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# The safety assessment of foods from transgenic and cloned animals using the comparative approach

L. Kelly

Food Standards Australia New Zealand, P.O. Box 7186, Canberra BC, ACT 2610, Australia

**Summary**

The comparative approach to food safety assessment is based on the idea that the safety of a new food can largely be determined by its comparison to the benchmark of commonly consumed foods already in the food supply (also called the 'conventional counterpart'). Any differences between the new food and its conventional counterpart are evaluated to determine their relevance to human health and safety. In this way it is possible to conclude whether a new food is 'as safe as' conventional food already in the food supply. This approach, first developed primarily for use in the safety assessment of food from transgenic plants, is now generally accepted for food from both transgenic and cloned animals as well. This article outlines the basic principles behind the comparative approach, discusses some of the potential food safety concerns associated with transgenic and cloned animals, and describes important elements of the comparative approach and how these might be applied to assessing the safety of food from animals.

**Keywords**

Cloning – Comparative approach – Compositional analysis – Epigenetic effect – Food safety – Molecular characterisation – Nuclear transfer – Substantial equivalence – Transgenesis – Unintended effect.

## Introduction

Animal biotechnology, specifically the production of transgenic and cloned animals, has many potential applications in fields such as agriculture, aquaculture and medicine. This article will focus on the application of animal biotechnology to food production, and in particular on issues relevant to the safety assessment of food from both transgenic and cloned animals.

Although perceived to be a fairly recent development, the experimental production of transgenic and cloned animals actually predates that of transgenic plants. While animal cloning and transgenesis are techniques that can be complementary for the purpose of animal production, they represent distinct technologies. Animal transgenesis involves the introduction of new genetic material into the genome of an animal, whereas animal cloning involves the production of animals that are near identical copies of the single 'parent' animal. Animal cloning per se does not involve any intentional modification to an animal's genome.

Experimentation with animal transgenesis was first reported in the literature in the 1970s (4, 26). The first transgenic animal (a mouse), developed using gene transfer of isolated deoxyribonucleic acid (DNA), was subsequently reported in 1980 (21), and this technology has since been applied, with varying degrees of success, to a number of different food animals, including cattle, sheep, goats, pigs, chickens, carp, salmon and tilapia. As yet, no transgenic animal has been used for commercial food production, although this may be imminent in the case of transgenic carp and salmon. The types of transgenic animals being produced for food production tend to fall

into one of two categories: those with improved production characteristics (e.g. improved feed conversion efficiency, disease or parasite resistance, improved growth rates, improved fibre production) and those with improved product characteristics (e.g. altered milk composition and profile, enhanced carcass characteristics). Such traits have been the goal of selective breeding for many years, but the use of transgenic technology offers new opportunities in terms of the range of genetic modifications, and hence traits, that may be possible.

In animal production, the term 'cloning' can actually refer to a number of different techniques, but in the present context refers almost exclusively to somatic cell nuclear transfer (SCNT), a technique first used in 1996 to clone sheep (61) and which has now also been applied to a number of other food animals such as cattle (7, 29), goats (1) and pigs (2), in addition to many non-food animals. The practice of animal cloning first commenced in the late 1970s, with the use of embryo-splitting techniques as an adjunct to embryo-transfer programmes (59). The more sophisticated nuclear transfer techniques were not developed until the following decade, beginning with the transfer of nuclei from embryonic blastomeres to enucleated oocytes (60). The SCNT, which involves the transfer of a nucleus from a fully differentiated somatic cell, has generated considerable interest and is believed to offer the most promise in terms of commercial application, as potentially unlimited clones can be made of the one individual. Although still in its infancy as a technology, SCNT has an advantage over other cloning techniques, such as blastomere nuclear transfer (BNT), because it allows the cloning of adult animals (either conventionally bred or transgenic), whose traits are already known, thereby enabling the preservation, indefinitely, of those individuals with superior traits. The techniques of embryo-splitting and BNT have been in commercial (although not extensive) use by the dairy industry since the 1980s (39), and hence the food products from such animals have been in the food supply since that time. This is not the case with SCNT cloning, where, at present, a voluntary moratorium on the commercial release of food products is being observed in a number of countries, until such time as certain policy and regulatory issues are resolved.

Recent and ongoing advances in the production of transgenic and cloned animals mean that not only are these animals a scientific reality but that their entry into the food supply may soon become a commercial reality, at least in some countries. As is often the case with any new or rapidly advancing technology, concern has been expressed about the potential hazards associated with such products and their entry into the food supply and so greater attention is now being paid to the safety assessment of such foods. This paper will:

*a)* outline the basic principles of food safety assessment

*b)* discuss some of the potential food safety concerns that may be associated with both transgenic and cloned animals

*c)* describe the important elements of safety assessment and how they might be applied to food from animals.

