

# The food safety risk assessment of GM animals

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## 1. Introduction

### 1.1. General

Over 98% of all genetically modified organisms (GMOs) that have been introduced into the environment worldwide are genetically modified plants. Micro-organisms and animals constitute only a very limited portion of all GMOs that have been 'field'-tested so far. This situation may, however, change as there are already a number of examples of genetically modified (GM) animals. Of these, GM fish varieties are perceived as closest to marketing. So far no GM animals have been introduced into either the US or EU market, but depending on regulatory developments and public opinion the number of different GM animals bred and marketed world wide may increase.

Two types of GM animals can be distinguished. The first group has been genetically modified to enhance overall performance and have, therefore, added value in an agronomic and/or economic sense. The whole animal will eventually be available for the food market. The second group of GM animals has been transformed to produce specific substances in milk, eggs or blood or serve as medical research model. The goal of this later GM technology is the production and isolation of the specific substance or tissue as a marketing material or to use the animal for medical research purposes or for toxicity testing and ; they are not intended for food production. However, animal disposition of these GM animals is still a concern. In addition, a distinction should be made between germ-line modified animals and somatic cell -modified animals. In the latter case only specific tissues will have incorporated the new trait(s), whereas the rest of the animal will remain genetically unaltered.

When discussing the food safety aspects of GM animals two scenarios should be considered: 1) the intentional introduction of GM animals into the food production chain and 2) the unintentional introduction of GM animals. Although in the latter case precautions will be taken to avoid GM animal materials entering the food production chain, such an unintentional event should nevertheless be part of the risk evaluation process.

### 1.2 Regulatory aspects

In recent years considerable expertise has been gained in the food safety assessment of GM plants. Although work is on-going to optimise current safety assessment approaches, it can be stated that solid strategies have already been developed that minimize the possibility of adverse health effects for the consumer of GMO-derived plant products. In fact, no adverse effects have been observed that can be related to an approved GM plant variety. These food safety assessment strategies for crop plant products are based on a series of expert reports initiated by the IFBC (1990), that was taken over and carried further by OECD (1993,1996) and FAO/WHO (1991, 1996, 2000) in order to come to globally agreed safety assessment approaches (FAO/WHO, 2001; Codex Alimentarius, 2003). Current safety assessment strategies address a number of different issues related directly to the genetic modification as well as to potential unintended side effects of the genetic modification on the food organism. In practice, information is asked on 1) the process of the genetic modification, 2) the safety of the newly introduced proteins, including information on potential allergenicity, 3) occurrence and potential implications of unintended side effects of the genetic modification, 4) possible effects of gene transfer and recombination, 5) the role of the new

food in the diet and 6) the influence of food processing (Kuiper et al., 2001). Based on this dossier additional questions of food safety may be asked.

The safety assessment of GM animal-derived food materials has also been subject to discussion in a number of expert meetings by OECD (1992,1993) and FAO/WHO (1991,1996, 2000) and the US National Research Council (NRC, 2002). It was concluded that the minimal food safety assessment of GM animals should comprise (Kleter and Kuiper, 2002) 1) the molecular characterisation of the inserted foreign DNA, 2) the safety assessment of the introduced genes and their products, 3) any unintended effects of the insertion of foreign DNA in the organism, and 4) the effect of disease resistance brought about in transgenic food animals on consumer's exposure to disease-causing agents. Depending on the method of gene transfer used, additional questions of food safety will have to be answered with relation to the infectivity of the vector, the assessment of potential effects of vector regulatory elements on the host cell and the possibility of recombination with endogenous viral sequences.

Experience in the safety assessment of GM animals is still very limited, but it is clear that evaluators of GM animal-derived food products will benefit from experiences with GM plants as basic approaches for GM plant materials also apply to GM food animals. This working paper will draw upon the experience in the food safety assessment of GM plants to discuss the food safety assessment of GM animal –derived food products.

### **1.3 Risk assessment approach and limitations**

The traditional paradigm for conducting a risk assessment includes four steps: hazard identification, hazard characterization, exposure assessment and risk characterization (NAS report, 1983). With GM animals and derived foods all four steps of the risk assessment must be undertaken on a case-by-case basis within this still evolving area. Thus the authors propose a comparative safety assessment to enable the final risk characterization. Drawing from the experiences with GM plants, the safety assessment is often a two-tiered approach where initially information is gathered to identify parameters and the magnitude of these parameters that distinguish the GMO from its traditional counterpart. The next phase is then to gain further insight into the toxicological and nutritional relevance of the detected differences. These characterization steps, when matrixed with the exposure of the hazard, will allow for the final risk assessment.

