SCIENTIFIC OPINION

Draft Guidance on the risk assessment of food and feed from genetically modified animals including animal health and welfare aspects[1]

EFSA Panels on Genetically Modified Organisms (GMO) and

Animal Health and Welfare (AHAW)[2, 3]

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ABSTRACT

This draft document provides guidance for the risk assessment of food and feed containing, consisting or produced from genetically modified (GM) animals as well as for the health and welfare assessment of these animals, submitted within the framework of Regulation (EC) No 1829/2003 on GM food and feed. The assessment strategy seeks to deploy appropriate approaches to compare GM animals and derived food and feed with their respective comparators. The health status of a food/feed producing animal has traditionally been considered as an important indicator of the safety of derived foods/feed and therefore comparative analysis of the phenotypic characteristics of the GM animal with the traditionally bred animal, including health and physiological parameters is considered an important component in the risk assessment. The document addresses the molecular characterisation, which provides information on the structure and expression of the insert(s) and on the stability of the intended trait(s); the toxicological and allergenicity assessment, of the novel protein(s) as well as of the whole food derived from the GM animal; and the nutritional assessment to evaluate whether food and feed derived from a GM animal is as nutritious to humans and/or animals as traditionally bred animals. This guidance document also addresses the scientific requirements for the assessment of health and welfare of GM animals bred for food and feed use. The assessment is made in terms of the effective functioning of their body systems e.g. immuno-competence and resistance to various stressors in a given environment. The document does not cover the environmental risk assessment of GM animals which will be addressed in a stand alone guidance under development by the EFSA GMO Panel.

[1] On request from European Commission, Question No EFSA-Q-2007-069, endorsed for public consultation on 16 June 2011 and 7 July 2011. Available from http://www.efsa.europa.eu/en/calls/consultations.htm

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KEY WORDS

GM animals, guidance, applications, Regulation (EC) No 1829/2003, food safety, feed safety, risk assessment, comparative approach, animal health and welfare assessment

SUMMARY

The European Commission asked the EFSA Panel on Genetically Modified Organisms (EFSA GMO Panel) and the EFSA Panel on Animal Health and Welfare (AHAW Panel) to provide guidance for the risk assessment of food and feed containing, consisting or produced from genetically modified (GM) animals, submitted within the framework of Regulation (EC) No. 1829/2003 on GM food and feed. The present version of this draft guidance document was endorsed by both the GMO and AHAW Panel, on 7 July 2011 and 16 June 2011 respectively, in order to be launched for public consultation. The document does not cover the environmental risk assessment of GM animals which will be addressed in stand-alone environmental risk assessment (ERA) guidance documents developed by the EFSA GMO Panel.

The risk and health and welfare assessment strategy for GM animals and derived food and feed proposed seeks to deploy appropriate approaches to compare GM animals and derived food and feed with their respective comparators. In the case of risk assessment, the underlying assumption of this comparative assessment approach for GM animals is that traditionally bred animals have a history of safe consumption as food and feed for the average consumer or animal to which the animal-derived products are fed. These traditionally bred animals can serve as a baseline for the food/feed safety assessment of GM animals or products from GM animals and their welfare.

The document outlines the principles of the risk assessment of GM animals and derived food and feed, providing an overview of the comparative approach and definitions of the different steps and objectives of the risk assessment process. The health status of a food/feed producing animal has traditionally been considered as an important indicator of the safety of derived foods/feed. Therefore, the most important component in the risk assessment, addressed in this document, is an extensive comparative analysis of the phenotypic characteristics of the GM animal, including health and physiological parameters. The document addresses the details of the other components as well: the molecular characterisation, which provides information on the structure and expression of the insert(s) and on the stability of the intended trait(s); the toxicological assessment, which addresses the impact of biologically relevant change(s) in the GM animal and/or derived food and feed resulting from the genetic modification; the assessment of potential allergenicity, of the novel protein(s) as well as of the whole food derived from the GM animal; and the nutritional assessment to evaluate whether food and feed derived from a GM animal is as nutritious to humans and/or animals as traditionally bred animals.

In general, this guidance document explains why it is not valid for the safety assessment of food and feed products derived from GM animals with non-heritable traits, although some aspects may also be applicable in these cases.

This guidance document also addresses the scientific requirements for the assessment of health and welfare of GM animals bred for food and feed use. The question to be answered is whether there are problems in the health and welfare of GM animals. The assessment is made in terms of the effective functioning of their body systems e.g. immuno-competence and resistance to various stressors in a given environment. More precise information may be gained by comparing health and welfare of GM animals with those of their comparators. Where no comparator can be identified, an assessment of health and welfare of the GM animal itself will be considered.

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BACKGROUND AS PROVIDED BY EFSA

The genetically modified (GM) organisms that have been introduced into the environment and the food and feed chain so far are mostly genetically modified plants and micro-organisms. However, this situation may change in the future as genetically modified animals with added value in the food and other markets are under commercial development.

In that context, the Codex Alimentarius Commission has already adopted Guidelines addressing the safety and nutritional aspects of foods consisting of, or derived from, animals that have a history of safe use as sources of food, and that have been modified by modern biotechnology to exhibit new or altered expression of traits (Codex Alimentarius, 2008). EFSA provided scientific and technical advice to the Commission with respect to the preparation of these guidelines.

Following a request of the European Commission (DG Environment and DG SANCO, ENV.B3 D(2007) 2004, DG SANCO, SANCOJK/dj D5 D(2010) 450066), EFSA initiated the development of Guidance for the safety assessment of GM animals that would address both food and feed and environmental safety and animal health and welfare issues related to GM animals.

To address the request of the European Commission with respect to environmental safety issues the following work plan and timeline of deliverables were foreseen:

- By the end of 2008 an external open call on GM fish was launched, which was followed in the beginning of 2009 by open calls on GM insects, GM birds and GM mammals. By the beginning of 2011, external contractors provided deliverables in the form of separate reports in which environmental risk assessment criteria for GM fish, insects, birds and mammals are discussed;

- Within Working Groups (WG) of the EFSA GMO Panel on environmental risk assessment of GM animals *de novo* guidance document for applicants is being developed on the environmental safety assessment of GM animals using background information from the reports provided by external contractors. Several seminars and workshops may be implemented to support this work. Due to the broad scope, the complexity and novelty of the subject, the intended final deliverable will be available by mid 2012.

Regarding food and feed safety and animal health and welfare issues, the following work plan and timeline of deliverables were followed:

- At the beginning of 2009, EFSA implemented a WG of the GMO Panel on the molecular characterization and the food and feed safety assessment of products derived from GM animals. In 2010, EFSA implemented a WG of the AHAW Panel to deal with animal health and welfare aspects of GM animals. By the end of 2011 on the basis of deliberations in these WGs on GM animals and on existing guidance documents on the subject (e.g., Codex Alimentarius Commission guidelines) as well as any other relevant background information available in the EU and elsewhere and comments received during the public consultation, the EFSA GMO and AHAW Panel will deliver the guidance on the safety assessment of food and feed products from GM animals including animal health and welfare issues regarding these animals.

The present draft document provides detailed guidance for the safety assessment of genetically modified animal derived food and/or feed containing, consisting of or produced from these animals prepared, endorsed by the EFSA GMO Panel and guidance on health and welfare assessment of these animals prepared, endorsed by the EFSA AHAW Panel and is published for public consultation.

The EFSA GMO and AHAW Panels will consider all comments received before preparing a revised guidance document by the end of 2011. The GMO and AHAW Panels will not consider issues related

to risk management (traceability, labelling, coexistence). Ethical and socio-economic issues are also outside the remit of the Panels. EFSA will regularly review this guidance in the light of experience gained, technological progress and scientific developments. By establishing a harmonised framework for risk assessment, this document should provide useful guidance both for applicants and for risk assessors.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION (DG SANCO AND DG ENVIRONMENT)

On 13 February, 2007, EFSA received a mandate from the European Commission (DG Environment and DG SANCO, ENV.B3 D(2007) 2004) with the request to develop, building on work done in the context of Codex Alimentarius Commission, a guideline on the safety evaluation of GM animals that would address both food/feed and environmental safety of modern biotechnology . In these letters, EFSA presented its work plan informing the European Commission that the environmental safety and the safety assessment of food and feed products derived from GM animals will be addressed in parallel (Ref. CGL/SR/DC-SM/cz(2007) 2269682; Ref. CGL/PB/SM-YD/md(2008) 3187837; Ref. CGL/PB/SM-YD/md (2008) 3187581; Ref.RM/PB/EW/shv/lg(2009)3701902).

On 25 March, 2010, the European Commission (DG SANCO, SANCOJK/dj D5 D(2010) 450066) requested that the guidance for the human health and environmental risk assessment of GM animals, should be revised to include animal health and welfare aspects impacting on food and feed safety, in order to produce a final comprehensive package of information on the use of GM animals.

ASSESSMENT

A. INTRODUCTION

The working definition for GM animals in this document is animals whose genetic material has been altered in a **heritable** way through the techniques of genetic modification included in Annex I*A, part 1 of the* Directive 2001/18/EC and referred to in Article 2(2), all of which allow for the combination and/or introduction of genetic material into host animal genomes in a way that does not occur naturally by mating and/or natural recombination. This definition does not preclude the possibility that this guidance document can also be applied to animals produced by other (existing or novel) techniques that require a safety assessment.

Developments and scientific activities in the area of GM animals indicate that future applications may include traits related to: i) more efficient or increased production of food/feed of animal origin, ii) enhanced nutritional characteristics and wholesomeness of these foods, iii) lower emissions to the environment and iv) the improvement of the health characteristics of the GM animal, including better resistance to abiotic stressors and pathogens, improved fertility and lower mortality. Other applications may be used for ornamentation and production of pharmaceuticals and biomaterials or xeno-transplantation, but in these cases, if the resulting animals are solely developed for this particular goal, the market introduction of these animals for food and feed production would be inadvertent.

In animal production, standard animal hygiene procedures in combination with other quality control aspects during the production phase or, for instance, in the slaughterhouse or in the dairy, have been implemented to guarantee the quality of the resulting animal product, especially in terms of food safety aspects (hygiene for food of animal origin[4]). Specific procedures for safety assessment are necessary for the various animal species (e.g. cattle, sheep, goat, pigs, poultry, fish and their products) and categories (e.g. cattle for milk or meat; poultry for laying or meat).

These standard procedures will form the basis for the safety assessment of GM animals and derived food and feed products. The aim of the safety assessment is to ascertain that the new GM animal derived food and feed products are at least as safe as comparable products that are already on the market. Where no comparator(s) can be identified, a comparative safety assessment cannot be made and a comprehensive safety and nutritional assessment of the GM animal-derived food/feed *per se* should be carried out.