# General principles of food safety assessment

The safety assessment of food from transgenic animals has been under discussion for many years (15, 28, 30, 40, 41, 63), although in the early years most attention was given to food from transgenic plants, which started to be commercialised during the 1990s. More recently, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) jointly hosted an expert consultation to develop appropriate strategies for the safety assessment of food from transgenic animals (16). Similar consideration has also recently been given to the safety and assessment of food from cloned animals (17, 37, 44). The consensus from these discussions is that the safety assessment of food from both transgenic and cloned animals can be conducted in a similar way to the safety assessment of food from transgenic plants. Fundamental to this assessment is the use of the comparative approach.

The basic principle of comparative assessment – known originally as the concept of substantial equivalence – had its genesis in the early 1990s (40, 63) and has subsequently been further developed and refined as experience has been gained in its use. A recent joint FAO/WHO expert consultation on genetically modified (GM) foods of plant origin re-evaluated the usefulness of the concept of substantial equivalence in the safety assessment of GM foods, concluding that there were presently no alternative strategies that would provide a better assurance of safety (65).

The comparative approach is based on the idea that the safety of GM foods can be assessed, to a large extent, by comparison with the benchmark of commonly consumed foods already regarded as safe (the traditional or conventional counterpart). This approach was adopted because it was recognised that the traditional toxicological approaches for assessing the safety of discrete chemical substances (i.e. animal toxicity testing) could not easily be applied to whole foods, which are complex mixtures of substances (42). Feeding animals high levels of a single food produces both adverse physiological effects as well as nutritional imbalance, both of which are unrelated to potential toxicity. Historically, foods prepared and used in traditional ways have been considered safe on the basis of long-term experience, even though some may have

contained harmful agents (natural toxicants, anti-nutritional substances, microbiological hazards) (40).

The safety assessment applied to GM foods is designed to identify whether any hazard is present, whether it has a nutritional basis or is related to the presence of a chemical, and if so, to gather information on its nature and severity (8). But rather than try and identify every single hazard that may be present in the food, the focus of the assessment is to identify new or altered hazards relative to the conventional counterpart. The comparative approach has been developed as a tool to facilitate the identification of similarities and differences in the food. Any identified differences (either intended or unintended) then become the focus of further assessment, and their relevance to human health and safety is established. The overall intent of the assessment is thus to determine if the new food is 'as safe as' the conventional food.

While this approach was developed initially to assess foods derived from transgenic plants, there is nothing that precludes its application to food from transgenic animals, and in fact this approach may be generally applicable to any food produced using a new technology, for which a comparator exists, including food from cloned animals.

# Potential food-related safety concerns

## Food from transgenic animals

The potential food safety concerns in relation to transgenic animals, which have previously been elaborated in detail (16, 28, 36), relate mainly to the presence of transferred DNA (the transgene) in the host genome. While the desired outcome of transgenesis is usually the stable integration of a transgene or transgenes into the genome, without disrupting any essential host functions, often the introduced genetic material does not behave in a predictable manner and its insertion and expression may result in a number of unexpected outcomes. Because of this, there are really two types of potential food safety concerns to consider: those arising directly from the expression of the transgene (the intended effect of the modification); and those arising as an indirect consequence of the insertion and expression of the transgene (the unintended effects).

## Potential food safety concerns directly related to the intended effect

With a few exceptions, most of the transgenic animals produced so far have been modified to express a novel protein. Examples of some of the different types of proteins being expressed are given in Table I. While the vast majority of proteins forming part of the normal human diet are ingested without any adverse effects, a small number have the potential to impair health. Therefore, in cases where a novel protein has been expressed, the main focus of any safety assessment should be to determine whether its presence raises any food safety concerns. Proteins can produce a range of adverse effects including toxic, allergenic, and other physiological effects.

Allergenicity is a potential concern because the process of transgenesis enables the transfer of food allergens from one species to another, as well as the introduction of new and potentially allergenic proteins into the food supply. While a protein from a known allergenic source can be directly tested for its allergenicity using sera from allergic individuals, it is much harder to predict with certainty the allergenic potential of a protein that comes from a non-allergenic source because such sera will not exist. In this latter case, more indirect methods must be used to try and predict the allergenic potential (8).

