		Hazard to Animal	Food Consumption Hazards
				Construct	Product		
Retro/lenti virus derived* (Kay et al., 2001, Park et al. 2000; Naldini et al. 1996)	Integrated	Retroviruses require dividing cells lentiviruses do not require dividing cells	Not expected beyond target cells	Stable in target cell, but target cells may be cleared	May decrease over time due to silencing	Insertional mutagenesis of construct at target cells; local or systemic toxicity from interaction w expression product(s)	None from vector. Direct and indirect hazards arising from expression product or perturbation of animal's physiology.
Transposon derived*	Integrated	Chemical or physical introduction only; either dividing or quiescent cells	Not expected beyond target cells	Stable in target cell, but target cells may be cleared	Often stable unless silenced. Cells may be cleared.	Insertional mutagenesis of construct at target cells; local or systemic toxicity from interaction w expression product(s)	None from construct; may have persisting excipient(s). Direct and indirect hazards arising from expression product or perturbation of animal's physiology.
Naked DNA ***	Integrated	Chemical or physical introduction only; either dividing or quiescent cells	Not expected beyond target cells	Stable in target cell, but target cells may be cleared	Often stable unless silenced. Cells may be cleared.	Insertional mutagenesis of construct at target cells; local or systemic toxicity from interaction w expression product(s)	None from construct; may have persisting excipient(s). Direct and indirect hazards arising from expression product or perturbation of animal's physiology.
Adenovirus derived* (Kafri et al. 1998)	Episomal	Receptor mediated. Dividing or non-dividing cells	Not expected beyond target cells	No long term stability documented; clearance from cell, cells may	Linked to persistence of construct unless silenced	No insertional mutagenesis. Local or systemic toxicity from interaction w expression products(s). May have potentially significant	Transient immunogenicity (via oral exposure) if edible products containing applied virally-derived delivery vector are consumed (excipient effect). Direct

Table 1: Characteristics of Methods of Generating recombinant-DNA (rDNA) Animals and their Potential Food Consumption Hazards (*adapted from Thomas et al. 2003*)

Type of Construct	Integrated/ Episomal	Primary delivery	Transmissibility**	Fate/Persistence		Hazard to Animal	Food Consumption Hazards
				Construct	Product		
				cleared		immune response to viral proteins.	and indirect hazards arising from the rDNA construct, expression product, or perturbation of animal's physiology.
AAV derived* Nakai et al. 2001	90% episomal/ 10% integrated‡	Receptor mediated. Dividing or non-dividing cells	Not expected beyond target cells	No long term stability documented; clearance from cell, cells may be cleared	Linked to persistence of construct unless silenced	No insertional mutagenesis. Local or systemic toxicity from interaction w expression products(s). Less inflammatory than Adenovirus-derived packaging vectors.	Transient immunogenicity (via oral exposure) if edible products containing applied virally-derived delivery vector are consumed (excipient effect). Direct and indirect hazards arising from the rDNA construct, the expression product, or perturbation of animal's physiology.
Herpes virus derived*	Episomal	Strongly neurotropic	No	Persistent	Linked to persistence of construct unless silenced	No insertional mutagenesis. Local or systemic toxicity from interaction w expression products(s). May be inflammatory due to presence of persisting viral proteins.	Limited to consumption of neuronally derived tissues. Direct and indirect hazards arising from the rDNA construct, the expression product, or perturbation of animal's physiology in those tissues.

Artificial chromosomes	NA	Usually to cells used as donors for nuclear transfer	No for surrounding cells; may be heritable	Persistent	Linked to persistence, unless silenced	No insertional mutagenesis. Local or systemic toxicity from interaction w expression product(s)	None from vector. Direct and indirect hazards arising from expression product or perturbation of animal's physiology.
Heritable Construct		Requires generating new animal	NA	Stable, unless lost	Stable, unless silenced	Insertional mutagenesis of construct at target cells; local or systemic toxicity from interaction w expression product(s	Direct and indirect hazards arising from expression product or perturbation of animal's physiology.