This guidance document also addresses the scientific requirements for the assessment of health and welfare of GM animals bred for food and feed use. Such an assessment should be made in terms of the effective functioning of their body systems e.g. immuno-competence and resistance to various stressors in a given environment. Standard animal production and husbandry procedures are being implemented to promote animal health by preventing and reducing the incidence of animal diseases (Community Animal Health Policy – New strategy 2007-2013). Similarly, EU minimum standards set up for the protection of animals bred or kept for farming purposes (Council Directive 98/58/EC) are being implemented by EU directives on the welfare of calves (EU Directive 91/629/EEC as amended by Directive 97/2/EC and Commission Decision 97/182/EC), pigs (EU Directive 91/630/EEC as amended by Directive 2001/88/EC and Directive 2001/93/EC), laying hens (EU Directive 99/74/EC).

B. GENERAL PRINCIPLES GOVERNING THE COMPARATIVE APPROACH FOR THE RISK ASSESSMENT OF FOOD/FEED FROM GM ANIMALS INCLUDING ANIMAL HEALTH AND WELFARE ASPECTS.

This guidance document addresses the assessment of the safety for humans and animals of GM animal derived food and feed that may be placed on the market within the framework of the Regulation (EC) No 1829/2003 including guidance on animal health and welfare aspects of GM animals. Guidance for the deliberate release of GM animals into environment will be addressed separately in line with the

[4] http://europa.eu/legislation_summaries/food_safety/veterinary_checks_and_food_hygiene/f84002_en.htm

Directive 2001/18/EC. The scope of this document includes primarily GM animals with new, heritable traits to be placed on the market and details the scientific requirements for the risk assessment of food and feed derived from these GM animals. GM food and feed derived from GM animals with non-heritable traits are not covered by this guidance. In general, it will be difficult to develop a structured food and feed safety strategy for this latter type of products and therefore a case-by-case approach should be followed with respect to their food and feed safety assessment (see Section 2.4.1). Ethics and socio-economic aspects are not addressed in this guidance document.

Animals that were taken into consideration when drafting the document include all husbandry animals, and fish, as well as crustaceans and molluscs. Insects and other invertebrates were not taken into account, with the exception of honey bees that are used in agricultural practice. It is only possible to develop *general* guidance for the large diversity of different type of animals being bred for food and feed use, whilst specific requirements for the safety assessment of food and feed derived from each GM animal species, and indeed GM animal line, and associated AHAW aspects, will have to be determined on a case-by-case basis.

In general, parallels can be drawn to the safety assessment of GM plants, for which guidance has already been developed (EFSA, 2011a). The risk assessment strategy for GMOs seeks to deploy appropriate methods and approaches to compare the GMO and derived products with their comparator(s). The underlying assumption of this comparative assessment approach for GM animals is that traditionally bred animals have a history of safe consumption as food and feed for the average consumer or animal to which the animal-derived products are fed. This equally applies to AHAW assessment. These traditionally bred animals can serve as a baseline for the food/feed safety assessment of GM animals or products from GM animals and their health and welfare. To this end the concept of substantial equivalence was developed by WHO (WHO, 1991) and OECD (OECD, 1993) and elaborated by WHO/FAO (WHO/FAO, 2000) for the assessment of the food safety of GMOs. This concept is also taken into consideration in the "guideline for the conduct of food safety assessment of foods derived from recombinant-DNA animals" prepared in the context of Codex Alimentarius (Codex Alimentarius, 2008).

The first step of the food and feed safety assessment of GM animals and derived food and feed products will be a comprehensive molecular characterisation of the GM organisms in question with the objectives to characterise the intended effect of the genetic modification and to identify potential unintended effects. Subsequently, a comparative safety assessment (Kok and Kuiper, 2003) of the phenotypic and compositional characteristics should be performed i.e. the practical implementation of the concept of substantial equivalence is essential. Application of this concept serves the purpose of identifying similarities and differences between the GM animal-derived food/feed and its conventional counterpart(s) [and/or other non-GM comparator(s)]. The outcome of this comparative analysis will further structure the subsequent assessment procedure, which may include further specific safety and nutritional testing. This approach should provide evidence on whether or not the GM animal-derived food/feed is as safe as the comparator(s).

The implementation of this comparative approach will by default be different in the case of GM animals compared with to GM plants. The health and welfare status of a food/feed producing animal has traditionally been considered as an important indicator of the safety of derived foods/feed. The practice of only allowing animals with known and acceptable health and welfare status to enter the human/animal food/feed supply is considered to be an essential step to ensuring safe food/feed. This approach will also be used for the safety assessment of GM animals.

Thus, the most important step in the approach to the case of GM animals is an extensive comparative analysis of the phenotypic characteristics including health and physiological parameters of the organisms in question. In addition, in most cases, this phenotypical analysis will be accompanied by a comparative biochemical composition analysis of relevant tissues/organs/fluids and of the composition of derived food and feed. Such comparisons should be made between the GM animal or products from such animals (e.g. milk, eggs, honey) and its comparators.

The risk assessment then focuses on food/feed safety issues and the nutritional impact issues of any identified differences resulting from intended or unintended effects. Where no comparator(s) can be identified, a comparative safety assessment cannot be made and a comprehensive safety and nutritional assessment of the GM animal-derived food/feed per se should be carried out. Similarly, for the assessment of the health and welfare of a GM animal, where no comparator can be identified an assessment of the health and welfare of the GM animal itself will be considered.

Intended and unintended effects

Any type of genetic modification may result in intended and possibly unintended effects in the modified organism. The risk assessment is focussed on the identification and characterisation of such effects with respect to a possible impact on human and animal health as such or following the consumption of GM animal-derived food or feed products.

Intended effects are those that are expected to occur from the introduction of the genetic modification(s) in question and which fulfil the original objectives of the genetic modification process. Unintended effects are considered to be consistent differences between the GM animal and its comparator(s), which go beyond the primary intended effect(s) of the genetic modification.

Alterations in health and welfare of a GM animal may be identified through clinical observations and examinations to detect deviations from normal health and behaviour. Alterations in the phenotype may be identified through a comparative analysis of for example, visual characteristics, growth performance, developmental characteristics, digestive and reproductive capacity, disease resistance and health (including immunological) parameters. Alterations in the composition of specific parts of a GM animal or in products such as milk and eggs compared with the same parts and products of its comparator(s), may be identified by measurements of a set of constituents which represent components of important metabolic pathways in the organism, taking into account natural variation. The components should include key nutrients, i.e. proximates, macronutrients and micronutrients, and bioactive compounds, if relevant. Analytical methods used should meet specific quality and validation criteria.

Therefore, to identify both intended and unintended effects, comparative phenotypic and targeted compositional analysis should be carried out when applicable. Identified differences between the GM animals and their comparator(s) should be assessed with respect to their potential safety, welfare and nutritional impact.

FOOD AND FEED RISK ASSESSMENT

1. The objectives of the different steps of the risk assessment procedure for GM animals and derived food/feed and issues to be considered

1.1. Objectives of the different steps of the risk assessment

1.1.1. Hazard identification

Hazard identification is the identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food and feed or group of foods and feeds (Codex Alimentarius, 2007). In the case of GM animals used to produce food and feed hazard identification should include those capable of causing adverse health to the GM animals themselves. Hazard identification is the first step in risk assessment and in case of GM-animal derived food/feed products is focused on the identification of differences between the GM animal and its comparator(s) by using the molecular characterisation and the comparative analysis of compositional and phenotypic characteristics. Identification of differences will determine the additional studies required to characterise these differences with respect to possible impact of GM animal-derived food and feed products on human and animal health.

1.1.2. Hazard characterisation

Hazard characterisation is defined as the qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents which may be present in food/feed. In principle a dose response assessment should be performed. This step is focussed on a possible quantification of the toxicological or nutritional potential of identified differences between the GM animal and derived food/feed and the comparator(s).

Useful information with respect to the hazard characterisation may be obtained from studies using (laboratory) animals. An appropriate test model (animal species) and suitable test material and test design should be used in order to generate data identifying the onset of adverse effects, and a possible dose-response relationship.

1.1.3. Exposure assessment

The aim of the exposure assessment is the quantitative estimation of the likely exposure of humans and animals to the GM animal-derived products including any new constituents. With regard to humans and animals, an exposure assessment characterises the nature and size of the populations exposed to a source and the magnitude, frequency and duration of that exposure. It is necessary to identify every significant source of exposure. In particular it is of interest to establish whether the intake of the GM animal-derived products and new constituents is expected to differ from that of the conventional product which it may replace. In this respect specific attention will be paid to GM-animal-derived food/feed which is aimed at modifying nutritional quality. This category of GM-animal-derived food/feed may require post-market monitoring to confirm the conclusion of the exposure assessment.

1.1.4. Risk characterisation

Risk characterisation is defined as 'the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment' (Codex Alimentarius, 2007).

1.2. Elements to be considered for the risk assessment of GM animal-derived food/feed products

The following elements should be considered for the risk assessment of GM animals and products:

a. the characteristics of the donor and recipient organisms;

b. the genetic modification and its functional consequences in the GM animal, intended as well as unintended;

c. the phenotypic characteristics of the GM animal including health and physiology;

d. the compositional characteristics of relevant animal tissues, organs, fluids and/or derived products;

e. the influence of processing on the characteristics of the GM animal-derived food and feed;

f. the potential toxicity, bioactivity and allergenicity of gene products, metabolites and the derived GM animal food and feed;

g. the potential for nutritional impact of GM animal-derived food and feed;

h. the potential for changes in dietary intake as a result of the introduction of the GM animal-derived food or feed.

2. Information required for risk assessment of GM animal- derived food and feed

2.1. Hazard identification and characterisation

2.1.1. Information relating to the recipient or (where appropriate) parental animals

The applicant should provide the following information.

a. Complete names; (a) family, (b) genus, (c) species, (d) subspecies, (e) breeding line or strain, (f) common name.

b. Geographical distribution and husbandry of the animal(s), including its distribution and/or husbandry in Europe, and information on natural predators, parasites, competitors, and symbionts, where appropriate.

c. Information on the recipient or parental animals relevant to their safety assessment, including information on any known toxicity and allergenicity of constituents and susceptibility to pathogens.

d. Data on the past and present use of the recipient organism. This information should include the history of safe consumption as food or feed, information on how the animal is typically bred, reared, transported and housed and whether special processing is required for safe consumption of derived food or feed. In addition, the normal role of the animal-derived product in the diet should be described (e.g. which part of the animal is used as a food/feed source, whether its consumption is important in particular subgroups of the population, which macro- and/or micro-nutrients in the product make nutritionally significant contributions to the diet).

e. Information on the ploidy of animal(s).

2.1.2. Molecular Characterisation

The applicant should provide sufficient information on the genetic modification to identify the nucleic acid intended for transformation and related vector sequences potentially delivered to the recipient animal, and to characterise the DNA actually inserted in the animal and expression and stability of the intended trait(s).

2.1.2.1. Information relating to the genetic modification

2.1.2.1.1. Description of the methods used for the genetic modification

The applicant should provide information on the following:

a. the method and the steps of genetic alteration including relevant bibliographic references, the production method of the vector/fragment used for transformation and methods and criteria used for selection;

b. the recipient animal material;

c. the helper plasmids, if used during the genetic transformation process, including a detailed description of the *cis/trans* acting system;

d. the purity of the preparation containing the construct prior to introduction into recipient animals or cells;

e. when microorganisms are used, additional information on pathogenicity to humans or the animal and the relationship to known human or animal pathogens.