Another potential concern relates to the expression of proteins that are bioactive. The concern in this case is whether these proteins could retain their bioactivity after consumption (36). The most common of these is growth hormone, but other bioactive proteins are also being

**Table I**
**Examples of novel proteins being expressed in transgenic food animals**

| Protein | Purpose | Species | Reference |
|---|---|---|---|
| Lysostaphin | Resistance to mastitis | Goats | 14 |
| Bovine α-lactoglobulin | Improved milk production | Pigs | 38 |
| Growth hormone | Increased growth rate and feed conversion efficiency | Sheep, pigs, various fish species | 56 |
| Insuline-like growth factor 1 | Increased wool growth | Sheep | 51 |
| Monoclonal antibodies | Resistance to gastroenteritis | Pigs | 46 |
| *Escherichia coli* phytase | Phytate utilisation | Pigs | 20 |
| Envelope from avian leukosis virus | Increased disease transmission | Chicken | 11 |
| Cecropin peptide | Increased disease resistance | Catfish, medaka | 13, 27, 47 |

considered for various uses, for example the bactericidal protein lysostaphin for the control of mastitis (14) and the antimicrobial peptide cecropin-melittin for the control of fish pathogens (13, 27, 47). It has been suggested that such proteins may raise food safety concerns by altering the balance of intestinal flora in the human gut or causing the emergence of resistant strains of human pathogens, for example a lysostaphin-resistant strain of *Staphylococcus aureus* (36).

The possibility that the expression of a novel protein may have toxic or anti-nutritional effects is also an issue, although in the case of food animals these potential effects are considered to be of low food safety concern (36). Probably the most important factor in assessing the safety of a novel protein is the source of the gene from which it is expressed. Typically, with animal transgenesis, most transgenes are derived from other food animals. In such cases, the expressed protein would already have a history of safe human consumption and so should raise few concerns with regard to potential toxicity. Unlike plants, food animals also rarely produce toxins, although some notable exceptions are to be found among aquatic species, for example tetrodotoxin is present in puffer fish and thiaminase, an anti-nutrient, is found in the viscera of certain other fish species (30). Proteins from sources not previously used for human food would raise greater concern and so should receive greater scrutiny with regard to their potential toxicity.

In considering the potential food safety concerns arising from the expression of novel proteins in transgenic animals it is important to remember that, unlike many other substances added to foods, most proteins have a predictable metabolic fate in the digestive system, that is, they are typically broken down into their constituent amino acids and then assimilated. In addition, most animal food products will either be cooked or pasteurised prior to consumption. Such processing would generally denature most proteins, destroying any biological activity. These considerations may be pivotal in determining whether a particular food safety issue, e.g. toxicity, would ever actually become a real concern.

### Potential food safety concerns related to the occurrence of unintended effects

The genetic modification of an animal by the insertion of a transgene to achieve a specific phenotype (the intended effect) carries with it the possibility of unintended effects and these may be many and varied (16, 28, 36, 37). The occurrence of unintended effects is not limited to the production of transgenic animals; they are also well documented from conventional animal breeding. Double muscling in cattle (22) and porcine stress syndrome in pigs (57) are two such examples. Some of the mechanisms that could give rise to unintended effects in transgenic animals are listed in Table II.

Many of the unintended effects that occur are detected during the early stages of research and development when multiple transgenic lines are being produced. Transgenic lines exhibiting unintended effects are typically discontinued and would rarely ever be considered for commercialisation. The main issue therefore is whether there may be subtle effects, which may not be readily apparent in an otherwise healthy animal, and which may nonetheless have implications for the safety of a food. For instance, pleiotropy could result in a metabolic process being altered, leading to the production of toxic metabolites in edible tissues (36). Similarly, a bioactive protein might be intended for expression in a non-edible animal tissue, but problems with ectopic expression could lead to its unintentional presence in edible tissues, e.g. expressed in the mammary gland and subsequently secreted into milk.

Many unintended effects will probably be predictable based on knowledge of the new protein and its metabolic connections, or indeed the site of transgene insertion (8, 65). Over time, as knowledge of biological systems and transgenesis increases, it seems reasonable to expect that the predictability of many unintended effects will be increased, making it easier to screen for such effects, and that many will also be prevented or minimised by means of improved vector design, for example (16). Unintended effects such as pleiotropy, however, will probably always occur. As unintended effects may be harmful, beneficial or even neutral with respect to the health of the animal or the safety of the foods derived from them, each case or occurrence needs to be evaluated separately to determine its significance in relation to food safety.