## **2. GM animals**

### **2.1 General**

There are two types of GM animals: those whose alterations are stably incorporated throughout their genomes and those with non-heritable transgenic constructs. The former are often referred to as “transgenic animals” while the latter techniques are often referred to as “gene therapy”. Gene therapy modifications are not limited to modifications intended only to therapeutically treat animals. In fact, the distinction between heritable and non-heritable modifications is not dependent on the intent of the modification. Rather, it is a function of the technology chosen for the intended modification.

### **2.2 Methodologies used for gene transfer**

#### *2.2.1. Non-Heritable Modifications*

Animals containing non-heritable changes are produced by the introduction of the gene of interest in a vector that targets the somatic cells of the animal. There are two types of vectors preferentially used: those based on viral sequences and those based on transposable elements.

Viral-based technologies take advantage of the integrative properties of retroviruses and adenoviruses. The integrative function is the ability of viruses to “cut in” to the sequence of host DNA. Such interruptions may be benign or hazardous. Transposon-based technologies have also been developed. Transposons are often referred to as “jumping genes” because of their ability to catalyze their own movement within the genome of the animal. Transposons were first discovered in the plant kingdom, but have recently been identified in animals, including humans.

Any gene therapy technique may give rise to insertional mutagenesis or unintended gene activation or silencing. The risk scenario for both viral and transposon-based vectors also includes concern for recombining with existing viruses in the intended target animals, in humans who are exposed to them, or in other animals that may be exposed to the target animals or their wastes. Recombination may give rise to viruses with increased host ranges (swine viruses becoming capable of infecting humans), increased virulence (innocuous viruses causing serious illness), or generation of entirely new, pathogenic viruses.

### *2.2.2. Heritable Modifications*

GM animals are produced as the result of the stable incorporation of genetic constructs in their nuclear chromosomes or mitochondrial genomes. In general, transgenic animals are produced by injecting early embryos with solutions of DNA that contain constructs that have all of the requisite information for directing the expression of the gene(s) of interest, but rely on the cell's internal recombinatory enzymes for integration. Scientists have also used viral vectors or transposon-based vectors to produce transgenic animals with heritable traits.

Production of a transgenic line of animals is usually a two-step process. Mosaic transgenic animals are produced by the introduction of the transgenic construct into early stage embryos. The expectation is that most of the cells of that developing embryo will contain the gene of interest, including some germ cells. These animals are considered "mosaics" as they are composed of two or more genetically distinct cells. Mosaics are then bred and the offspring tested to find animals with 100% transgenic cells (i.e., derived from a transgenic germ cell in the mosaic). A founder animal, in which all cells carry the transgene, is selected and bred to propagate the transgenic line.

## **2.3 Genetically Modified Animals and Their Products**

### *2.3.1 Laboratory Models*

GM animals are now common tools used to investigate the mechanisms of both normal physiology and the pathophysiology of humans and animals. An example is the pig model for human retinitis pigmentosa, a progressive disease that begins with night blindness. This model is intended to help develop pharmaceutical strategies to slow the onset and progression of the disease.

### *2.3.2 Biopharm Modification in Food-Animals*

#### *2.3.2a Human therapeutic agents*

GM animals can be developed as bioreactors for the production of therapeutic proteins. In general, these protein products will be produced in the animal's milk (cows, sheep, and goats), eggs (chickens), semen (swine), or blood (large farm species). The advantages of producing these products in animals rather than cell or tissue cultures include high yields, mammalian glycosylation pattern and lower post-development costs (Ziomek, 1998). Examples of therapeutic products from GM animals include alpha-1-antitrypsin (ATT) in goat milk. This human blood protein is intended to treat hereditary emphysema (ATT deficiency), cystic fibrosis, and chronic obstructive pulmonary disease (Colman, 1999). Other examples include antibody production in GM animals for diagnostic and medicinal purposes from milk or blood (Houdebine, 2002).

#### *2.3.2b. Xenotransplantation*

The field of xenotransplantation covers many procedures, ranging from implantation of single cells to treat Parkinson's disease to the transplantation of organs to treat organ failure. GM animal organ transplantation has yet to be successfully implemented in humans, although transplants of smaller tissues and individual cells are currently under active clinical investigation. Because of their physiological similarities to humans, pigs are attractive as a potential organ donor species. Because tissue rejection appears to be the primary medical barrier, pigs have been modified to knock-out 1, 3-galactosyl transferase, a protein linked to acute human tissue rejection.

#### *2.3.2c Industrial Products*

The use of GM animals in the production of industrial products provides a novel "manufacturing" source, and a number of challenges to manufacturers, regulators, and the public. Perhaps the best known example is transgenic goats producing spider silk proteins in their milk. These proteins could

be used in the manufacture of body armor. The larger part of this category of transgenic animals will be kept in containment and it is essential that they should not enter the food production chain. Nevertheless the unintended entry into the food supply chain should be part of the risk assessment procedure prior to the breeding of these GM animals.