2.1.2.1.2. Nature and source of vector(s) used for transformation

The applicant should provide the following information:

a. a table identifying each component of the plasmid/vector, including the region intended for insertion, its size, its origin and its intended function;

b. a physical map of the functional elements and other plasmid/vector components together with the relevant information needed for the interpretation of the molecular analyses (e.g. restriction sites, the position of primers used in PCR, location of probes used in Southern analysis). The region intended for insertion should be clearly indicated;

c. description of the technologies which remove part of the insert, if used;

d. description of technologies which limits the chance of mobilization of the insert, if used;

e. description of the technology to drive the trait through the population, if used.

2.1.2.1.3. Source and characterization of nucleic acid used intended to be inserted

The applicant should provide information on the donor organism(s) and on the nucleic acid sequence(s) intended to be inserted in order to determine whether the nature of the donor organism(s) or the nucleic acid sequence(s) may trigger any safety issue. Information regarding the function of the nucleic acid region(s) intended for insertion should comprise the following elements:

a. the complete nucleotide sequence intended to be inserted, including information on any deliberate alteration(s) to the corresponding sequence(s) in the donor organism (s) and on the techniques used for producing these changes (site-directed mutagenesis, gene shuffling, production of synthetic nucleotide sequences);

b. in case of synthetic nucleotide sequences with no gene counterpart in existing organisms, information on the design and assembly of the synthetic nucleotide sequences introduced.

Information regarding each donor organism should comprise its taxonomic classification and its history of use regarding food and feed safety.

Information regarding the DNA region(s) intended for insertion should comprise the following elements:

- history of safe consumption of the gene product(s) arising from the regions intended for insertion;
- data on the possible relationship of the gene products with known toxins, anti-nutrients, allergens and other bioactive compounds with potential adverse health effects;
- if viral vectors, transposons or known zoonotic organisms have been used, information on their natural hosts, target organs, transmission mode and stability, pathogenicity, and potential for recombination with endogenous or exogenous pathogens, e.g. viruses;
- information related to the occurrence of transposons or viruses in the recipient animals which are related to the construct used and which might be able to provide *trans* acting transposase or act as helper virus.

2.1.2.2. Information relating to the GM animal

2.1.2.2.1. General description of the trait(s) and characteristics which have been introduced or modified

The introduced trait(s) and the resulting changes to the phenotype including metabolism of the GM animal should be described.

Information provided should also include a description of the generation of the GM animal to be marketed from the initial GM founder animals including the breeding strategy and information on whether the initial GM animal was mosaic.

2.1.2.2.2. Information on the sequences actually inserted/deleted or altered

The applicant should provide the following information:

a. the size and copy number of all detectable inserts, both complete and partial. The analysis should cover sequences that could be inserted into the host animal, such as any parts of the plasmid/vector or any carrier or foreign DNA. The analysis should span the entire transgenic locus/loci as well as flanking sequences;

b. the organisation and sequence of the inserted genetic material at each insertion site;

c. size and function of the deleted/modified region(s), in the case of intended deletion/modification(s);

d. sub-cellular location(s) of insert(s) (integrated in nuclear or mitochondrial genome, or maintained in a non-integrated form) and methods for its determination;

e. sequence information for both 5' and 3' flanking regions at each insertion site, with the aim of identifying interruptions of known genes, presence of genes in the vicinity of the insert and possible deletions in the recipient DNA. Bioinformatics analysis should be conducted using up-to-date databases with the aim of performing both intraspecies and interspecies homology searches. The characteristics and versions of the databases must be provided;

f. open reading frames (ORFs)[5] present within the insert and spanning the junctions. The ORFs should be analysed between stop codons, not limiting their lengths. Bioinformatics analyses should be conducted to investigate possible similarities with known toxins or allergens using up-to-date databases. In case of allergens, it is recommended to use different similarity searching strategies in combination with more than one database to obtain a higher degree of confidence in the outcome. The characteristics and versions of the databases should be provided;

g. depending on the information gathered, further analyses may be needed to complete the risk assessment.

2.1.2.2.3. Information on the expression of the inserted/modified sequence

The applicant should provide information to demonstrate whether the inserted/modified sequence results in the intended change(s) at the protein, RNA and/or metabolite level(s). In many cases the intended genetic modification will lead to the expression of new protein(s), therefore protein expression data will be the most relevant. In other cases (e.g. silencing approaches or where biochemical pathways have been intentionally modified) the analysis of specific RNA(s) or

[5] **O**pen **R**eading **F**rames should be defined as any nucleotide sequence that contains a string of codons that is uninterrupted by the presence of a stop codon in the same reading frame.

metabolite(s) may be the most informative. The analysis of risk associated with a change of protein and metabolite level(s) is covered in Section 2.1.3.4.

Data should be related to the conditions in which the animals are bred, fed and reared. Information should be provided on tissues of the animal where the inserted/modified sequence is expressed and tissues where the expressed products are localised. Data on expression levels from those parts of the animal that are used for food/feed purposes and relevant to the scope of the application are considered necessary in all cases. Where tissue-specificity is intended, information on expression and presence of expression products in different tissues, fluids and other compartments relevant for the risk assessment. The requirement for information on developmental expression should be considered on a case-by-case basis taking into account the promoter used, the intended effect of the modification and the scope of the application.

If the genetic modification results in newly expressed protein(s) and where the analytical method has been shown to be specific, no comparator is necessary. In other cases, such as silencing approaches or where the modification is intended to modify the levels of specific proteins or metabolites, the experimental design should include a non-GM comparator in order to compare the levels of relevant endogenous RNA(s), protein(s) and/or specific metabolite(s). Where appropriate, the impact of specific treatments linked to the trait should also be assessed.

The applicant should provide the following information:

a. description of the method(s) used for expression analyses. The specificity of the methods used should be demonstrated;

b. the range of concentrations of newly produced proteins or endogenous animal proteins deliberately modified in the GM food(s) and feed(s) to be placed on the market, together with the raw datasets;

c. when justified by the nature of the insert (e.g. gene silencing through RNA interference), information on the expression of targeted gene(s) and on possible effects to other endogenous genes (to be selected by *in silico* analysis) should be provided;

d. on a case-by-case basis expression of genes situated near the inserted/modified sequence.

For applications which include the use of living organisms in the scope, the above requirements for food, feed, import and processing should be met (including trial design). Depending on the trait and scope of the application, information may also be required for the assessment of impacts on other organisms. In such cases, information on expression in various parts of the animal during development is required (see the ERA guidance documents to be developed). Data should be related to the conditions in which the animals are bred, fed and reared in Europe.

2.1.2.2.4. Genetic stability of the inserted/modified sequence and phenotypic stability of the GM animal

Information should be provided to demonstrate the genetic stability of the locus/loci altered by the genetic modification and the phenotypic stability and inheritance pattern(s) of the introduced/modified trait(s).

Applicant should provide data on the stability of the introduced/modified nucleotide sequences and associated phenotypes in the offspring for at least two sexual generations. The source of the material, the sampling design and the number of animals used for the analysis should be specified and clearly indicated on the breeding diagram.

The applicant should also consider the safety implications of the loss of function of specific genes from multi-gene expression cassettes after their insertion into an animal.

2.1.2.3. Conclusions of the molecular characterisation

- The molecular characterisation should provide data on the structure of the genetic modification, expression and stability of the intended trait(s) and the applicant should indicate whether it raises safety concerns.

- It should be specifically indicated whether the molecular characterisation of the genetic modification(s), raises safety concerns with regard to the potential production of proteins/products other than those intended.

- The molecular characterisation should specifically aim to identify whether the genetic modification(s) raise(s) any issues regarding the potential for mobilization of the insert and producing new toxins or allergens.

- The potential unintended changes identified in this section should be addressed in the relevant complementary part(s) of the safety assessment.

2.1.3. Comparative analysis

The comparative analysis of composition and phenotypic characteristics including health status and physiology represents, together with the molecular characterisation, the starting point to structure and conduct the risk assessment of GM animals and their derived food and feed products. It aims at:

- identifying similarities and differences between the GM animal and its comparator(s) in phenotypic characteristics (intended and unintended alterations), including data on health status, physiology and reproduction; and

- identifying similarities and differences in composition between the GM-animal derived food/feed and its comparator(s).

Where no appropriate comparator(s) can be identified, a comparative safety and welfare assessment cannot be made, and thus a comprehensive safety and nutritional assessment of the products from the GM animal *per se* should be carried out. This would be the case where the GM animal is not closely related to an animal with a history of safe consumption as food and feed or where a specific trait or specific traits are introduced with the intention of bringing multiple substantial changes in the composition of the animal-derived food and feed products and the GM animal itself.

2.1.3.1. Choice of the comparator(s)6

Regulation (EC) No 1829/2003 defines a conventional counterpart as '*a similar food or feed produced without the help of genetic modification and for which there is a well-established history of safe use*' (Art. 2.12). The EFSA GMO Panel recommends the use of the term "conventional counterpart" only when referring to the comparator(s) with a genetic background that is as close as possible to that of the GM animal and have a history of safe consumption as food and feed.

Information on the breeding scheme (pedigree) applied to both the GM animal and the comparator(s) and justification for the use of the selected comparator(s) should be provided. The term "comparator" should be used in all other cases, i.e. cases in which the comparative assessment includes genotypes which do not fit with the definition of conventional counterpart as provided above.

2.1.3.2. Animal trials for comparative analysis - experimental design & statistical analysis

Production of animal material for comparative analysis is performed in order to assess similarities and differences between the GM animal and its appropriate comparator(s). For any particular endpoint,

[6] As defined in Article 2(12) of Regulation (EC) No 1829/2003, "conventional counterpart" means a similar food or feed produced without the help of genetic modification and for which there is a well-established history of safe use.

there should be a difference test between the GM animal and the conventional counterpart. Where there is sufficient and appropriate animal material (see below), an equivalence test with a null-hypothesis of non-equivalence may be applied, using the methodology described in EFSA, 2010a. Equivalence should be considered as the absence of differences other than those expected naturally through variation between traditionally-bred animals with a history of safe consumption as food and feed. Hazard characterization should be used to place identified differences into biological context. Such differences may point to biological changes caused by the genetic modification which should subsequently be further assessed for their toxicological and/or nutritional relevance.

For certain animal species it is recognized that the available number of samples may be limited, so it may be impractical, or even impossible, to include enough traditionally bred animals with a history of safe consumption as food and feed in the experiment to obtain a sufficiently good estimate of variation from concurrent data to set the appropriate equivalence limits (EFSA, 2010a). Only if concurrent data is unavailable should consideration be given to the setting of equivalence limits using data from previous experiments, historical data from appropriate compositional databases, or data from the scientific literature and research reports, but the validity of such data should then fully justified.