## Food from cloned animals and their progeny

The potential food safety concerns associated with cloned animals have also previously been described (36, 37, 44). Although various techniques can be used to clone animals, most concern in relation to food safety has been reserved for SCNT. This is principally because of the genomic reprogramming that must occur when a nucleus from a differentiated cell is transferred to an enucleated egg and then forced to direct the development of a new embryo (36). Such reprogramming is frequently incomplete or otherwise aberrant and so high rates of 'epigenetic effects' are observed where cloned embryos exhibit abnormal patterns of DNA methylation and gene expression, leading to high rates of embryonic, foetal, perinatal and neonatal deaths, as well as offspring with various abnormalities

**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) |

(37). Such unintended effects, however, are not unique to cloning. Many of the same abnormalities (e.g. large-offspring syndrome) have also been observed, albeit at a lower frequency, with techniques such as *in vitro* fertilisation and other related assisted reproductive technologies (49, 53, 54, 66, 67), which suggests that some of the observed effects may not be solely the result of cloning by nuclear transfer per se but may also be due to the use of *in vitro* embryo culture techniques.

Cloned animals exhibiting overt signs of deformity, or which are otherwise unhealthy, would generally not be considered fit for human consumption and therefore, arguably, are unlikely to enter the food supply directly. However, as with transgenic animals, the question remains as to whether more subtle physiological effects may occur in otherwise healthy cloned animals and whether, and to what extent, these may impact on the safety of any food products (36, 37, 44). These more subtle effects would most likely consist of changes in gene expression patterns, which could result in a change in the abundance of a cell constituent such as a metabolite, protein, lipid or carbohydrate, leading to a compositional change in animal tissues and food products (36, 37). It has also been suggested there may be changes in immune function which could alter the potential for food-borne disease or changes in reproductive function which could result in elevated hormone levels in milk, for example (44). At this point in time, adequate data does not exist to be able to determine if such effects routinely occur.

Little, if any, reprogramming is required in the case of embryo splitting (36). However, in the case of BNT, where blastomeres from embryos of greater than eight cells are typically used, full nuclear reprogramming by the cytoplasm of the oocyte is necessary for successful development (31). As a consequence, abnormalities similar to those observed in SCNT clones have also been observed in BNT clones (19, 62). However, since food products from both embryo splitting and BNT clones have a history of prior human consumption without any apparent adverse effects, they are considered to pose minimal, if any, food safety concerns. Nevertheless, it has been recommended that the composition of food products derived from BNT clones should be evaluated to confirm that they fulfil existing standards for food products (36).

Less concern about food safety exists in the case of the offspring of cloned animals. It is believed that the process of gametogenesis naturally resets the epigenetic signals for gene expression, effectively clearing the genome in the progeny of any incomplete or inappropriate signals that

may be present in the cloned parent (17, 43). Reports of healthy progeny resulting from the natural mating of cloned animals (24, 34), even when the cloned animals have themselves exhibited abnormal phenotypes (48, 52), would tend to support this hypothesis.

It is considered more likely that the progeny of cloned animals, rather than the cloned animals themselves, will enter the food supply, at least in the first instance. The reasons for this are primarily economic, i.e. the cloned animals that currently exist have been extremely expensive to produce and are too valuable as breeding animals to be slaughtered for meat (44), although it is feasible that milk products would be available. It has been concluded that edible products from the progeny of healthy clones are likely to be as safe to eat as similar products from the progeny of conventional animals, based on underlying biological assumptions, evidence from the mouse model system, and limited data in the species evaluated (17).

# Use of the comparative approach

The comparative approach is often only considered in terms of the compositional analysis of the new food relative to conventional food. Certainly, in the case of food from transgenic plants, most emphasis has been on this aspect. From some of the early publications on the subject of substantial equivalence, however, it is clear the comparative approach was always intended to go beyond mere compositional analysis. In fact, the comparative approach was considered to comprise three elements:

*a)* molecular characterisation of the introduced DNA

*b)* phenotypic analysis of the organism

*c)* compositional analysis of the food (15, 64) (Fig. 1).

A combination of these three elements can be used to structure the safety assessment and determine where the point of focus should be for each case under evaluation.

## Molecular characterisation

The notion that molecular characterisation is an important part of the comparative approach is rarely recognised, as it is often considered to stand apart from the comparative approach. Molecular characterisation, which includes knowledge of the source and function of the introduced DNA, is, however, critical to comparative assessment, because it helps to define the inserted DNA, and with it the intended effect. The intended effect is usually one of the most important differences that will be identified, and further assessment will be required to determine its impact on the safety of the food. In the case of cloned animals, molecular characterisation will be relevant only if the animal is also transgenic.

Characterisation of the intended effect is the most logical place to start because it first focuses the assessment on a deliberately engineered difference and points towards the relevant parameters of the intended effect that need to be examined, e.g. the level and site of expression of the novel protein. While molecular characterisation is predominantly used for analysing the intended effect, it may also provide important clues about possible unintended effects. For example, it might be possible to determine the site of insertion and hence if any host genes have been disrupted; or whether a mutation has occurred in the coding region of the novel protein during insertion, which could alter its function or specificity.