### **2.3.3 Agronomic Modification in Food Animals**

#### *2.3.3a Animal Health and Productivity*

The most well-known products in this category are those incorporating growth hormone (somatotropin) genes into the genomes of the same or other species. Aquaculture provides several good examples. The main traits to be altered are growth rate, cold tolerance, disease resistance and sterility. Transgenic salmon, catfish, carp and tilapia have been developed to reach market weight sooner than their non-transgenic counterparts by using fish-derived somatotropin. (However, earlier research involved somatotropins from other sources. The promoters used can be either tissue-specific or constitutive (Maclean, 2003). For cold tolerance antifreeze proteins, such as winter flounder-derived delta-9-desaturase, have been tested, but have not yet proved successful (Maclean, 2003).

Disease resistance in animals can also be enhanced using GM techniques. Lysostaphin, a bacteriocidal enzyme, has been introduced into cows to decrease the incidence of mastitis caused by *Staphylococcus aureus*. Moth cecropin, a broad spectrum antimicrobial peptide, has been transgenically incorporated into catfish to decrease their susceptibility towards a broad range of bacterial diseases (Zhang et al., 1998).

#### *2.3.3b Enhanced animal nutrition*

Enhanced animal nutrition and growth performance by modification is possible. For example, bovine lactalbumin and insulin-like growth factor-1 (IGF-1) have both been introduced into sow milk for the improvement of the growth characteristics of the piglets (Wheeler et al., 2001). Attempts in fish are ongoing to alter the carbohydrate metabolism of especially salmonoids in order to be able to use vegetable products in the aquacultural systems (Maclean, 2003). The "Enviropig" is another example of GM that affects the nutrition of the pig. In this specific case, phytase is introduced into pigs to allow them to make better use of the phosphorus in their feed. This not only allows the farmer to decrease phosphate supplements, but also decreases the amount of phosphorus in pig manure. (Golovan et al., 2001)

#### *2.3.3c Human Foods*

Foods derived from GM animals can be altered with respect to functionality and composition. For example, cows can be modified to make a more desirable milk: (1) producing milk more digestible for lactose intolerant individuals by lowering its lactose content, or (2) increasing the amount of a naturally occurring antimicrobial enzyme to increase the shelf life of milk. Although the meat industry also has increasing interest in the improvement of the sensory and nutritional quality of their meat products (Garnier et al., 2003), few GM experiments are currently performed in this area as yet.

Fish can also be modified to provide better, more nutritious food. One example is the transgenic modification of rainbow trout to increase the amount of the omega-3 fatty acid that they produce and store.

## **3. Comparative safety assessment**

### **3.1 Principle of substantial equivalence, applied**

The principle of substantial equivalence was originally described by the FAO/WHO (1991), and subsequently named and detailed by the OECD (1993). The rationale behind the principle is that many food products we eat today are derived from organisms that we can not consider inherently safe. Nevertheless, we have been consuming these products for decades without any obvious deleterious effects. Because of this history of safe use, it is generally acknowledged that traditional food products should serve as a baseline for comparison and that novel GMO-derived food products should be at least as safe as the traditional products that they may replace in the diet. The

principle has led to much debate in recent years as interpretation of the principle differed between countries. Nevertheless, the basic idea of comparing new GMO-derived products with closely related traditional counterparts to assess the safety of the newly developed organisms is unchallenged. Substantial equivalence should represent a starting point of the assessment rather than the end point (Kuiper et al, 2002) and should not be confused with being an absolute safety standard.

Application of the principle is usually a tiered approach, a Comparative Safety Assessment (CSA, Kok and Kuiper, 2003) where the initial step is comprised of a thorough comparison with the closely related traditional counterpart. This comparison includes both phenotypic characteristics as well as a compositional analysis. The phenotypic analysis should also include factors such as disease resistance to common diseases. Information should be supplied on:

- the transformation process of the genetic modification, including the sequence of the inserted material,
- the copy number and place(s) of insertion,
- stability of the integration,
- the safety of any newly introduced proteins, including allergenicity,
- occurrence and implications of unintended effects,
- potential effects of gene recombination,
- the role of the new GM animal food in the diet and
- the influence of processing on the new GM food product.

Within Europe, sequence analysis of the place(s) of insertion is also part of initial phase of the CSA. More precise criteria for the molecular characterisation are currently being discussed in the OECD.

### **3.2 Hazard Identification and Characterization**

The hazard identification step is typically the first step in any risk assessment. However, for complex GMO-derived foods, the hazard identification step will not be as readily completed as in the case of well-characterised single chemical compounds. Similarly, the hazard characterization is not as readily determined with complex GMO-derived foods. The variety and magnitude of unintended effects when testing complex food products, whether GMO-derived or not, may preclude straightforward hazard identification and characterization. The differences found as a result of the CSA serve as comparable to the hazard identification and hazard characterization steps in a traditional risk assessment paradigm.