* Assumes replication competence of virus is removed during vector construction.
** From target to surrounding cells/tissues
*** Assumes that DNA is delivered via chemical or physical means (lipsomes, transfection, biolistic techniques, etc.) and does not contain structures required for replication (see Artificial Chromosomes).
‡ Many of the characteristics described here are based on observations in humans (Thomas *et al*. 2003). Individual references indicate particular citations describing a particular characteristic described for that type of vector.
NA= Not applicable

Table 2: Overview of Differences between rDNA Animals with Heritable Constructs (HCs) and Non-Heritable Constructs (NHCs): Implications for Food Safety Assessment

rDNA Construct Characteristics	HCs	NHCs	Implications of Differences for Food Safety Assessment
Nature of the construct	Usually linearized DNA containing regulatory regions and the gene of interest. May also contain sequences from the propagating vector backbone, e.g., origin of replication, selectable marker(s) and other prokaryotic or eukaryotic sequences.	May be linearized DNA, autonomously replicating unit or something else (see Table 1). May also contain sequences from the propagating vector backbone, e.g., origin of replication, selectable marker(s) and other prokaryotic, viral or eukaryotic sequences.	No difference.
Method of construct introduction	Direct transfer by liposomes and other non-biological vectors, or by microinjection.	May use biologically-derived vectors, although direct transfer by liposomes and other non-biological vectors may also be used.	Excipients in rDNA animals with NHCs may pose direct and indirect food safety risks.
Initial cell(s) into which the construct is introduced	Germ cell, embryo or donor cell for nuclear transfer.	Any somatic cell.	No difference. Regardless of whether HC or NHC, indirect adverse outcomes may occur when they are introduced into the genome, as a result of interruption of regulatory or coding sequences. No such effects would be expected for episomally maintained NHCs.

Table 2 (continued): Overview of Differences between rDNAAnimals with Heritable Constructs (HCs) and Non-Heritable Constructs (NHCs): Implications for Food Safety Assessment

rDNA Construct Characteristics	HCs	NHCs	Implications of Differences for Food Safety Assessment
Construct location, stability and prevalence in the rDNA animal	All cells; once stabilized, should be constant (unless small foci of loss or rearrangement occur).	NOT all cells (focal to dispersed; affected by route and method of introduction).	Although there may be differences in the location and amount of rDNA construct in animals with NHCs, because there are no direct food consumption risks linked to the construct itself, there are no differences in food consumption risk.
Construct expression: cell type, amount and duration	All cells possible, but may be cell, tissue or developmental stage specific. Amount likely consistent among rDNA animals of the same line. Duration likely stable.	Only a subset of cells; may have ectopic expression if non-specific promoters are used. Amount may differ among cells/tissues and among animals.	Although expression product dose can be estimated in rDNA animals with both HCs and NHCs, the variance may be higher in the latter, affecting sampling requirements. Some cells, tissues or organs have no construct at all (NHCs) or no construct expression (tissue specific HCs or NHCs).

Table 2 (continued): Overview of Differences between rDNA Animals with Heritable Constructs (HCs) and Non-Heritable Constructs (NHCs): Implications for Food Safety Assessment

rDNA Construct Characteristics	HCs	NHCs	Implications of Differences for Food Safety Assessment
Generation of more rDNA animals	Breeding or nuclear transfer.	Introduction of NHCs (see Table 1).	rDNA animals containing HCs: once the genome is established, predictions for additional rDNA animals of the same "genome" should follow Mendelian expectations and food safety assessment can be done on "prototypic" rDNA animal. rDNA animals containing NHCs: each animal may be considered as unique and, unless protocols are tightly controlled and worst-case assumptions are made for all animals developed via one protocol, food safety will need to be evaluated individually.

Table 3 summarises the main conclusions of the Consultation in response to the two questions asked about recombinant-DNA animals carrying heritable versus non-heritable constructs. In most respects it was concluded that there are no major differences with respect to food safety concerns depending on whether the recombinant-DNA constructs are integrated into germline cells, but rather the concerns are a function of the recombinant-DNA construct, its integration status, and its corresponding product. Thus the differences with respect to food safety are found between integrated and non-integrated (i.e. episomal) recombinant-DNA constructs. The major difference between recombinant-DNA animals bearing HCs and NHCs with respect to food safety is thus not due to the transgene but rather to the potential presence of the excipients used to transfer the recombinant-DNA construct in the non-heritable application as the primary animal would be the animal entering the food chain and thus there may be exposure to humans consuming the food. Based on the methodology contained in Figures 1 and 2 and Tables 1 and 2, Table 3 summarizes the potential food safety issues at the level of hazards to the animal, food consumption hazards, and food consumption risks.