2.1.3.2.1. Principles of experimental design

General recommendations for experimental design may be found in (Cochran and Cox, 1957). For animal experiments the principles of experimental design should be followed from ILAR Journal, 2002, particularly the papers of (Festing and Altman, 2002) and (Johnson and Besselsen, 2002). In the statistical theory of the design of experiments the causes that are thought to contribute towards the value of the variables measured by the experiment are often termed 'factors', especially when they are controllable in the experiment and take a limited number (termed 'levels') of different values. 'Treatment factors' are those of primary interest and relate directly to the questions the experiment is designed to address. For example, experiments to inform risk assessment might have a treatment factor with two levels: a GM animal and a conventionally-reared counterpart. 'Blocking' is the arranging of experimental units in groups (blocks) that are similar to one another. Typically, a 'blocking factor' is a source of variability that is not of primary interest to the experimenter. An example of a blocking factor might be the husbandry conditions that the animals are kept under. Usually an experimental unit is represented by a single animal. However, these will often be kept within a group of animals (as for poultry and fish) and one of the blocking factors will be the housings for those groups (such as cages, tank and pens). The blocking factors in the design should be chosen to be appropriate for the experimental units and should help to maximise the statistical power of the experiment to detect treatment effects (Richardson et al., 2004). The experimental design should ensure that the principal comparisons of interest are performed with a sufficient number of degrees of freedom for the experimental error. If animals are kept in groups then computerized individual feeding techniques when feasible should be established to enable feed conversion rates to be calculated. It is important to keep animals that are being compared under the same (conventional) conditions. On a case-by-case basis, it should be considered whether to include different husbandry practices as a blocking factor(s) within the experimental design, to assess whether the effects of the genetic modification are influenced by such practices. Similarly, and on a case-by-case basis, it should be considered whether to include in the design other factors where appropriate, such as age, sex, parity, lactation, laying cycle, etc. The chosen experimental design and husbandry conditions should ensure that any confounding of the main effect of GM versus comparator(s) with other factors is minimized. The applicant should explain the choice of conditions to rear the animals, as well as other distinctive factors included, or excluded, in the experimental design.

Certain genetic modifications may result in husbandry conditions that are appropriate for the GM animals but being suboptimal or non-permissive for the conventional counterpart, and vice-versa. An example of this is cold-tolerant GM fish that express antifreeze proteins; these can be farmed at locations where some comparator(s) cannot be reared. In such cases, the conditions for rearing the GM animal and its counterpart should be as closely representative as possible of typical commercial practice, with conditions that approximate as closely as possible to those that produce a food/feed

product with a history of safe consumption, but still under the same conditions for both types of animal (GM animal and counterpart). Another example is rapidly-growing GM animals that reach maturity or marketable sizes earlier than their counterparts. In that case, a counterpart with the same size or weight rather than the same age may have to be chosen in order to represent an appropriate comparator, especially for the developmental stage at which they are marketed as ready for consumption. It is recommended that the experimental design represents a range of husbandry conditions to reflect those commonly used in Europe, including feeding regimes suitable for the GM and its comparator, but such that both the GM and comparator can be reared without unacceptable risk of mortality or adverse health and welfare issues. Both GM and comparator must be reared under those selected conditions.

The selection of appropriate comparator animals may be aided by considering genetic distance and pedigree and it should be ensured that between-animal variation is representative of the genetic variability present in populations of traditionally-bred animals of that species (Taylor, 1985). All test materials, the GM animal and any comparator(s), should be fully and properly randomized to the experimental units. Care should be taken to choose an experimental design that does not suffer unduly from unexpected loss of animals during the trial.

It is important to consider whether the use of extra comparators might help to place any effects of the genetic modification into context by allowing the assessment of whether any husbandry or other management practices influence the expression of the studied endpoints (EFSA, 2010).

For each study the applicant should ensure that the design is such that the main effect for the difference test (assuming there are no interactions between the main effect and other factors) has sufficient statistical power to provide a reasonable level of credible evidence and should seek to attain as close to 80% power for a 5% size of test as is feasible. For each study, the applicant should state explicitly the size of the treatment effect that the study is designed to detect for the standard difference test and provide an analysis that estimates the statistical power for each difference test on each endpoint, based on the stated effect size and assuming a 5% type I error rate. The analysis should be done at the planning stage of the study. The power analysis should use only information verifiable as available prior to the study; under no circumstances should data from the study itself be used.

2.1.3.2.2. Principles of statistical analysis of the data

Recommended procedures for statistical analysis involving difference and equivalence tests are discussed in (EFSA, 2010a); applicants should follow if possible the recommendation to calculate a confidence interval for each endpoint and to display all endpoints on the same graph(s). Care must be taken that the analysis is appropriate if the experimental unit is a group rather than an individual animal.

It is recommended that the applicant prepares a statistical analysis protocol for each study (see Perry et al., 2009 for a checklist). Data transformation should be considered to ensure normality and to provide an appropriate scale on which statistical effects are additive. If an equivalence test is done, its form should follow that termed ‘average equivalence’ in the sense used in the drug testing literature (Wellek, 2002). For studies that use extra comparators, the analysis should encompass separate difference tests (between the GM and each of its different comparators) and separate equivalence tests (between the GM and each of its different comparators), and these should be reported similarly.

Consideration should be given to the possible need to analyse males and females separately, where appropriate. Allowance should be made, usually through analyses involving statistical mixed models, for possible temporal autocorrelation when repeated measurements are taken from the same animals. Rejection of outliers should only be done for biological reasons, which should be explained why they are so categorised. Statistical tests for outliers should never be applied for automatic outlier removal. Any discarded outliers should be identified and analyses should be provided both with and without outliers.

2.1.3.2.3. Information required

The applicant should provide any data analysed and all programming code used for analyses and simulation, in an editable form, together with a detailed description of the statistical model used, listing any assumptions made, the factors used and the interactions tested. In addition, the applicant should provide a table or graph categorised by the factors in the experimental design, giving, for each (possibly transformed) endpoint, the means and standard errors of means of the GM animal and its comparator(s) , and any other test material, where applicable. Laboratory analytical methods should be documented and limits of detection provided. The husbandry conditions selected, including the composition of feed used during field trials, should be comprehensively described and fully justified. The use of all veterinary drugs should be described fully and the reason for their administration.

The applicant should list explicitly all the questions that each trial is designed to address, in words. In addition, each of these questions should be re-stated in formal terms, in the form of the precise null hypothesis that was tested to answer the question. Any departures from the experimental design and statistical analysis protocols specified prior to the study and referred to above should be specified.

For a size of difference test of α%, a proportion of α in 100 of these tests is expected to yield a significant result by chance alone. However, the applicant should report and discuss all significant differences observed between the GMO, its comparator(s) and, where applicable, any other test material(s), focussing on the biological relevance of these differences within the context of risk characterisation. If statistical interactions are found, the possible reasons for their existence and the implications for the inferences drawn from the trials should be discussed.

A full and explicit justification should be given for the choice of animals, including any traditionally-bred animals with a history of safe consumption as food and feed employed to test equivalence and/or any extra comparators included within the design. When an equivalence test is done but concurrent data are not used to set the equivalence limits, detailed and explicit justification should be given concerning the derivation and validity of these limits.

2.1.3.3. Comparative analysis of phenotypic characteristics including health, physiological and welfare parameters

Phenotypic characteristics including health and physiological parameters are important components in the comparative approach. The phenotypic comparison aims at identifying similarities and differences between the GM animal and its comparator(s) which may be due to intended and / or unintended effects of the genetic modification. Unintended effects on the GM animals may manifest themselves through, for example, changes in susceptibility to biotic and abiotic stresses, through morphological, biochemical, physiological, developmental or reproductive changes or, on a case-by-case basis, through modified responses to husbandry and dietary regimes. Evaluation of the health and welfare status of the GM animals may also give information about possible toxicity and bioactivity (endocrine, pharmacological or immunological activity) of the newly expressed substances. An evaluation of health involves the monitoring of an animal over the course of its commercial lifetime which varies substantially according to the animal species, and the collection of data on its health status throughout important developmental stages, e.g. the juvenile period and post-pubertal maturation as well as other common breeding parameters, where applicable. Health records to be compared should include the results of physical inspection, clinical and physiological examination, the records of illnesses and therapies, feed intake, performance parameters as growth and development, weight gain, feed efficiency, and reproduction. Survival of the perinatal period provides primary evidence for normal physiological development of the GM animal. Therefore, GM animals should be already observed in the prenatal period and birth weights should be recorded if applicable. Reproductive functionality is one of the most important parameters for evaluating the health and functionality of GM animals. Proper reproductive functionality indicates that the complex interrelated physiological systems required for the foetal development and delivery and pregnancy maintenance have developed appropriately. Evaluation of the vaccination response can provide additional evidence for adequate immune competence of GM animals. Clinical chemistry and haematology evaluations are selected

based on clinical indications. They serve to confirm clinical diagnosis, or aid in differential diagnosis. In case of evaluating the health of GM animals they may serve as indicators of normal functioning. It is important to compare the measured parameters and their confidence interval (e.g. immune responses, biochemical and haematology values) with those obtained in conventional populations.

2.1.3.4. Comparative analysis of compositional characteristics

2.1.3.4.1. Selection of material for compositional analysis

Analysis of the composition is crucial when comparing the GM animal derived food/feed product with its conventional counterpart. The material to be used for the comparative assessment should be selected while taking into account the uses of the food and feed products from GM animals and the nature of the genetic modification. Analysis should normally be carried out on the unprocessed animal materials as this usually represents the main point of entry of the material into the food/feed production and processing chain. On a case-by-case basis, i.e. where physiologically relevant, animal derived products may need to be sampled at different stages in the productive life of the animal. Specific samples from the animal body (from tissues or organs) or products from animals (e.g. milk, eggs, honey, see Table 1) may be taken for analysis. Additional analysis of processed products (food/feed, food ingredients, and feed materials) may be necessary on a case-by-case basis (see Section 2.1.3.4.3). The preparation of the tested material and the analyses must be carried out according to appropriate quality standards. Examples of material to be tested for analysis on a case-by-case basis are given in Table 1.

Table 1: Examples of materials to be tested for comparative analysis and further safety relevant studies of food/feed from GM animals

Types of samples	**Mammals**	**Birds**	**Aquaculture (e.g. fish, molluscs)**	**Insects (honey bees)**
Samples from the animal body for food and feed	Tissues: -Meat, muscle (M.long.dorsi; M.bic.femoris) -Body fat -Blood -Some organs (liver, kidney, spleen, brain, etc.) -Residue body (Meat and bone meal as feed)	Tissues: -Meat, muscle (breast, thigh) -Abdominal fat -Blood -Some organs (liver, kidney, spleen etc.) -Residue body (Animal body meal as feed)	-Edible fraction (e.g. fillet) -Residue body (e.g. fish meal as feed)	none
Samples for food and feed produced by animals	Milk	Eggs	Eggs	Honey

2.1.3.4.2. Selection of compounds for compositional analysis

Besides the analysis of the level of the newly expressed proteins (see Section 2.1.4.3), the compositional analysis should be carried out for an appropriate range of constituents. In each case, key measures will include macro- and micro-nutrients as well as bioactive compounds (if identified as important e.g. hormones and growth factors), and allergens (if identified in the animal species of interest). In very specific cases there may also be anti-nutritional or toxic compounds that need to be included in the comparative compositional analysis. The comparative study on the level of common allergens may be performed in connection with specific allergenicity studies (see Section 2.1.5).