### **3.3 Gene Transfer**

The DNA construct used to change the genetic make-up of the animal should be considered within an assessment especially if the gene or its promoter is derived from a viral source since horizontal transfer or recombination may occur. Additionally, bacterial host-derived materials may contain additional sequence fragments unrelated to the target gene. Because such fragments can be heterogenous in size and sequence, they are difficult to detect. This is particularly a problem with retroviral vectors. Host cells often contain large numbers of endogenous viruses and virus-like sequences (Chakraborty et al 1994; Scadden et al 1990, NAS hazard report). Inadvertant introduction of such sequences into the germline of a GMO not only has the potential for creating unintended genetic damage but can also contribute by recombination to the generation of novel infectious viruses. A well known example is the generation of a replication-competent murine leukemia virus (MLV) during the growth of a vector containing a globin gene (Purcell et al., 1996). In a similar way prions may be introduced to the GM animal or derived products (Faber et al., 2003).

Furthermore, there is potential for horizontal transfer of the gene construct: food-ingested foreign DNA may not be completely degraded in the gastrointestinal tract of mice (Schubbert et al., 1997; Schubbert et al., 1998). It was shown that phage M13 mp18 DNA following oral ingestion by mice may reach peripheral leukocytes, the spleen and liver via the intestinal wall mucosa and was covalently linked to mouse DNA (Schubbert et al., 1997). Similar results were obtained when a plasmid containing the gene for the green fluorescent protein was fed to mice (Schubbert et al.,

1998). However, these results have been criticized due to the complication of artifacts within the methodology (Beever and Kemp, 2000). For the food safety assessment it is prudent to assume that DNA fragments may survive the human gastrointestinal tract and be absorbed by either the gut microflora or somatic cells lining the intestinal tract.

Commonly used marker genes are genes that code for antibiotic resistance. Risk assessment of these selectable genes should focus on gene transfer to microorganisms residing in the gastrointestinal tract of humans or animals. There is general agreement that transfer of antibiotic resistance genes from plants to human gut micro flora is unlikely to occur and impact antibiotic efficacy (FAO/WHO 1996, 2000; Van den Eede et al.). Similarly, the likelihood of such transfer from GM animals to human gut microflora will also be low. However, as the potential of gene transfer can not be completely ruled out, the safety assessment should also consider information on the role of the antibiotic in human and veterinary medical uses. Furthermore, within the EU the use of antibiotic resistance marker genes in newly developed GMO-derived food products is not allowed.

### **3.4 Safety of the gene product**

The safety of the gene product must be assessed on a case-by-case basis. Depending on the knowledge on the expressed product the assessment may range from a limited evaluation process of the available data on the protein, such as amino acid sequence and expression rates in different tissues, to, in the case of less well-documented proteins, extensive toxicity testing including animal studies. In theory, the advent of GM animals may lead to the introduction of many new proteins without a history of safe use into the human diet. The assessment of the novel proteins should be based on current knowledge of toxic substances, including a search for sequence homology with known toxins, and the function of the novel protein. In the case of unknown proteins a full classic toxicological safety assessment procedure will form part of the evaluation.

In this respect a distinction should be made between GM animal-derived food products that were developed, to improve agronomic characteristics and GM animal-derived food products developed for veterinary, pharmaceutical or industrial purposes. So far the number of different genes that is used for the production of GM food animals is still rather limited when compared with plants, but this situation may change with the progress of genome sequencing programmes that are likely to provide a wealth of data on important animal physiological pathways.

### **3.5 Allergenicity**

Food whether developed by conventional means or through biotechnology is a potential source of allergens. All food allergies are mediated by antigen-specific IgE and are characteristic of type-I reactions. In the case of new proteins being expressed in the GM animal, the allergenic potential of the protein will need to be established. In the case of production of specific well-characterised (medicinal) proteins by the GM animal, it needs to be established whether the post-translational modifications are comparable to the same substances being produced by more traditional sources in order to assess potential altered toxicological or allergenic properties of the newly synthesized proteins (Dyck et al., 2003).

Efforts to characterize the mechanisms of allergies at both cellular and molecular levels, have produced only a limited understanding of the characteristics that allow a protein to induce sensitisation or a full allergic reaction. Because of these complexities, it has long been recognized that there is no single parameter that can predict the allergenic potential of a substance. Recently, the strategy to address allergenicity of biotechnology products has been formulated (FAO/WHO, 2001; Metcalfe et al, 1996, Codex Alimentarius, 2003), which relies on the following parameters: source of the gene, sequence homology, serum testing of patients known to be allergic to the source organism or to sources distantly related, pepsin resistance, the prevalence of the trait and animal models.