For hazards to the animal that may impact food safety, the principal differences between recombinant-DNA animals with HC and NHCs are that: (1) episomally maintained constructs do not cause insertional mutations; and (2) issues due to the presence of excipients. An inter-related issue is that both horizontal gene transfer and recombination events are theoretical pathways for the propagation of new hazards in the animals. Recombinant-DNA constructs maintained as episomes may be more accessible for horizontal gene transfer events, but that does not preclude the possibility for horizontal gene transfer involving an integrated construct. It is also noted that the potential for recombination may be related to the presence of viral, bacterial, or other sequences present in the recombinant-DNA construct.

For food consumption hazards, again the differences between food derived from recombinant-DNA animals with HC and NHCs are a function of: (1) the integration of the construct; and (2) excipient effects in recombinant-DNA animals with NHCs. Although the level of recombinant-DNA expression product may differ between NHCs and HCs, the food safety implications are not a function of whether the recombinant-DNA construct is heritable or not. Excipients in recombinant-DNA animals with NHCs may pose direct and indirect food consumption hazards, because with NHCs there is more opportunity for exposure to the excipients than with recombinant-DNA animals with HCs as the excipients would be lost over generations. An inter-related issue is that both horizontal gene transfer and recombination events are theoretical pathways for the propagation of new hazards in the animals. Recombinant-DNA constructs maintained as episomes may be more accessible for horizontal gene transfer events, but that does not preclude the possibility for horizontal gene transfer involving an integrated construct.

For food consumption risks, the only qualitative difference is greater opportunity for exposure to the excipients in food from animals carrying NHCs than with recombinant-DNA animals with HCs. Excipient-related risks only occur when the cells or tissues being consumed contain the excipients and, as noted above, the excipients are not present in animals with HCs. Quantitative differences in food consumption risks may arise based on expression pattern and amount of the construct, not its heritability.

Table 3. Summary of Hazards and Risk in recombinant-DNA(rDNA) Animals with Heritable (HC) and Non-Heritable Constructs (NHC)

	The Same	**Different**
Hazards to Animals	Insertional mutagenesis for integrated rDNA constructs. Expression product hazards. Local effects, including recombination and its sequelae, if similar viral vectors used to introduce NHCs and HCs. Key factors influencing likelihood of an adverse outcome from horizontal gene transfer and recombination are the origin of the sequences in the construct (e.g., bacterial, viral or other eukaryotic sequences), not its heritability.	No insertional mutagenesis for episomally maintained NHCs. Excipient effects.
Food Consumption Hazards	May arise from insertional mutagenesis in the rDNA animal. The level of expression product may differ between NHCs and HCs, but qualitatively remains the same. Key factors influencing likelihood of an adverse outcome from horizontal gene transfer and recombination are the origin of the sequences in the construct (e.g., bacterial, viral or other eukaryotic sequences), not its heritability.	No insertional mutagenesis for episomally maintained NHCs. Excipients in rDNA animals w NHCs may pose direct and indirect food consumption hazards. With the NHCs there is more opportunity for exposure to the vectors than with rDNA animals w HCs due to "dilution effects".
Food Consumption Risks	Depends on the expression pattern and amount of the construct, not the heritability of the rDNA construct.	Excipient-related risks are a function of potential exposure (whether cells or tissues being consumed contain the excipients). With the NHCs there is more opportunity for exposure to the vectors than with rDNA animals w HCs due to "dilution effects".

6. Conclusions

6.1 Conclusions on marker and reporter genes

- ***What developments have occurred in the development and use of reporter and selectable marker genes?***

  - At least three major types of marker genes are used to screen and/or select for the success of introduction of foreign DNA into animal cells.

  - Most of the representatives of these 3 classes of markers were developed as basic research tools. At this time, there is insufficient information on the food safety of recombinant-DNA animals with these markers genes. Nevertheless, information from other species can be used as a starting point for further research on the safety aspects of marker genes

  - Marker genes for both positive and negative selection will become increasingly important in combination with nuclear transfer from somatic cells or pluripotent cells in the generation of recombinant-DNA food animals.

- ***Are there non-antibiotic resistance marker or reporter genes that have been demonstrated to be safe to humans in food products, and if so, what are they?***

  - Although many non-antibiotic resistance marker or reporter genes exist, few are currently used for producing recombinant-DNA animals intended for food.

  - Experience on the utility, stability and performance of these marker genes is available from studies of animal models in the laboratory. However, the limited number of studies that has been performed on the safety of non-antibiotic marker genes in recombinant-DNA food animals, has yielded inconclusive results.

- ***When removal of specific DNA sequences is desired, are reliable and safe techniques available to do this on a routine basis?***

  - Site-specific recombination systems can provide a functional means for removing marker genes provided measures are taken to minimize off-target effects.