Key nutrients are those components of diet that have a major impact on human/animal health, i.e. proteins, carbohydrates, lipids/fats, vitamins and minerals. The vitamins and minerals selected for

analysis should be those which are present at levels which are nutritionally significant and/or which make nutritionally significant contributions to the diet at the levels at which the animal-derived product is consumed. The specific analyses required will depend mainly on the type of derived food/feed product although in the case of products intended as animal feeds the analyses will also depend on the target species. A detailed assessment appropriate to the intended effect of the genetic modification should always be included. In most situations analyses providing more detailed information than that provided by measuring total amounts of fat, protein etc. will be required. This may include a detailed fatty acid profile of the lipids present and an amino acid profile (individual protein amino acids and main non-protein amino acids). In some circumstances depending on the aim of the genetic modification comparison of the profile of individual proteins present may be additionally required.

Anti-nutritional compounds and toxic compounds, if identified in the species under assessment should be studied to the respective animal species and the proposed use of the food/feed product. Compounds other than the key nutrients, toxins, and anti-nutrients and allergens may be included in the analyses on a case-by-case basis. For example, in pigs that have been genetically altered to express phytase in their digestive juice to enhance the uptake of phosphorus from organic compounds, the uptake of other trace elements (e.g. Cu, Zn) and heavy metals (e.g. Cd) in different (edible) tissues would also need to be assessed. Thus, the characteristics of the introduced trait may trigger further analysis of specific compounds including metabolites of potentially modified metabolic pathways. On a case-by-case basis a comparison of the concentrations of hormones and growth factors or other bioactive compounds should be included.

2.1.3.4.3. Effects of processing

Almost all food/feed produced by animals will require some form of processing before consumption. Processing includes, for example, physical separation of constituents (e.g. fat from milk), pasteurization/sterilization, or fermentation. Processed products may be assessed together with the assessment of the original food/feed from the GM animal, or a processed product may be assessed independently. The applicant has to provide the scientific rationale for the risk assessment of these products. On a case-by-case basis, experimental data may be required. If assessed together with the original GM animal-derived food/feed, the applicant should assess whether or not the processing and/or preserving technologies applied are likely to modify the characteristics of GM animal-derived foods/feeds compared with their respective unprocessed counterparts. This would require the description of the different processing technologies in sufficient detail. If however a processed product is assessed independently then the comparison should be with the equivalent processed food/feed from non-GM animals.

2.1.3.5. Conclusions of the comparative analysis

The conclusion of the comparative analysis should clearly state:

a. whether phenotypic characteristics including health and physiological parameters of the GM animal are, except for the introduced trait(s), significantly different from the characteristics of its comparator(s);

b. in case an equivalence test was performed, whether phenotypic characteristics including health and physiological parameters of the GM animal are, except for the introduced trait(s) equivalent or not to the reference breeds, taking into account natural variation;

c. whether compositional characteristics of the GM animal-derived food/feed are, except for any introduced trait(s), significantly different from the characteristics of its comparator(s);

d. in case an equivalence test was performed, whether compositional characteristics of the GM animal-derived food/feed are, except for any introduced trait(s)equivalent or not to the reference food or feed, taking into account natural variation.

The identified differences should be assessed with regards to the possible impact to the human and animal health (see below).

2.1.4. Toxicological assessment

The health of a food/feed producing animal has traditionally been used as an important indicator of the safety of derived foods. The practice of only allowing animals with known and acceptable health status to enter the human food supply has been - and continues to be - an essential step to ensuring safe food. In addition, the potential impact of any changes resulting from the expression of introduced genes or any other type of genetic modification, e.g. gene silencing or over-expression of an endogenous gene, should be assessed.

The purpose of performing toxicological studies of compounds, using either experimental animals and/or *in vitro/silico/situ* systems, is to characterise any hazard linked to their presence and to determine exposure levels that do not result in adverse effects to humans and animals, using uncertainty or safety factors. These factors take into account differences between test animal species and humans, and inter-individual variations among humans. This internationally accepted approach is similar to the one applied for testing chemicals in foods described elsewhere (Renwick et al., 2003; Smith, 2002).

Toxicological assessment should be performed:

a. to demonstrate that the intended effect(s) of the genetic modification of the GM animal and derived food or feed has no adverse effects on human and animal health upon consumption of these products. Risk assessment of deviations from the characteristics of the comparator(s) may require different toxicological approaches and varying degrees of testing;

b. to demonstrate that unintended effect(s) of the genetic modification(s) which have been identified or assumed to have occurred based on the preceding comparative phenotypic, compositional or molecular analyses, have no adverse effects on human and animal health upon consumption of the GM animal and derived food or feed.

The requirement for toxicological testing should therefore be considered on a case-by-case basis and will be determined by the outcome of the molecular and comparative analysis, i.e. the differences identified between the GM product and its conventional counterpart, including intended as well as unintended changes. In principle, the assessment should consider (a) the presence of newly/differently expressed proteins, (b) the potential presence of other new constituents (c) the possible changes in the levels of natural constituents beyond normal variation and/or (d) the impact of changes in composition due to the genetic modification. The specific information requirements and testing strategies are outlined in the following sections.

In the case of newly/differently expressed bioactive substances, GM animals should be evaluated for potential effects of those substances as part of the overall animal health evaluation. It is possible that such substances may be active in humans. Moreover, in some cases there may be an increased expression of known substances such as sexual hormones, growth hormones, etc. Consideration should therefore be given to potential dietary exposure to the newly expressed substances or to altered levels of naturally occurring compounds, whether these substances are likely to be bioactive following consumption by humans or animals, and, if so, their potential to exert adverse effects.

In case the applicant considers that a conclusion on safety can be reached without conducting some of the tests recommended in this chapter and/or that other tests are more appropriate the applicant should

state the reasons for not submitting the recommended studies and/or for carrying out studies other than those mentioned below.

Any adverse effect(s) on individuals that could be due to their exposure to GM food/feed material as part of their professional activities e.g. farming or carcass processing should be reported by the applicant. Appropriate studies should be performed to further characterise these indications of potential adverse effects.

2.1.4.1. Standardised guidelines for toxicity tests

The applicant should use for toxicity testing internationally agreed protocols and test methods described by the OECD[7] or in accordance with the requirements of Article 13 of Regulation (EC) No 1907/2006 (EC, 2002). The most up-to-date version of any test guideline should be followed. Adaptations of these protocols or use of any methods that differ from these protocols should be justified. It is essential that facilities in which toxicological tests are performed, apply appropriate quality assurance systems in order to ensure that the results are of high quality. Such principles are laid down by Directive 2004/10/EC of the European Parliament and Council of 11 February 2004 on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances (EC, 2004). If such tests are carried out outside the Union, they should follow the OECD Principles of Good Laboratory Practice[8] (GLP). With regard to studies other than toxicological studies, they should be conducted under ISO or GLP standards or other appropriate quality assurance. A non-exhaustive list of validated test protocols which, where necessary, should be used in a possibly adapted form for GMO toxicological testing is provided in Tables 2 below.

Table 2: Non-exhaustive lists of OECD guidelines for testing of chemicals which may be selectively applied for (geno)toxicological testing relevant for GMO risk assessment

No. OECD	Title
402	Acute Dermal Toxicity
406	Skin Sensitisation
407	Repeated Dose 28-day Oral Toxicity Study in Rodents
408	Repeated Dose 90-Day Oral Toxicity Study in Rodents
410	Repeated Dose Dermal Toxicity:21/28-Day
415	One-Generation Reproduction Toxicity
416	Two-Generation Reproduction Toxicity Study
417	Toxicokinetics
421	Reproduction/Developmental Toxicity Screening Test
424	Neurotoxicity Study in Rodents
471	Bacterial reverse mutation test
473	*In vitro* mammalian chromosome aberration test
474	Mammalian erythrocyte micronucleus test
475	Mammalian bone marrow chromosome aberration test
476	*In vitro* mammalian cell gene mutation test
479	*In vitro* sister chromatid exchange (SCE) assay in mammalian cells
482	DNA damage and repair, unscheduled DNA synthesis in mammalian cells *in vitro*
487	Draft guideline on: *In vitro* mammalian cell micronucleus test

It is emphasized that in general toxicological testing of most of the GM animal- derived food and feed and specific food and feed constituents will not be necessary. Application of test protocols depends on the type of GM animal-derived food/feed, type of the genetic modification and resulting intended and

[7] OECD guidelines for the testing of chemicals available from http://www.oecd.org/document/40/0,3746,en_2649_34377_37051368_1_1_1_1,00.html
[8] OECD Principles of Good Laboratory Practice available from http://www.oecd.org/findDocument/0,3770,en_2649_34381_1_1_1_1_1,00.html

unintended alterations, intended use and exposure/intake, and the available knowledge and should be scientifically justified and documented. Some of the tests are relevant for the assessment of risks at the workplace.

Toxicology studies designed to evaluate risks to human and/or animal health complement each other. Most studies recommended for the assessment of the safety of the GM food are relevant for the assessment of GM feed. Testing methodologies are basically the same and the same level of data quality is required.

2.1.4.2. Phenotypic comparison

Analysis of the GM animal health and welfare status including aspects of physiology may provide an indication for potential adverse health effects of the newly expressed proteins, other new constituents and/or changed levels of natural constituents (intended changes), including effects due to specific biological activities of the respective constituents, e.g. endocrine, pharmacological or immunological activity (see Sections 2.1.4.3 to 2.1.4.5). Moreover it may also provide information on the occurrence of unintended effects of the genetic modification. Therefore, the health and welfare status of the GM animal should be carefully observed and compared in detail to the health and welfare status of closely related comparator(s). If the genetic modification has no negative impact on the GM animal, this is a strong indication that consumption of the GM animal-derived products will not have adverse effects on the health of the consumers. Any negative impact on the general health status of the GM animal has to be further assessed with regard to potential adverse effects on the health of humans/animals upon consumption of GM animal-derived products.

2.1.4.3. Assessment of newly expressed proteins

All newly expressed proteins should be assessed. The studies required to investigate the potential toxicity of a newly expressed protein should be selected on a case-by-case basis, depending on the knowledge available with respect to GM animal's health (see Section 2.1.4.1.) and the source, function/activity and history of human/animal consumption of the protein. In the case of proteins expressed in the GM animal where both the animal and the newly/differently expressed proteins have a proper use and safe consumption as food and feed and where no negative impact of the genetic modification on the GM animal's health or welfare status is observed, specific toxicity testing may not be required.

If specific testing is required, it is essential that the tested protein is equivalent to the newly/differently expressed protein as it is expressed in the GM animal. If, due to the lack of sufficient amount of test materials, a protein produced by microorganisms is used, the structural, biochemical and functional equivalence of this microbial substitute to the newly/differently expressed animal protein should be demonstrated. Comparisons of the molecular weight, amino acid sequence, post-translational modification, immunological reactivity and, in the case of enzymes, the enzymatic activity, are needed to provide evidence for equivalence. In case of differences between the GM animal-expressed protein and its microbial substitute the significance of these differences for the safety studies should be evaluated.