The source of the introduced protein should be part of the background material available to conduct an allergenicity assessment. Allergenic sources of genes would be defined as those organisms for which reasonable evidence of IgE mediated oral, respiratory, or contact allergy is available.

Knowledge of the source of the introduced protein allows the identification of tools and relevant data to be considered in the allergenicity assessment. (Codex Alimentarius, 2003)

Sequence homology is the initial step in the allergenicity assessment. When sequence homology to a known allergen is demonstrated, the product is considered allergenic and no further testing is typically undertaken. The FAO/WHO panel recommended using an amino acid window for the sequence homology that was scientifically justifiable. Research reports showed that an amino acid window size of less than eight amino acids may result in a high rate of false positives (Hileman, 2002).

Specific serum screening is then undertaken irrespective of the prevalence of allergy to the source material in question when the source is a known food allergen. When no sequence homology has been found between the expressed protein and an allergen, targeted serum screening (direct source and related organisms) is undertaken. The use of larger numbers of sera is advocated whenever possible to increase the confidence associated with the results.

Additional assessment of the potential allergenicity of expressed proteins may be performed by pepsin degradation analysis or by using animal models. Pepsin digestion stability is believed to impart on the allergen an increased probability of reaching the intestinal mucosa intact where absorption of significant quantities may lead to sensitization. Protein stability in itself is, however, not sufficient to exclude potential allergenic properties as exceptions are known of less stable proteins that are allergenic. There are several animal models including the intraperitoneal (IP) murine model and the Brown Norway rat model. Failure to elicit IgE antibody production after IP administration to the laboratory mice where immunogenicity is evident on the basis of IgG response may provide some reassurance that the protein lacks a significant potential to provoke allergic sensitization. In practice the predictive value of these systems for proteins that are new to the human diet may, however, be limited (König et al.).

### **3.6 Unintended effects**

Potential unintended effects represent a significant concern with GMOs including GM animals and these effects highlight the difficulty of establishing uniform considerations instead of case-by-case considerations. Unintended effects can be divided into insertional effects, related to the place of insertion of the transgenic fragment, and secondary effects, related to the nature of the expression products of the introduced genes. The major approach to detect any unintended side effects in the GM animal is a phenotypical and compositional analysis to compare the new food organism with the traditional counterpart. Whereas, there are databases on plant species describing the current knowledge (including a listing of (natural variation in) macro and micronutrients, natural toxins and other anti-nutritional factors) (OECD, 2003), a comparable database is not as readily available for food animals.

For GM animal-derived food products the same approach should apply. The edible tissues of the GM animal under investigation and comparable tissues from a genetically related non-GM animal should be phenotypically and compositionally analysed and screened for differences that may have toxicological or nutritional relevance. Similar to the GM plants, the key constituents of the tissue would have to be established. Because of the likeness between animals and humans, few animal tissue constituents can be considered anti-nutrients or natural toxins, but there are exceptions, such as thiaminase in different fish species and tetrodotoxin in puffer fish (Kleter and Kuiper, 2002). An important difference with GM plants is the average number of off-spring from one GM animal. The number of GM animals derived from a single GM founder animal will in general be much lower compared to GM plants. As the associated costs will be considerable, the selection process of the initial founders will be very limited compared to the plant breeding situation where thousands of GM calluses are screened for incorporation of the transgenic fragment and subsequently monitored for their phenotypic characteristics. This means that the information on the variation range between animals with the same genetic modification will be rather limited and that detected differences between individual animals will be difficult to interpret. In theory, the consequence of the smaller number in animal breeding may be that the selection process is less stringent with GM animals

which may lead to higher chances for unintended effects. On the other hand, however, GM animals may be more vulnerable to smaller changes in their physiology and therefore selected transgenic organisms without obvious phenotypic aberrations may show relatively few physiological alterations when compared to GM plants. Further research may shed more light on these aspects with relation to the safety assessment.

As the number of key nutrients and/or anti-nutrients is limited in any species, a targeted compositional analysis will have its limitations in the information that can be provided. For animal products where there is no tradition of composition analysis, unbiased profiling methodologies that are currently being developed may become a valuable addition to the present targeted approaches as part of the food safety assessment strategy, once they are validated (Kuiper et al., 2003). The issue of sampling is crucial for both the targeted and profiling approach. For comparative compositional analyses, it is very important that the conditions for breeding of the animal and sampling of the edible animal parts are highly similar to avoid the detection of differences that are unrelated to the genetic modification. Animal tissues have to be analysed before any processing has taken place. At the same time, any potential effects of the subsequent processing steps should also be included in the overall risk evaluation.

There is likely to be expanded work in profiling food derived from GMOs including GM animals for safety evaluations as part of a CSA. The profiling approach can be roughly divided into holistic approaches on three different integration levels: genomics, proteomics, and metabolomics.