  - Only limited scientific information relevant to food safety aspects of site-specific excision systems used in food animals is available.

6.2 Conclusions on non-heritable applications

- ***Are there relevant differences from a food safety perspective between animals with heritable and non-heritable traits, and if so what are they?***

  - The differences in food consumption hazards posed by recombinant-DNA animals are a function of (a) the integration status (and origin and composition of sequences) of the construct, not its heritability, and (b) excipient effects, which need to be evaluated in recombinant-DNA animals with NHC.

- There are no qualitative differences between heritable or non-heritable constructs regarding the nature of the hazards and risks when the constructs are chromosomally integrated.

- Quantitative differences in the safety of foods derived from recombinant-DNA animals containing heritable or non-heritable recombinant-DNA constructs may arise from the expression pattern and amount of the construct, not its heritability.

- The potential for horizontal gene transfer to occur is a function of whether the recombinant-DNA construct is integrated into the genome of the recipient cells or maintained episomally, and not of the heritability of the construct. Episomal recombinant-DNA (heritable and non-heritable) may be more readily transferred or taken up than integrated recombinant-DNA by bacteria or somatic cells of animals or humans consuming food products derived from recombinant-DNA animals. This may pose animal health risks, but the degree to which such potential horizontal gene transfer poses human health risks via food consumption risks is not clear.

- ***Are there specific food safety questions (e.g. with regard to types of vectors) that should be considered relative to the assessment of safety of foods from animals containing heritable and non-heritable traits?***

- Quantitative differences in the safety of foods derived from recombinant-DNA animals containing heritable or non-heritable recombinant-DNA constructs may arise from the extent to which the vectors may contain viral sequences. In this case, recombination with endogenous viral sequences could result in health risks to the recombinant-DNA animals. The degree to which these animal health risks pose human food consumption risks is a function of, among other things, the host range of the resulting recombined viruses.

7. Recommendations

7.1 Recommendations on marker and reporter genes

- The continued validation and development of gene excision systems is strongly encouraged to allow the controlled removal of specific DNA sequences in recombinant-DNA animals. This is in line with the outcome of the 2004 FAO/WHO expert consultation which recommended avoiding the use of unnecessary DNA sequences in the gene construct, including marker genes (FAO/WHO, 2004).

- Further research with focus on studies relevant to food from recombinant-DNA animals is needed to evaluate the safety of non-antibiotic resistance marker genes and gene excision systems.

- It is desirable to develop novel non-antibiotic resistance markers that facilitate efficient positive and negative selection of transgenic cells.

- To minimize the potential for off-target effects, recombinant-DNA animals intended for food use should be free of the introduced gene excision system.

7.2 Recommendations on non-heritable applications

- Some potential animal health hazards associated with the use of viral sequences were identified, including the potential for recombination and subsequent expression, altered pathogenicity, and reverse transcription of RNA viral sequences. These issues should comprise the basis of a guideline on the safe use of virally-derived vectors for non-heritable applications for animal health and production. The recent developments on nonviral episomal vectors provide a means to overcome many of the concerns associated with viral-based vector systems. These guidelines should take into account the principles of guidelines developed for human gene therapy. A suitable venue for developing such a guideline would be the OIE.

- In order to minimize the likelihood of an adverse event posing an animal health risk to recombinant-DNA animals *via* horizontal gene transfer to prokaryotic organisms, the coding region(s) of the genes in recombinant-DNA constructs that are integrated into the recombinant-DNA animal's genome should contain introns. (Bacteria do not contain the cellular machinery to splice out introns and therefore would not be able to produce a functional product should horizontal gene transfer occur.)

- Care should also be taken that the health of the recombinant-DNA animal is not compromised in the course of developing a safe food product. The animal health issues should form the basis of a guideline on the health of recombinant-DNA animals similar to the one being developed for animal clones by OIE. A suitable venue for developing such a guideline would be the OIE.

- Further direct research should be encouraged to help elucidate whether food safety hazards are affected by
  a. potential horizontal gene transfer to prokaryotic or eukaryotic cells
  b. recombinant-DNA made with viral sequences that are part of NHCs (e.g., viral recombination).

- Because recombinant-DNA animals containing NHCs may have an increased inter-animal variance in the expression products of the recombinant-DNA constructs, there is a need to establish a guideline on statistically appropriate sampling strategies for assessing potential exposure and risks from eating food derived from recombinant-DNA animals with NHCs. Appropriate venues for creating such guidelines would be international standard setting bodies with interests in animal health and food safety (e.g. FAO, WHO, OIE).