To demonstrate the safety of newly expressed proteins, the applicant should provide the following:

a. molecular and biochemical characterisation of the newly expressed protein, including the amino acid sequence, molecular weight, studies on post-translational modifications and a description of the function. In the case of newly expressed enzymes, information on the enzyme activities including the temperature and pH range for optimum activity, substrate specificity, and possible reaction products should also be provided. Potential interactions between the newly expressed proteins and other animal constituents should be evaluated with respect to safety impact;

b. up-to-date search for homology to proteins known to cause adverse effects, e.g. toxic proteins. A search for homology to proteins exerting a normal metabolic or structural function may also contribute valuable information. The database(s) and the methodology used to carry out the search should be specified;

c. Information on the stability of the protein under the relevant processing and storage conditions for the food and feed derived from the GM animal. The influences of temperature and pH changes should be examined. Potential modification(s) of the proteins (e.g. denaturation) and/or production of stable protein fragments generated through such treatments should be characterised;

d. data concerning the resistance of the newly/differently expressed protein to proteolytic enzymes (e.g. pepsin), e.g. by *in vitro* investigations using appropriate and standardised tests. Stable breakdown products should be characterised and evaluated with regard to the potential risks linked to their biological activity;

e. In specific cases: repeated dose toxicity studies using laboratory animals, unless reliable information demonstrating the safety of the newly expressed protein (including its mode of action) can be provided, and it is demonstrated that the protein is not structurally and functionally related to proteins adversely affecting human or animal health. The repeated dose 28-day oral toxicity study in rodents with the newly expressed protein should be performed according to OECD guideline 407[9] (see Table 2). It is recommended to use a sufficient number of animals per test group in order to obtain an adequate statistical power. Depending on the outcome of the 28-day toxicity study, further targeted investigations may be required.

Acute toxicity testing of the newly expressed proteins of GM animals is of little additional value for the risk assessment of the repeated human and animal consumption of GM food and feed derived from GM animals and is, therefore, discouraged.

2.1.4.4. Assessment of new constituents

Identified new constituents other than proteins, for instance bioactive compounds with, for example, endocrine, pharmacological or immunological activity, should be evaluated. This evaluation may include toxicological testing on a case-by-case basis, taking into consideration the assessment of their toxic potency and occurrence in the GM food/feed. Here also the health status of the GM animal physiologically producing the bioactive compounds, should be carefully observed as a basis for the subsequent food and feed safety assessment strategy. To establish the safety of new constituents having no history of safe consumption as food and feed, information analogous to that described in the "Guidance on submissions for food additive evaluations by the Scientific Committee on Foods" (SCF, 2001) and Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives[10] should be provided. This implies the submission of information on a core set of studies and the consideration of whether or not any other type of study might also be appropriate. Normally, the core set includes information on metabolism/toxicokinetics, sub-chronic toxicity, genotoxicity, chronic toxicity, carcinogenicity and reproduction and developmental toxicity (see Table 2). Genotoxicity test protocols are given in Table 2.

2.1.4.5. Assessment of altered levels of food and feed constituents

This section applies if the intended or unintended effect of the genetic modification is that the content of natural food and feed constituents is altered beyond the natural variation.

[9] http://www.oecd.org/dataoecd/50/41/37477972.pdf
[10] OJ L 133, 22.5.2008, p.1.

Natural food and feed constituents comprise a large variety of substances: macro- and micronutrients, anti-nutrients, and bioactive compounds as well as other secondary animal metabolites. To demonstrate the safety of the altered content of natural food and feed constituents, a detailed risk assessment based on the knowledge of the physiological function and/or toxic properties of these constituents and the anticipated change in their intake levels should be submitted. Here also the health status of the GM animal physiologically producing the bioactive compounds, should be carefully observed as a basis for the subsequent food and feed safety assessment strategy. The result of this assessment will determine if, and to what extent, toxicological tests are required.

2.1.4.6. Assessment of the whole GM food/feed derived from GM animals

The risk assessment of the GM animal- derived food/feed is primarily based on molecular characterisation, comparative phenotypic, health status, and comprehensive compositional analysis, and the toxicological evaluation of the identified intended and unintended effects. When these types of analyses indicate a reason to perform an animal study to check whether the GM animal derived food/feed is as safe as the conventional counterpart, a 90-day rodent feeding trial specific tissues and/or organs of the GM animal may be considered. Thus, toxicological testing of whole GM-animal derived food/feed should be considered if there are indications or remaining uncertainties for the potential occurrence of unintended effects based on the comparative analysis, including the extensive phenotypic and compositional comparison, and the molecular characterisation, which could not be toxicologically assessed as described above.

Since the amount of GM animals-derived materials that can be included in rodent diets will be limited, feeding trials should therefore only be conducted if the sensitivity of the proposed experiment is deemed sufficient to detect adverse effects.

Design and performance of a 90-day feeding study in rodents

The design of the toxicity study with whole food and feed derived from a GM animal should be performed according to the principles of OECD guideline 408[11] (see Table 2) following a protocol adapted for whole food and feed (EFSA, 2011b).

Special attention should be paid to the selection of doses and the avoidance of problems of nutritional imbalance. Normally a minimum of two test dose levels and a negative control is used (same diet but using a of the same background). The highest dose level should be the maximum achievable without causing nutritional imbalance; the lowest dose level should contain the tested food and/or feed in an amount at least equivalent to the one consumed by humans or animals. It will need to be assessed on a case-by-case basis whether sufficiently high dose levels can be achieved in the case of the different types of test material (GM animal-derived, animal parts, food/ feed products) for the study to be meaningful. Stability of test diets and nutritional equivalence between control and test diets are important aspects to consider. When such studies are conducted, the control diet(s) should include the appropriate comparator(s). It is recommended that, whenever possible, information on natural variation of test parameters is derived from historical background data rather than from the inclusion of reference varieties, consisting of commercially available food and feed derived from non-GM animals with a history of safe use, in the experiments. The statistical analysis should focus on the detection of possible differences between the test material and its control. Detailed discussion is available in the opinion of the EFSA Scientific Committee on 90-day feeding trial protocol (EFSA, 2011b).

Depending on the outcome of the 90-day feeding trial further studies may be considered (e.g. studies on reproductive/developmental effects, chronic toxicity).

[11]http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en

Supplemental information to 90-day feeding studies in rodents on the possible occurrence of unintended effects may be obtained from comparative growth studies conducted with young rapidly growing animal species (broiler chicks as animal model for non-ruminants; lambs for ruminants; or other rapidly growing species; see Sections 2.1.6.1 and 2.1.6.2). Studies of this type are limited to those animal derived materials suitable for inclusion in their diets and which can be nutritionally matched to a suitable control diet. Performance of these studies would have to be scientifically justified and documented by the applicant. Livestock feeding studies with target animal species shall be considered by the applicant, on a case-by-case basis and be hypothesis driven. The focus shall be on the safety of newly/differently expressed constituents, on the identification and characterisation and impact of unintended effects, and on the nutritional impact of any intentional and/or substantial compositional modifications of the GM animal (see Section 2.1.6).

In cases where the extensive phenotypic and compositional comparison, and the molecular characterisation, have demonstrated no difference between the GM animal derived food/feed and their comparator(s), except for the inserted trait(s), and have not indicated that unintended effects may occur, or if they do occur they are not harmful, the performance of animal feeding trials with rodents or other (target) animal species is of little additional value if any, and is therefore not recommended.

2.1.4.7. Conclusion of the toxicological assessment

The conclusion of the toxicological assessment should indicate whether:

a. the information provided and the testing strategy used to assess the intended and/or unintended changes of the GM food/feed are considered adequate;

b. potential adverse effects identified in other parts of the safety assessment have been confirmed or discarded;

c. the available information on the newly/differently expressed protein(s) and other new constituents resulting from the genetic modification gives indications of potential adverse effects; in particular, whether and at which dose levels adverse effects were identified in specific studies;

d. the information on natural constituents of which the levels are different from those in its comparator(s) provides indications of potential adverse effects, in particular, whether and at which dose levels adverse effects were identified in specific studies;

e. toxicologically relevant adverse effects have been identified in the animal studies made on the whole GM-animal derived food/feed compared to their comparator(s).

The results of the toxicological characterisation should be evaluated in the light of anticipated intake of the GM food/feed (see Section 2.2).

2.1.5. Allergenicity

Food allergy is an adverse reaction to food and represents an important public health problem. Food allergy is different from toxic reactions and intolerance. Allergy is a pathological deviation of the immune response to a particular substance which affects only some individuals where a combined effect of variations in the environment and genetic predisposition has resulted in allergic sensitisation. In allergic individuals, sometimes minute amounts of a food that is well tolerated by the vast majority of the population can cause serious symptoms and death. It is not the allergen *per se*, but the allergic person's abnormal reaction to the allergen that causes the adverse health effect. Food allergy can be caused by various immune mechanisms. However, IgE-mediated food allergy represents the main form of food allergy, that causes the most severe reactions and the only form causing life-threatening reactions. This IgE-mediated food allergy has been the focus in the risk assessment of allergenicity of GMOs. Importantly, food allergy consists of two separate phases: first *sensitisation* where no

symptoms occur while the capacity of the immune system to react increases dramatically, and later *elicitation (provocation)* with clinical manifestations. When ingested, the allergen(s) i.e. the sensitising food or food component is to some extent degraded by digestive enzymes, absorbed by the gut mucosa (small amounts even by the oral mucosa), processed in specialised cells of the immune system and then presented to the reactive immune cells that produce an immune response. Sensitisation can also occur if the food allergen comes into contact with the skin or is inhaled.

The majority of the constituents that are responsible for allergenicity of foods are proteins. Some protein breakdown products, i.e. peptide fragments, may conserve part of the allergenicity of the native protein and thus can also be considered as allergens. The specific allergy risk of GM animals is associated i) with exposure to newly/differently expressed protein(s) that can be present in edible parts of the animals. This point is related to the biological source of the transgene; and ii) with alterations to the allergenicity of the whole animal and derived products, e.g. due to over-expression of natural endogenous allergens as an unintended effect of the genetic modification. This point is related to the biology of the recipient organism itself.

2.1.5.1. Assessment of allergenicity of the newly expressed protein

Allergenicity is not an intrinsic, fully predictable property of a given protein but is a biological activity requiring an interaction with individuals with a pre-disposed genetic background. Allergenicity therefore depends upon the genetic diversity and variability in atopic humans. Frequency, severity and specificity of allergic reactions also depend upon geographic and environmental factors. Given this lack of complete predictability, it is necessary to consider several aspects in the risk assessment process to obtain a cumulative body of evidence which minimises any uncertainty with regard to the protein(s) in question.

When studying the structural characteristics and the biological and physicochemical properties of a newly expressed protein, it is essential that the tested protein is equivalent with respect to structure and activity to the newly expressed protein in the GM animal. Studies carried out using purified target proteins prepared by expression in suitable expression systems are acceptable as long as the properties of the substitute protein are identical to those of the protein expressed in the animal, taking into account the post-translational modifications that specifically occur in mammals/vertebrates.