Detailed guidance on molecular characterisation has been developed in the case of transgenic plants (8), but is not yet available for transgenic animals, although it is anticipated that the approach to transgenic plants and animals will have much in common. Because animals are considered to be more biologically and physiologically complex than plants, and the techniques used to transform them are more complicated, a recent report of a workshop conducted by the Canadian government suggested that expanded data and more extensive characteristics will need to be examined in the case of animals, taking into account the following characteristics:

– stability
– transformation process
– potential to increase disease/pathogen susceptibility
– transgene copy number
– homozygosity (23).

## Phenotypic analysis

The rationale behind the use of phenotypic analysis is that the phenotype of an organism can often provide important clues as to what unintended changes, if any, have occurred to derived food products. Currently, this is best illustrated using plants, where, for example, reduced yield and leaf chlorosis could be an indication of reduced magnesium levels (3), or where stunting and slow growth might indicate a deficiency in the production, transport or accumulation of an important micronutrient such as niacin (9). Phenotypic analysis has thus always played an important part in the screening of transgenic plants during their development, but it is a tool that is rarely used or relied upon by the safety assessor. This may in part stem from the fact that many plants can be phenotypically normal and healthy, yet still produce compounds (e.g. natural toxicants and anti-nutrients) that are harmful

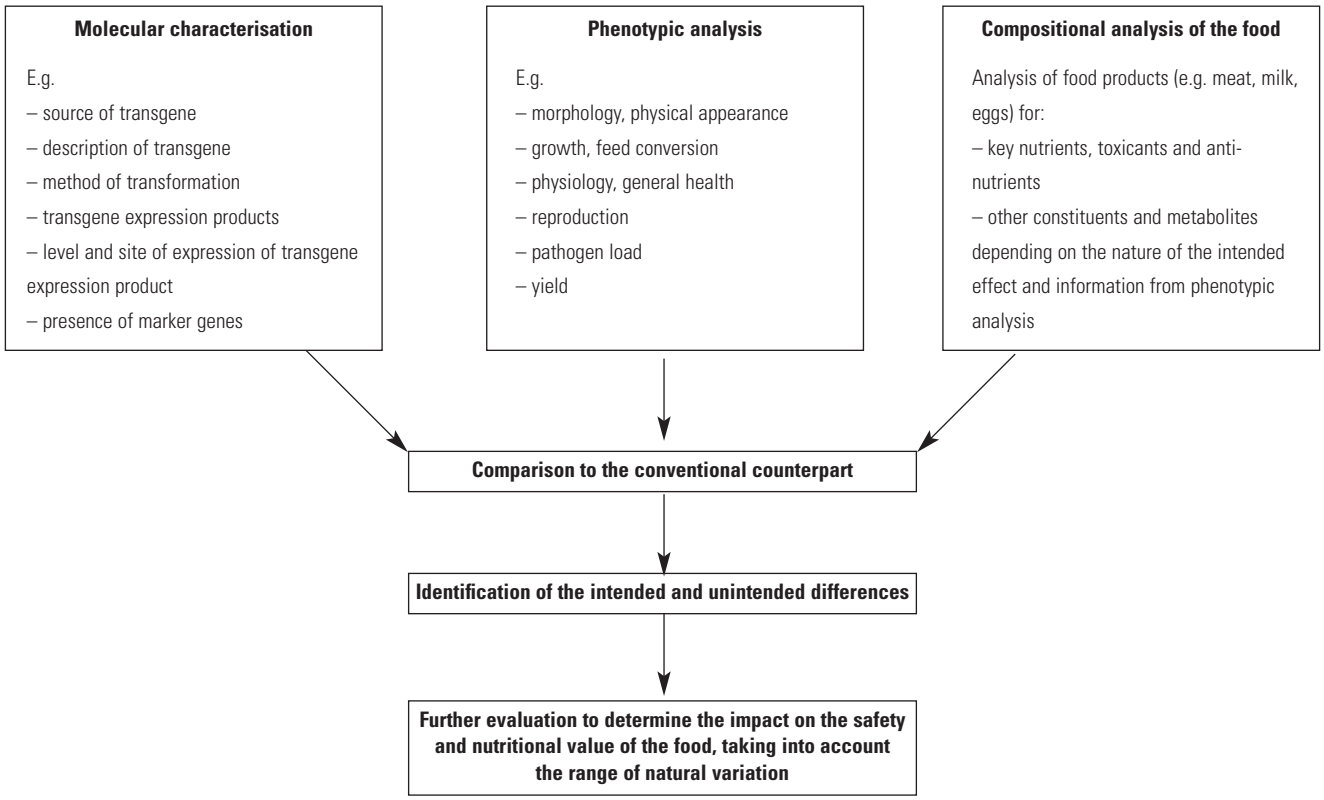


**Fig. 1**
**The three elements of the comparative approach**

to human health. These are best evaluated through compositional analysis of the food.