### *3.6.1 Genomics*

Microarray technology is a powerful tool to study gene expression. The technology allows comparison of expression profiles of a large number of genes under different environmental or developmental conditions. CDNA or EST (expressed-sequence tag) libraries can be established of any organism under investigation (Kok and Kuiper, 2003). If alterations in gene expression are found, the nature of the related gene will provide initial information on the toxicological or nutritional relevance of the alteration. Detected differences should be confirmed by additional targeted analysis preferably aiming also at the corresponding proteins or metabolites. The main advantage of the gene expression microarray approach over proteomic and/or metabolomic approaches is the scale of study. Where proteomics or metabolomics are likely to include at best 10% of the proteome or metabolome, respectively, the gene expression microarray makes it feasible to study whole transcriptomes of the organism.

### *3.6.2 Proteomics*

Proteomics is the direct counterpart to transcriptomics. In general, correlation between mRNA expression and protein levels is rather poor as the rates of degradation of mRNA and proteins differ (Gygi et al., 1999). Therefore, understanding the biological complexities underlying gene function is facilitated by analysis of many proteins simultaneously. Methods used for analyzing differences in protein patterns include SDS-PAGE followed by peptide mass fingerprinting. There are, however, limits to what 2DGE can analyse as, in general, only highly expressed proteins will be detected (Gygi et al., 2000). Another approach that is currently being tested is the use of isotope-coded affinity tags to analyse fragmented proteins or multidimensional liquid chromatography coupled to mass spectrometry (<http://www.foodstandards.gov.uk/science/research/NovelFoodsResearch/g02programme/g02projectlist/g02001>). Also a protein microarray approach to accomplish the same end is under development based on the interactions of individual proteins with their substrates or with other proteins. This development may lead in time to 'whole proteome' approaches that may reduce the necessity for initial gene expression profiling (MacBeath et al., 2000; Templin et al., 2002).

### *3.6.3 Metabolomics*

Continuing down the cascade from genome and proteome is the metabolome. The metabolome consists of the metabolites that occur within a biological entity. A multi-compositional analysis of biologically active compounds (metabolites) may also indicate the presence of unintended effects. Metabolites can be determined by traditional chemical techniques including gas chromatography

(GC), high pressure liquid chromatography (HPLC), coupled to mass spectrometry (MR) or nuclear magnetic resonance (NMR). These methods are capable of detecting, resolving, and quantifying a wide variety of compounds in a single sample. This type of chemical fingerprinting provides more details than can be obtained by single compound analysis. Once differences are observed these differences should be further analyzed by in vitro or in vivo testing. Before chemical fingerprinting can be readily exploited to determine substantial equivalence as related to GMO-derived foods, efforts to standardize sample collection and extraction are needed. Once again, background data on non-GMO comparator sources should be collected to acquire knowledge on the natural variability of the species.

### **3.7 Toxicology**

In general, it will not be possible to test complex animal products by classical toxicological animal studies in the way they are routinely used to test single compounds. Classical studies measuring physiologic response relative to dose are complicated if the laboratory animal is receiving doses of the GM animal's edible tissue. If the genetic modification would result in the expression of novel proteins or if the compositional analysis revealed an alteration in an endogenous protein product or metabolite, the traditional toxicological approach would require the concentration of the product to be elevated in the laboratory animal's diet to the extent that the diet will often become unbalanced. This might result in toxicological observations that are unrelated to the product under investigation. To avoid this scenario, the concentration of the product can only take place within the limits of national and international recommendations on optimal laboratory animal diets, thereby limiting the sensitivity of the tests (Barlow et al., 2002; Cockburn, 2002). On occasions where the genetic modification results in an increase in a specific (exogenous) protein, for instance directly derived from the gene construct, traditional testing would still be valid to assess that portion of the derived food. Alternatively, there may be instances wherein endogenous protein levels in the GM food are increased well above physiologic level in the given animal species and it might be prudent in specific cases to (also) test this elevated protein in animal studies.

### **3.8 Nutritional analysis**

The nutritional analysis should focus on the potential replacement of nutritionally important food products by the novel GM animal-derived food products with possibly altered characteristics. The information for the nutritional analysis will largely be derived from the initial CSA, including the compositional analyses (especially macro-, micro- and anti-nutrients) and the estimated consumption rates. Detected alterations in the GM animal-derived food products compared to the traditional counterpart will be assessed by evaluating the significance of the compositional differences for the consumer in general and also, in specific cases, for specific consumer groups. Nutritional aspects of GMO-derived foods may become of increasing significance when the number of compositionally altered food products on the market increases. Therefore, the nutritional assessment of GM animal-derived food products is dependent on current consumption data of animal-derived food products in distinctive consumer groups and with respect to geographical and demographical differences. Special consumer groups perhaps worthy of special consideration include children, pregnant or lactating women and elderly persons.