The source of the transgene should be considered carefully to make clear whether or not it encodes an allergen. Information should specify at what stage of the development of the animal and in what organs of the animal the potential allergenic protein may be expressed.

In line with the recommendations of EFSA (EFSA, 2010b) and the Codex alimentarius ad hoc Intergovernmental Task Force on Foods Derived from Biotechnology (Codex Alimentarius, 2003), an integrated, case-by-case approach, i.e. so called weight-of-evidence approach, shall be used in the assessment of possible allergenicity of newly expressed proteins.

- Amino acid sequence homology comparison between the newly expressed protein and known allergens: In every case, a search for sequence homologies and/or structural similarities between the newly expressed protein(s) and known allergens should be performed to identify potential IgE cross-reactivity between the newly expressed protein and known allergens. The quality and the comprehensiveness of the databases used should be considered. Improvement and harmonisation of the algorithms that are used should be sought. The alignment-based criterion involving 35 % sequence identity to a known allergen over a window of at least 80 amino acids is considered a minimal requirement (EFSA, 2010b). All sequence alignment parameters used in the analysis should be provided including calculation of percent identity (PID). It is recommended that the calculation of PID is performed on a window of 80 amino acids with gaps so that inserted gaps are treated as mismatches. In some cases, for assessing short peptidic fragments such as ORFs, a search for sequences of contiguous identical or chemically similar amino acid residue can be conducted. However, this search is not

recommended routinely for the identification of potential linear IgE binding epitopes because of its poor sensitivity or specificity.

- Specific serum screening: When there is indication of sequence homology or structure similarities, an important procedure for assessing the potential that exposure to the newly expressed protein(s) might elicit an allergic reaction in individuals already sensitised to cross-reactive proteins, is based on *in vitro* tests that measure the capacity of specific IgE from serum of allergic patients to bind the test protein(s). It is noted that there is inter-individual variability in the specificity and affinity of the human IgE response. In particular the specificity of the IgE antibodies to the different allergens present in a given food/source and/or to the different epitopes present on a given protein may vary amongst allergic individuals. In order to optimize the sensitivity of the test, individual sera from well-characterised allergic individuals should be used rather than pooled sera. Specific serum screening should be performed in the following cases:

    - If the source of the introduced gene is considered allergenic even if no sequence homology of the newly expressed protein to a known allergen is demonstrated or if the source is not known to be allergenic but there is any indication of relationship between the newly expressed protein(s) and a known allergen, based on sequence homology and/or structure similarity, specific serum screening should be undertaken with sera from individuals with a proven allergy to the source or to the potentially cross-reacting allergen using relevant immunochemical tests. IgE-binding assays (such as Radio or Enzyme Allergosorbent Assay (RAST or EAST), Enzyme Linked Immunosorbent Assay (ELISA) and electrophoresis followed by immunoblotting with specific IgE-containing sera) are adequate methods.

- Pepsin resistance and in vitro digestibility tests. Stability to digestion by proteolytic enzymes has long been considered a characteristic of allergenic proteins. Although it has been established that no absolute correlation exists between the stability of a protein to digestion and its allergenicity (Fu et al., 2002; EFSA, 2010b), resistance of proteins to pepsin digestion is still proposed as an additional criterion to be considered in an overall risk assessment. The pepsin resistance test is generally performed under quite standardized conditions (Thomas et al., 2004), at low pH values and high pepsin:protein ratios. It is recognized that the pepsin resistance test does not reflect the physiological conditions of the digestion. The digestibility of the newly expressed proteins in specific segments of the population such as infants and individuals with impaired digestive functions may be assessed using in vitro digestibility tests using different conditions (EFSA, 2010b). Also, since the protein encoded by the newly introduced genes will be present in the product as a complex matrix, the impact of the possible interaction between the protein and other components of the matrix as well as the effects of the processing should be taken into account in additional *in vitro* digestibility tests. Depending on the outcome of the *in vitro* digestibility test, it could also be useful to compare intact, heat-denatured and pepsin-digested proteins for IgE binding, since an altered digestibility may impact on the allergenicity of the newly expressed protein(s).

- Although additional tests including in vitro cell based assays or in vivo tests on animal models have not been validated so far for regulatory purposes, they may be considered useful to provide additional information e.g. on the potential of the newly expressed protein for *de novo* sensitisation.

2.1.5.2. Assessment of allergenicity of the tissues, organs & products from the GM animal

In general, foods of animal origin are common food allergens. In addition, it is noted that the most important ones (e.g. milk, eggs) mainly affect young children for whom those food allergies are the first way of sensitisation, whereas plant food allergens generally affect older individuals, sometimes after airway sensitisation to cross-reactive pollen allergens.

For each allergenic food of animal origin, there are generally many allergenic proteins and for each of them several isoforms that may differ by small changes in the amino acid sequence or post translational modifications. The allergen profiles qualitatively and quantitatively vary between the breeds, between the animals and for each individual animal depending on the age/physiological status and environment. Also animal products are complex food matrices in which interactions between proteins and other constituents occur and that are frequently processed (e.g. cooked) before being consumed. Such interactions, treatments and their combinations may alter the allergenicity of the whole food in an unpredictable manner.

In parallel with this complexity and variability in the allergen composition of animal-derived foods, a great variability in the intensity and specificity of the human allergic responses is observed.

The weight-of-evidence approach does not apply here and these characteristics make it difficult to routinely perform a reliable and conclusive comparison of the allergenicity of whole foods derived from GM vs non-GM animals.

It can be noticed that, in this particular situation, the hazard is clearly identified but that in most cases hazard and risk characterisation would not be feasible because of the lack of detailed information on exposure of at risk groups of the population and essentially also because no data on dose-response relationship (i.e. threshold doses) are available.

Therefore it is recommended that when the recipient of the genetic modification is an animal whose products are known common food allergens (e.g. milk, eggs, fish) and when there are no indications of possible interaction(s) between the metabolic pathway(s) involved in the expression of the trait protein encoded by the transgene and those involved in the biosynthesis of endogenous allergenic proteins naturally present in the conventional animal tissues, the same management measures as for the non GM animal products should be applied. If there are indications that the allergenicity of the whole GM animal-derived food might be dramatically/extensively, qualitatively or quantitatively, changed because of interactions between the metabolic pathways of synthesis of the newly expressed protein(s) and of endogenous or new allergens, the allergenic potential of GM food should be further and extensively investigated.

To confirm the absence of any increased risk of de novo sensitisation to the GM animal foods in the every day life conditions of exposure post market monitoring may be proposed (see Section B on Post Market Monitoring).

Should the genetic modification be aimed to reduce the allergenicity of the animal products, evidence should then be given by actual data obtained from experimental studies, including (human) clinical studies, to substantiate the claim in accordance with the regulation/procedure for assessment of health claims.

In addition, the applicant should provide, where available, information on the prevalence of occupational allergy (both food and respiratory allergy) in workers or in farmers who have significant exposure to GM animals and derived products.

2.1.5.3. Conclusion of the allergenicity assessment

The allergenicity assessment should clearly indicate whether the novel protein(s) is likely to be allergenic. When there is a likelihood of allergenicity of the whole food because the recipient of the genetic modification is an animal whose products are known common food allergens, appropriate conditions for placing on the market, including labelling, should be proposed. If there are indications that the allergenicity of the whole GM animal-derived food might be dramatically/extensively, qualitatively or quantitatively, changed because (of unintended effects) of the genetic modification, the allergenic potential of the GM food should be further and extensively investigated.

On a case-by-case basis, post market monitoring programmes can then also be proposed to confirm the absence of increased allergenic risk in actual conditions of exposure.

2.1.6. Nutritional assessment

Nutritional evaluation should be provided:

a. to demonstrate that introduction of the GM animal-derived food or feed into the market is not nutritionally disadvantageous to humans or animals, respectively. This evaluation should include an assessment of: (i) the nutritional relevance of newly expressed proteins and other new constituents; (ii) the changes in the levels of endogenous constituents in the GM animal derived food and feed; (iii) the potential alterations in the total diet for the consumers/animals.

b. to demonstrate that unintended effects of the genetic modification that were identified or that may be assumed to have occurred based on the preceding molecular, compositional or phenotypic analyses (see Sections 2.1.2. and 2.1.3.), have not adversely affected the nutritional value of the GM animal-derived food/feed.

Compositional analysis is the starting point and cornerstone for the nutritional assessment of food and feed material. The applicant should provide analyses of all the key components relevant to the genetic modification and the target species. Analyses of additional components should be determined on a case-by-case basis and depend on food/feed type and the introduced trait(s).

If the GM animal derived food and feed have been assessed as compositionally not different from its comparator except for the introduced trait(s) (see Sections 2.1.3), no further studies to demonstrate nutritional equivalence are required. If, on the basis of the comparative assessment it is not possible to conclude nutritional equivalence, further studies should be carried out (see Sections 2.1.6.1 and 2.1.6.2).

2.1.6.1. Specific considerations for the nutritional assessment of GM animal-derived food

The intended modification in GM animals may change the overall nutrient profile of derived animal products and this change could affect the nutritional status of individuals consuming the food and/or specific consumer groups. Unexpected alterations in nutrients could have the same effect. These aspects need to be assessed on a case-by-case basis.

The nutritional assessment of GM animal-derived food/feed should consider:

a. the composition of the GM animal-derived food/feed with regard to the concentrations of nutrients and anti-nutritional compounds (see Section 2.1.3);

b. the nutritional quality of the GM animal-derived food taking into account the potential influences of transport, storage and expected treatment of the foods;

c. the anticipated dietary intake of the GM animal-derived food (see Section 2.2) and resulting nutritional impact in a whole diet context.

When the comparative analysis has identified compositional characteristics of the GM animal-derived food that are different and/or not equivalent to the characteristics of its comparator(s), their nutritional relevance should be assessed on the basis of current scientific knowledge.

Further nutritional information relative to human food may be obtained from suitably designed studies using animal models (e.g. rodents, pigs) whereas additional nutritional data relative to animal feeds may require studies with the target species. Additional information may also be available where 90-

day feeding study in rodents has been carried out as part of the toxicology assessment (see Section 2.1.4.6). In specific cases, such studies, in addition to toxicological data, can provide valuable information on nutritional aspects since they start with juvenile animals in a rapid growth phase and are sensitive to effects on rate of weight gain and feed conversion efficiency.

The applicant should determine the necessity to perform nutritional studies. If the compositional data provide sufficient information on the nutritional characteristics of the new GM animal-derived food, it may not be necessary to perform additional nutritional studies in animals. If there are specific questions it may be informative to perform such a study, for instance if there are questions with relation to the bioavailability of specific food components as a result of the genetic modification. In that case the type and design of the nutritional study should be determined on the basis of the introduced trait(s), the outcome of the comparative analysis, and of the 90-day feeding study in rodents, where available. When nutritional studies are conducted, the control diet(s) should use the appropriate comparator(s).