Animals, in contrast, are regarded somewhat differently; the health of the animal is an important consideration in food safety and any adverse consequences from transgenesis are expected to be reflected in growth, development and reproductive capacity (63). The practice of allowing only healthy animals to enter the food supply is a mechanism that has long been used for ensuring the safety of animal food products. However, because some aquatic organisms are known to contain either exogenously or endogenously derived compounds that are toxic to humans, their apparent good health, by itself, is not considered to be a useful indicator of food safety (41). Nevertheless, phenotypic analysis has been recognised as an important element of the comparative approach as applied to both transgenic (15, 16, 63) and cloned animals (17, 44).

In the case of cloned animals, the 'healthy animal principle' has been further elaborated and extended into what has been termed the 'critical biological systems approach'; the first part of a two-pronged approach being proposed for the safety assessment of food from cloned animals (44). The critical biological systems approach involves the monitoring of an animal over the course of its lifetime and the collecting of data on its health status throughout important developmental stages, e.g. the juvenile period and post-pubertal maturation. If the physical examination and physiological measurements are consistent with historical measurements for appropriate comparators, there can be reasonable assurance that key organ systems are functioning appropriately and that the animal is healthy, thus posing no additional food risks compared to food from conventionally bred animals (44). An approach that links animal health to food safety is one that both the OIE (World Organisation for Animal Health) and the re-established Codex *Ad Hoc* Intergovernmental Task Force on Foods Derived from Biotechnology could investigate further.

Rudenko *et al.* (44) have elaborated in some detail the types of phenotypic data that might be collected, at least for terrestrial mammals. The important phenotypic parameters for other animal species, e.g. fish, would need to be similarly elaborated. Clearly, this sort of approach to phenotypic analysis would be equally applicable to transgenic animals and in fact there may be greater potential for such analysis as, unlike cloned animals, it is likely that multiple generations will be produced. In addition, some unintended effects arising from the presence of the transgene may be expressed as recessive mutations and hence may not become evident until the transgene is homozygous at the site of insertion (36).

## Compositional analysis of the food

A number of different effects may be evident in the animal as a result of either cloning or transgenesis, but these may not necessarily translate through to the food products, or if they do, they may not necessarily impact on its safety or nutritional value. While compositional analysis of food is really just an extension of the phenotypic analysis, the results are in many ways of greater value because they can be used quantitatively to address issues of food safety and nutritional value directly.

The compositional analysis of food is not a simple matter. Foods are highly complex matrices composed of many different chemicals, and their content can be influenced by factors other than the genetics of the organism from which the food is derived. In the case of animals, diet can also play an important part in influencing the composition of food products. For example, supplementation of the diet of chickens with omega-3 fatty acids has been used to increase the omega-3 content of eggs. The main importance of compositional analysis is often stated to be the detection of unintended effects. However, compositional analysis can also be important for evaluating the intended effect where, for instance, that has been to deliberately change the composition of food. Analysis of the food in these circumstances helps to confirm that the trait is being expressed appropriately and also helps to quantify the magnitude of the change, which may be important for assessing safety.

Most transgenic lines and clones exhibiting unintended or undesirable effects would typically be eliminated during the development and selection process. However, more subtle effects may occur, which may not be immediately apparent from molecular characterisation or phenotypic analysis. Such effects may be discernible only through careful analysis of food products. Rudenko *et al.* (44) alluded to this in the context of cloned animals, where for example a nutritional 'hazard' (such as altered calcium transport mechanisms) might be more readily detected by direct compositional analysis of the end product (milk), than by evaluating the health of the animal.

The classic approach to the compositional analysis of food is a targeted one; rather than analysing every single constituent, which would be impractical, the aim is to analyse only those constituents most relevant to the safety of the food or that may have an impact in the overall diet (8). The base set of constituents commonly analysed are therefore the key nutrients, toxicants and anti-nutrients, and these may vary from food to food. In the case of animals, some attempts have already been made to specify the key nutrients for both meat and milk (44), but this will need to be undertaken in a systematic way for each species, including aquatic species, as there may be species-specific differences. Analysis of elements other than the key constituents is generally not considered necessary unless there is an indication that there may be possible unintended effects, e.g. from the phenotypic analysis or molecular characterisation (in the case of a transgenic animal), or if the intended effect is expected to alter compounds other then the key constituents. The extent of the compositional analysis should therefore be guided by the nature of the intended effect and by the information from the molecular and phenotypic characterisation (15).