Micronutrients are vitamins and minerals that are essential for normal physiology and biochemical functioning. Both deficiency and excess of a micronutrient can cause health problems which emphasizes the importance of this class of compounds. Macronutrients include dietary lipids, proteins and carbohydrates and these classes of compounds are present in the food and diet in substantial quantities. Assessment of the replacement factor of important animal-derived sources of micro- and macronutrients by GM animal products in the event of altered composition with relation to these nutrients is therefore of major importance. Bioavailability of the important micro- and macronutrients from GM animal-derived tissues is also of significant importance in this respect.

## **4. Exposure Assessment**

To assess the amount of food or food ingredient an individual or group is exposed to, represents the goal of an exposure assessment. No exact criteria have been formulated so far for the factors considered in an pre-market exposure assessment of a complex novel food product. Some

exposure paradigms make assumptions based on per capita production while others use per capita distribution. An exposure assessment may also consider the cooking and food preparation process used. Some governments have instituted tracking of animal derived food and from this dataset, post-market consumption data may be determined. Exposure assessments will also include an estimate of the extent to which current food products will be replaced by the GM animal-derived novel food product. Thus, the accuracy of the exposure assessment for GMO-derived foods is dependent upon the available data on consumption patterns in consumer groups of interest and the validity of the underlying parameters (Kuiper 2001).

The potential exposure of children of different age groups to growth factors in GM fish-derived tissues is an actual example as this GM animal-derived food product is requesting market entry in the US. The exposure assessment will be based on available consumption data and our knowledge on the bioavailability of the growth factors upon consumption. Mathematical models for integrating food consumption and distributions may be used in a so-called probabilistic approach to estimate future exposures more precisely. Alternatively, biomarker based methodologies for quantifying exposure to food chemicals are garnering interest but this approach is not yet validated for traditional food additives much less for GMO-derived food (Kroes et al., 2002).

## **5. Risk Characterization**

Risk characterization typically refers to the probability that a hazard would produce a given adverse effect. Risk characterization is the stage of risk assessment that integrates information from exposure assessment and hazard characterization into advice suitable for use in decision making or risk management. It is prudent to highlight that risk characterization is typically viewed as an iterative and evolving process. With traditional food additives the risk characterization can take the form of establishment of an allowable daily intake level (ADI).

In the case of GMO-derived food the many facets of the CSA and the exposure assessment would need to be matrixed together. The baseline for the safety of novel food products derived from GMOs, including GM animals, in all cases will have to be the assessment that the novel GM animal-derived food products is at least as safe as its traditional counterpart. If any questions remain after the initial CSA with respect to the safety of the GM animal-derived food products additional tests may be required, including animal studies with the whole product or selected tissues/extracts. If, after a full safety assessment, the safety standard can not be satisfied the GM animal-derived product should not be approved for marketing. For food products derived from GM food animals this characterization should be established on a case-by-case basis.

### **5.1 Post-Marketing surveillance**

Closely related to the risk characterization is the issue of post-marketing surveillance. Post market surveillance could be useful in certain instances where a better estimate of dietary exposure and/or nutritional consequence of GMO-derived food are required. In general, potential safety issues should be addressed adequately through pre-market studies. However, given the complexities of food allergies it is conceivable that, for instance, allergenicity concerns could warrant post-market surveillance (Hlywka et al, 2003) as part of the risk management profile.

For GM animal-derived medicinal substances existing pharmacovigilance schemes will apply to monitor any unforeseen unintended side effects of the isolated medicinal substances. The same would apply in a veterinary sense with respect to the GM animal itself when modified with respect to the production of hormonal or disease-prevention substances: pharmacovigilance schemes could help to detect unintended side effects of the introduced expression product to the GM food animal that were not detected in the pre-market phase. To this end the GM animals should then be included in such pharmacovigilance schemes on the basis of 'internal' administration of the specific veterinary substance.

Post-marketing surveillance systems for GM animal-derived food products need the establishment of adequate traceability systems of the GM animal products in the food production chain. Here, the food animal sector has clear advantages over the crop plant sector where basic traceability

systems for individual farms, let alone plants, is still virtually lacking. In the animal production sector, such systems are already well-established for some animal food production chains in some countries and many other initiatives are ongoing in this field.

Traceability will in practice be most feasible for well-characterised GM animals dedicated for the production of specific substances or tissues that are kept in containment. Safety precautions should, however, be aimed at the prevention of any introduction of these GM animals into the food supply chain. The precautions should also include the development of analytical tests for detection and identification.

It is important to note that traceability and related control systems may be less straightforward in the case of chimeric organisms as different parts of the food animal will have different genetic constitutions and this may severely complicate analytical control of traceability systems.