- A comprehensive publicly available database should be established and maintained by appropriate international intergovernmental organizations such as FAO/WHO on all reported results arising from consumption of food derived from recombinant-DNA organisms, including the results of any subsequent investigations of those reports.

- A comprehensive publicly available database should be established and maintained by relevant international organizations such as OIE on the methods of introducing heritable and non-heritable recombinant-DNA constructs into animals, accompanied by a full bibliography.

- It would not be inappropriate to use the principles and methods outlined in the Draft Guideline[4] applied to assessing the food safety of recombinant-DNA animals, with the added caveats regarding excipients and episomes, for assessing animal health and food safety of animals bearing NHCs for production or other purposes to assess the food safety of animals treated with recombinant-DNA vaccines.

- Given the complexity and importance of the animal health and food safety issues raised by recombinant-DNA vaccines, these issues should be considered by a joint FAO/WHO/OIE expert group.

[4] Proposed Draft Guideline for the Conduct of Food Safety Assessment of Foods Derived From Recombinant-DNA Animals is currently being elaborated at Steps 3 and 4 of the Codex process (see ALINORM 07/30/34)

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9. Glossary

Conventional counterpart means an animal breed with a known history of safe use as food from which the recombinant-DNA animal line was derived, as well as the breeding partners used in generating the animals ultimately used as food, and/or food derived from such animals.

Construct means DNA used for transfer into a cell or tissue. The construct may be comprised of the gene or genes of interest, a marker gene and appropriate control sequences as a single package. A repeatedly-used construct may be called a cassette.

Episome (episomal) means an extrachromosomal genetic element (e.g. the F factor in *Escherichia coli*) which is maintained within a cell independently of the chromosome but may integrate into the host chromosome. The integration step may be governed by a variety of factors and so the term *episome* has lost favour and been superseded by the wider term plasmid.

Excipient is material that serves as a vehicle for the delivery of non-heritable constructs, e.g., liposome, gold beads used in biolistic application and calcium phosphate used for transfection.

Excipient effect is any direct or indirect effect resulting from exposure to the excipient.

Expression product is a specific RNA molecule which may encode a protein sequence and the resulting protein. Some RNAs do not encode a protein but provide a regulatory or biological function.

GM animal / genetically modified animal is used interchangeably to mean recombinant-DNA animal.

Horizontal gene transfer refers to the uptake of DNA molecules by cells independently of the normal reproductive processes.

Heritable construct is a construct that is stably integrated into the genome and transmitted from generation to generation.

Marker genes are used to determine if a piece of DNA has been successfully introduced into the animal cell. Marker genes are used either for selection and/or for screening.

Non-heritable construct is a construct which may be integrated into the genome of somatic cells but is not expected to be vertically transmitted, i.e., inherited across generations.

Recombinant-DNA animal means an animal in which the genetic material has been changed through in vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and recombinant ribonucleic acid (RNA) and direct injection of nucleic acid into cells and organelles.

Recombinases are a class of enzymes that are able to alter the arrangement of DNA sequences in a site-specific way. Some of these enzymes can excise DNA segments between specific DNA recognition sites.

Reporter gene is a gene that encodes a product that can be readily assayed. It is used as a marker to confirm the incorporation of a transgene into a cell, organ or tissue, and as a means of testing the efficiency of specific promoters.

Somatic cell nuclear transfer（SCNT） is the asexual production of organisms in which a somatic cell provides a donor genome and is reprogrammed to produce a genetic copy of the donor animal.

Trait is one of the many characteristics that define an organism. The phenotype is a description of one or more traits. Synonym: character.

Transgene refers to a heritable genetic construct that has been integrated into the germline of an organism.

Transgenic refers to an organism containing a construct.

Transfection is the introduction of a nucleic acid construct into a cell(s) so that it remains intact and maintains its function.

Vectors refers to vehicles for introducing recombinant-DNA constructs into recipient animals or cells, such as a plasmid, a virus or a bacterium.

ANNEX 1

List of participants

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ANNEX 2

List of documents

Application of genetic engineering for livestock and biotechnology products (by Anne MacKenzie)

Latest developments in relation to the use of reporter and selectable genes in animal biotechnology (by Louis-Marie Houdebine)

Heritable and non-heritable traits (by Larisa Rudenko)

Non-heritable applications of r-DNA animals (presented in a conference room document (CRD 2, pages 2-21) distributed for the 6th Session of the Codex Task Force, 2006)