The Organisation for Economic Cooperation and Development (OECD) has developed a series of Consensus Documents to aid in the compositional analysis of foods derived from transgenic plants (they can be consulted online at www.oecd.org/document/9/0,2340,en_2649_34391_1812041_1_1_1_1,00.html). These documents provide information on the key constituents for particular crops and also provide baseline data on the concentration range (i.e. natural variation) for each constituent. Baseline information is important because it assists in determining whether any identified compositional differences are outside the range of natural variation, and hence require further investigation. The OECD consensus documents are proving to be a valuable resource in the safety assessment of food from transgenic plants and it is anticipated that similar documents might also be developed to assist in the assessment of food from transgenic and cloned animals. The consensus documents for plants have been developed using data and information compiled from a number of sources including the general scientific literature and various public domain databases (e.g. the Nutrient Database for Standard Reference of the United States Department of Agriculture and the Crop Composition Database of the International Life Sciences Institute). It is not yet clear whether comprehensive baseline information will also be available for animal food products (16).

To date, very limited published information is available on the composition of food products derived from transgenic and cloned animals (Table III). In the case of transgenic animals, much of the compositional data is collected for the purpose of evaluating the intended effect and so the data presented is selective. There also tends to be little consistency between groups in the type of data collected or presented. The development of consensus documents outlining the data requirements for compositional analysis in important food animal species will promote consistency and favour the production of comprehensive data sets for analysis, similar to those that have already been produced for food from transgenic plants.

In addition, rather than relying on targeted approaches, so called non-targeted approaches, using profiling methodologies, such as DNA microarray, proteomics and metabolomics, have recently been advocated as a means of increasing the likelihood of detecting unintended changes (33, 65). The use of these approaches has recently been

**Table III**
**Examples of published food composition data from transgenic and cloned animals**

| Species | Modification | Data collected | Reference |
|---|---|---|---|
| Cattle | β- and κ-casein, cloned | Milk fat, lactose, minerals, β-casein, κ-casein, total protein | 5 |
| Pigs | Growth hormone – somatotropin | Lipid composition and cholesterol content | 50 |
| Pig | Bovine α-lactalbumin | Milk lactalbumin, milk protein, total solids, lactose | 38, 58 |
| Pigs | Fatty acid desaturase | Fatty acid profile | 45 |
| Atlantic salmon | Growth hormone | Carcass composition (protein, ash, lipid, dry matter energy) | 10 |
| Common carp | Growth hormone | Whole body amino acid profile | 18 |
| Common carp | Growth hormone | Protein, fat, moisture, amino acid profile, fatty acid profile | 6 |
| Arctic charr | Growth hormone | Muscle composition | 32 |
| Cattle | Cloned | Milk composition (total solids, fat, fatty acid profile, lactose, protein) | 55 |

comprehensively reviewed in the context of nutritionally improved transgenic plants (25). While such approaches could potentially provide an integrated analysis of gene expression, protein translation and metabolite formation in transgenic and cloned animals (with metabolite formation arguably having the most direct relevance to food composition), there remain a number of unresolved questions regarding their use for the detection of unintended changes. For example, it may not be possible to distinguish differences detected by such techniques from natural variation, as there is not yet enough information available on the extent of natural variation. Interpretation of any observed differences may also prove difficult, as their relevance to the safety or nutritional value of the food may not be easily determined, given the current knowledge of animal genomes. Further validation and standardisation will thus be required before such methods will be generally applicable to the safety assessment of foods. However, it has been suggested that such techniques might have greater utility if they were applied in a targeted way, e.g. they might be used to look for changes in metabolites within specified biosynthetic pathways or in degradative/catabolic pathways of interest (25). The profiling methodologies are therefore more likely to develop as a useful extension to targeted approaches, rather than as a substitute.

# Conclusion

Animal biotechnology is a rapidly advancing field that offers many potential benefits for both medicine and food production. As the commercialisation of foods from transgenic and cloned animals will soon be a reality it is important that methodology is in place to adequately assess the safety of such foods. Fortunately, over the last decade, much progress has been made in developing and refining such methodology for transgenic plants. While there are undeniably many biological and physiological differences between plants and animals, much can be learned from the experience gained over the last decade in assessing food from transgenic plants. Of critical importance to this assessment has been the use of the comparative approach, and probably one of the most important lessons to be learned is the remarkable versatility of this approach as a tool. Not only can it be applied to food from transgenic animals, but it can also be applied more broadly to food from cloned animals, and indeed any food produced using new technology.