Depending on the questions and risk management needs underlying the establishment of post-marketing surveillance systems the information conveyed to the consumer may, however, require adjustment. In order to enable consumers to relate potential adverse, for instance allergenic, effects to a GM animal-derived food product, it will be necessary to not only label the product as GMO-derived, but also provide information on the specific GM animal source, for instance by including in the label the unique identifier code specific for a single founder animal and its offspring.

## **6. Conclusions**

The food safety evaluation of GM animals and derived products can largely be performed along the lines that have already been established for the evaluation of GM plants and derived products for the consumer. This means that the initial step of the food safety evaluation will be a CSA of the GM animal with its traditional counterpart, if available. This approach identifies potential differences between the GM animal-derived food products and its traditional counterpart as the first phase. The next phase is then to gain further insight into the toxicological and nutritional relevance of the detected differences. As every GM (founder) animal at this moment will have a different genetic constitution with respect to the integration of the genetic construct, the safety evaluation should be carried out on a case-by-case basis, even if the same genetic construct was used for the genetic modification. If homologous recombination will reduce the possibility of insertional effects in the future, it may become more feasible to come to more harmonised approaches for the safety assessment of GM animals and products thereof.

Application of the concept of substantial equivalence allows for analysis of intended and unintended alterations in the GMO and is central to the CSA. The intended changes can be evaluated with knowledge of: the nature and source of the gene construct used in the modification, the process of the genetic modification, in situ characterization of the genetic modification in the animal, information on animal breeding and propagation of the GM animal, the amino acid sequence of expressed product from the gene construct, the expression rates in different tissues of the expressed product, and traditional toxicological testing of the expressed product.

In addition the food safety evaluation should focus on possible unintended side-effects of the genetic modification. Unintended effects can be divided into insertional effects, related to the place of insertion of the gene fragment, and secondary effects, related to the nature of the transcription products of the introduced genes. Allergenicity represents a possible hazard that most likely is an unintended effect of the modification of a food animal. To detect any unintended effects a comparative phenotypical and compositional analysis of the new food organism and the conventional counterpart should be carried out. This should currently be based on the known key micro- and macronutrients and anti-nutrients, if applicable, and may in the future also be based on unbiased profiling of the GMO-derived food and traditional counterpart. Techniques for the profiling approach are now under development and can be divided into three subsections: genomics, proteomics, and metabolomics to screen for differences in the GM animal with relation to the gene transcription products, proteins and metabolites, respectively. At this moment, however, none of these techniques is yet validated and ready for routine use in risk assessment. If applied,

depending on the identity of differences detected further toxicological testing may be required to assess the safety and nutritional impact of the observed differences.

A few major differences can be seen when comparing the GM animal to the GM plant situation. Firstly, the numbers of GM animals derived from a single GM founder animal will in general be much lower compared to GM plant genetic modification events and numbers available in subsequent plant generations. This will result in less animals being available for the comparative safety assessment. This will have major influence on the reliability of the results of the comparative safety assessment. Knowledge on the natural background variation in animal tissue constituents will even be more important compared to the plant situation as it will be less feasible to obtain statistically significant results from analysis of the GM animals versus the conventional counterpart. An additional difference is the omnipresence of natural toxins in plant products and the very few cases of animal products that have proved to contain antinutritional substances for the consumer.

A third difference relates to the traceability systems that are (will be) available in the animal production sector and not yet in the plant sector. The presence of these traceability systems will make proper post-marketing surveillance systems much more feasible compared to the plant situation. Post-marketing surveillance studies may be advocated in the case of uncertainties relating to the nutritional or exposure assessment of the product or, in exceptional cases, to the potential allergenicity of the newly introduced protein(s). Other health- and nutrition-related aspects should be sufficiently dealt with during the pre-market assessment. Depending on the questions underlying a post-marketing study it may, however, be necessary in order to meet the goal, of the study to add information on the GM source animal to the label and inform the consumer of this additional label information.

Current food safety regulations for traditionally food (or food additives) are less stringent compared to those applied to GM foods. Pre-market safety assessment of GMO-derived foods must provide sufficient safety assurances, also in the case of GM animals or products derived thereof. The use of post-marketing surveillance as an instrument to gain information on the long-term effects of food either GMO derived or traditional should be further explored, but the requirement of routine application will entail large costs for limited amounts of information and should therefore be limited to exceptional cases.

The fact that the physiology of animals has major resemblance to our own physiology may in some aspects make the assessment of (GM) animal-derived food products 'easier'. On the other hand animal-derived food products form an important part of the human diet. Relatively small compositional changes may therefore have considerable effects on the nutritional status of the consumer. With increasing numbers of genetically altered plant- and animal derived food products the nutritional aspects, beside the safety aspects, will increasingly gain weight. The new developments in the area of GM animals further necessitate a harmonised approach to maintain our current standard for a safe and nutritious food supply in the light of growing numbers of different (GMO-derived) foods and food ingredients and increasingly complex food supply chains.

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