For transgenic and cloned animals, greater weight can be given to phenotypic analysis as part of the safety assessment, since health status is likely to be a more sensitive indicator of food safety in animals than it is in plants. A greater emphasis on phenotype is exemplified by the two-pronged approach to the safety assessment of cloned animals proposed by Rudenko *et al.* (44). This approach has as its basis the hypotheses that:

*a*) a healthy animal is likely to produce safe food products

*b*) food from healthy animal clones and their progeny that is not materially different from the conventional counterpart food is safe.

While this approach is intended to be applied to the safety assessment of food from cloned animals, if a 'third prong' of molecular characterisation were also incorporated, such an approach would be equally applicable to transgenic animals.

What remains now is for the safety assessment of food from transgenic and cloned animals to progress from the theoretical/hypothetical to the actual. Ideally, this will require some degree of consensus on the important elements of the comparative approach as it applies to the assessment of food from animals. Detailed guidance will be required, particularly in the area of molecular characterisation, although some information will be transferable from transgenic plants. More work will also

need to be done to systematically determine, on a species-by-species basis, the parameters that will be important for phenotypic analysis, and the key constituents that will be important for compositional analysis. These analyses will ultimately depend on compiled information on what represents the normal range of values in the food supply for various constituents. As the data and information required for the safety assessment are likely to be collected during the research and development phase, it is important that agreement on information requirements be reached quickly so that timely and consistent guidance can be given to the product developers.

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# L'évaluation par l'approche comparative de la sécurité sanitaire des aliments issus d'animaux transgéniques et clonés

L. Kelly

**Résumé**

L'approche comparative appliquée à l'évaluation de la sécurité sanitaire des aliments repose sur l'idée que l'innocuité d'un nouvel aliment peut être en grande partie déterminée en le comparant à un produit de référence, c'est-à-dire son équivalent de consommation courante présent dans l'approvisionnement alimentaire (également appelé « homologue traditionnel »). Toute différence observée entre le nouvel aliment et son équivalent classique est évaluée pour déterminer son incidence en termes de santé humaine et de sécurité sanitaire. Il est ainsi possible de définir si un nouvel aliment est « aussi sûr » qu'un aliment traditionnel déjà présent dans l'approvisionnement alimentaire. D'abord élaborée pour servir essentiellement à l'évaluation de la sécurité sanitaire des aliments issus de plantes transgéniques, cette approche est désormais communément admise pour évaluer aussi les aliments issus d'animaux transgéniques et clonés. Le présent article énonce les principes fondamentaux qui sous-tendent l'approche comparative, examine certains des problèmes potentiels de sécurité sanitaire des aliments associés aux animaux transgéniques et clonés et décrit les éléments importants de l'approche comparative ainsi que les modalités de leur application pour l'évaluation de la sécurité sanitaire des aliments d'origine animale.

**Mots-clés**

Analyse de la composition – Approche comparative – Caractérisation moléculaire – Clonage – Effet épigénétique – Effet non intentionnel – Équivalence en substance – Sécurité sanitaire des aliments – Transfert nucléaire – Transgénèse.

# Evaluación de la inocuidad de los alimentos derivados de animales transgénicos o clónicos utilizando el método comparativo

L. Kelly

**Resumen**

El método comparativo se basa en la idea de que es posible determinar el nivel de inocuidad de un nuevo alimento comparándolo con otro producto de referencia que sea de consumo corriente en la cadena de aprovisionamiento alimentario (el llamado 'homólogo convencional'). Se analiza cualquier diferencia entre el nuevo alimento y su homólogo convencional para determinar su posible incidencia en la salud e higiene humanas. De este modo se puede saber si un nuevo alimento es 'tan inocuo como' otro alimento convencional que ya se esté consumiendo. Este método, elaborado en un principio para determinar la inocuidad de los alimentos obtenidos a partir de plantas transgénicas, se aplica hoy también a los derivados de animales transgénicos y clónicos. El autor repasa brevemente los principios básicos del método comparativo, examina algunos de los posibles problemas de inocuidad que pueden plantear los animales transgénicos y clónicos y describe una serie de importantes elementos del método comparativo y el modo en que éstos pueden aplicarse al análisis de la inocuidad de alimentos de origen animal.

**Palabras clave**

Análisis de composición – Caracterización molecular – Clonación – Efecto epigenético – Efecto fortuito – Equivalencia sustancial – Inocuidad de los alimentos – Método comparativo – Transferencia nuclear – Transgénesis.

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