In cases where an altered digestibility and/or bioavailability needs to be established and may raise concern for sub-population(s), the level of the nutrient in the food should be determined, taking into account all the different forms of the compound. The methods to test for digestibility and/or bioavailability should be selected on a case-by-case basis depending on the nutrient or other constituent, the food containing these constituents, as well as the health, age, nutritional status and dietary practices of the specific population(s) anticipated to consume the food. In some circumstances a study with suitable selected human volunteers may be needed.

2.1.6.2. Specific considerations for the nutritional assessment of GM animal-derived feed

Following the Bovine Spongiform Encephalopathy emergence in the EU in the 1980's-1990's, and in view of the role of the transmission of the disease through feed containing proteins of animal origin, since January 2001 the use of all processed animal protein in feeds for farmed animals has been banned throughout the EU with some exceptions (e.g. fish meal for non-ruminants) (EFSA, 2007). However, its use in other parts of the world continues. In the EU the use of proteins derived from animals in animal nutrition is regulated by two pieces of legislations, namely the Regulation (EC) No 1774/2002 (Animal By-Products Regulation, which will be replaced by a new Regulation during 2011 [EC, 2002]) and the Regulation (EC) No 999/2001 (TSE Regulation [EC, 2001]).

The applicant should determine the necessity and design of further nutritional studies on the basis of the introduced trait(s), the outcome of the comparative analysis, and the 90-day feeding study in rodents, where available.

In the case of GM animal-derived feed with intentionally or unintentionally altered nutritional characteristics, livestock feeding studies with target animal species can be considered taking account of current legal restrictions mentioned above, on a case-by-case basis to assess the impact of the feed. Where applicable, an appropriate control diet containing its comparator(s) should be formulated by supplementing it with the specific nutrient to the extent of the change effected in the GM animal-derived feed. Regarding co-products, from which the ingredient targeted by the genetic modification has been extracted, these should be compared with co-products derived from the comparator(s) and other conventional breeds as additional comparators (on the basis that all these products are low in the component targeted by the genetic modification). Target animal feeding studies should span either the growing and/or finishing period to slaughter for non-ruminants (e.g. chickens, pigs, fish) or a major part of the laying cycle for egg laying birds considering also the current legal restriction.

Various experimental designs might be necessary to demonstrate that nutritionally improved GM animal-derived feed fulfils the expected nutritional value as discussed in the Report of the EFSA GMO Panel Working Group on Animal Feeding Trials (EFSA, 2008). The exact experimental design and statistical approaches of feeding experiments in food producing animals to test the nutritional value of GM animal-derived feeds modified for enhanced nutritional characteristics will depend on a

number of factors and include choice of animal species, type of animal trait(s) studied and the size of the expected effect. The experimental diets need to be formulated in such a way that the key measured endpoints are responsive to a difference in the quantity and/or availability of the nutrient in question. Endpoint measurements will vary with the target species used in the study, but will include feed intake, body weight, animal performance and bioavailability of nutrients (EFSA, 2008; ILSI, 2007).

2.1.6.3. Conclusion of the nutritional assessment

The conclusion of the nutritional assessment of GM food/feed should indicate whether the GM food/feed is nutritionally equivalent relative to its conventional counterpart, taking natural variations into account.

The results of the nutritional assessment should be evaluated in the light of anticipated intake of the GM food/feed.

2.2. Exposure assessment - Anticipated intake/extent of use

An estimate of the expected intake is an essential element in the risk assessment of GM food/feed and also required for the nutritional evaluation. Information should be provided on the intended function, the dietary role, and the expected level of use of the GM animal-derived food/feed product(s).

On the basis of representative consumption data for products derived from the respective conventional animals, the anticipated average and maximum intake of the GM food/feed should be estimated. Probabilistic methods may be useful to determine ranges of plausible values rather than single values or point estimates. If possible, particular sections of the population with an expected high exposure should be identified and should be considered within the risk assessment. Any assumptions made in the exposure assessment should be described. Recent developments in methodologies and appropriate consumption data should be used. Data on import and production quantities may provide additional information for the intake assessment.

The concentrations of the newly expressed proteins, other new constituents and natural constituents, of which the levels have been altered as a result of the genetic modification (e.g. due to changes in metabolic pathways) in those parts of the GM animal intended for food or feed use should be determined by appropriate methods. Expected intake of these constituents should be estimated taking into account the influences of processing, storage and expected treatment of the food/feed in question, e.g. potential accumulation or reduction. In cases where the genetic modification has resulted in an altered level of a natural constituent, or if a new constituent occurs naturally in other food/feed products, the anticipated change in total intake of this constituent should be assessed considering realistic as well as worst case intake scenarios.

Information on known or anticipated human/animal intake of analogous food/feed and on other routes of exposure to the respective new and natural constituents, including amount, frequency and other factors influencing exposure, should be provided.

In case when, GM animal-derived foods are expected to provided health benefits or to pose risks to specific populations or sub-populations an exposure assessment should be performed.

2.3. Risk characterisation

2.3.1. Introduction

Risk characterisation of GM animals and derived foods/feed is based on data from hazard identification, hazard characterisation, and on exposure/intake data. A comprehensive risk characterisation should be carried out considering all the available evidence from several analysis including molecular analysis, comparative analysis of phenotypic characteristics (including health status, physiology and reproduction) and compositional analysis, toxicity and allergenicity testing. The

risk characterisation may give indications for the requirement of specific activities for post-market monitoring of GM food/feed.

Uncertainties identified at any stage of the risk assessment should be highlighted and quantified, to the extent possible (EFSA, 2006). Distinction should be made between uncertainties reflecting natural variation in ecological and biological parameters (including variations in susceptibility in populations), and variation reflecting differences in responses between species.

Depending on the issue to be addressed and the available data, risk characterisation may be only qualitative, but may also be quantitative. The estimated risk and associated uncertainties should be as precise as possible.

2.3.2. Issues to be considered for risk characterisation

Risk assessment of GM animal- derived food or feed should be carried out in an integrative manner and on a case-by-case basis depending on the type of genetic modification, taking into consideration husbandry practice of the GM animal and use of the derived foods/feed for human/animal consumption. To this aim, the applicant should take into account the different issues considered in hazard identification and characterisation and exposure assessment. The list of issues provided in this section is by no means exhaustive.

2.3.2.1. Molecular characterisation

Evaluation of the characteristics and previous use of the donor and the recipient organism is a key element to identify the need for specific analyses e.g. occurrence of specific toxins, or allergens in the unmodified recipient animal which may be unintentionally increased as result of the genetic modification.

Transformation protocols, molecular characterisation strategies and the specificity and sensitivity of the methods used should be discussed in relation to the intentional and possibly unintentional insertion and expression of nucleotide sequences.

Where molecular characterisation has identified potential hazards, additional molecular analysis, compositional and phenotypic analysis, and possibly animal feeding trials with the whole GM food/feed can be used to show that there is no safety issue.

2.3.2.2. Comparative assessment

An important issue to be evaluated is whether the comparative analysis between the GM animal and its comparator(s) with respect to phenotypic, health, and compositional characteristics has been carried out appropriately according to current guidelines, and what evidence is available that the conventional animal can be taken as a reference for safe breeding, rearing, and human/animal use. Protocols for performance of animal trials should be evaluated, and the data generated assessed to confirm they are representative for the proposed husbandry conditions of the GM animal.

The goal of the comparative safety assessment is to identify possible differences between the GM animal and its comparator(s). The choice of the comparator(s) is key and its selection should be justified in particular with respect to its history of safe consumption as food and feed. The risk characterisation should concentrate on statistically significant differences in the composition of the GM animal compared to its comparator(s) and whether these differences are likely to have an impact on food and feed safety or nutrition. The estimated risk and associated uncertainties should be as precise as possible and taken into account.

Unintended effects of the genetic modification are expected to result in differences or lack of equivalence between the GM animal and its comparator(s) that may be observed in one or more husbandry conditions. A difference or lack of equivalence that is consistently observed under any or all husbandry conditions can be an indicator of such unintended effects.

Unintended effects of the genetic modification are expected to result in differences between the GM animal and its conventional counterpart, or lack of equivalence between the GM and traditionally-bred animals with a history of consumption as food and feed, that may be observed in one or more husbandry conditions.

If statistically significant differences and/or non-equivalences are observed, using the methodology as described under Section 2.1.3.2, the following background data may be considered to put them into context with respect to their potential relevance for the human/animal health.

2.3.2.3. Data on variability inherent to the animal, the animal breed and the environment

Commonly considered is the range of levels observed for the compounds known to occur in the comparator(s) and in commercial breeds. This variability may be caused by differences that are genotype-dependent, environmentally dependent, or caused by genotype x environment interactions. In addition, the range of levels observed in a broad spectrum of food and feed representative for the human and animal diet may be taken into account. The rationale for considering this variability in the safety assessment is that it reflects the levels of the specific compound to which consumers may be exposed.

2.3.2.4. Information of variation of constituents from databases

The databases used for comparison should be specified and adequately assessed for their quality (e.g. type of material analyzed, analytical method used, sampling methods and strategies). No formal statistical analysis should be carried out, but ranges as well as mean values should be reported and considered. These data would indicate whether the GM breeds fall within the natural range in component concentrations found in non-GM comparators. The influence of environmental factors on phenotypical and compositional characteristics of animals should be taken into account when comparing analytical data from field studies with literature data.

2.3.2.5. Toxicological assessment

The data generated to estimate possible risks to human/animal health associated with the consumption of GM–animal-derived foods/feed should be evaluated with respect to the expression of new proteins/metabolites as well as significantly altered levels of original animal proteins/metabolites in GM foods/feed, taking also into account unintended effects of the genetic modification. If specific studies demonstrate that single constituents and/or whole GM food/feed were found to induce adverse effects, these should be addressed by applicants (e.g. dose response relationships, threshold levels, delayed onset of adverse effects, risks for certain groups in the population, use of uncertainly factors in extrapolation of animal data to humans).

The relevance of short-term toxicity data to predict possible long-term adverse effects of newly expressed proteins and/or new metabolites in the GM plant and derived food and feed should be discussed. The absence or inclusion of specific data on long-term studies (e.g. on reproductive and developmental toxicity) should also be discussed, when applicable. In the case of feeding studies with the whole food and feed the outcomes should be evaluated taking into account experimental limitations (e.g. dose range, dietary composition, confounding factors).

Data on the characteristics of the new compounds present in the GM animals, which may affect humans and animals, should be considered. If the compounds have known adverse health effects and maximum levels for their presence in the animal or derived products are laid down in specific legislations, these maximum levels should be taken into account. If these are not available, reference values for acceptable or tolerable levels of intake, such as the Acceptable Daily Intake (ADI) or Tolerable Upper Intake Level (UL), should be taken into account in relation to the anticipated intake. In cases where the compounds have been safely consumed in foods, the intake levels of consumers from a conventional diet can be implicitly considered as safe.

In cases where more complex genetic modifications are produced, e.g. via transfer of multiple genes in a single construct, re-transformation of pre-existing GM breeds, and trait stacking through conventional breeding of GM parents, strategies for the assessment of any risk(s) associated with possible interactions between the newly/differently expressed proteins/metabolites and original animal constituents should be discussed. A holistic approach for the assessment should be demonstrated considering all available information on e.g. the mode of action of the newly expressed proteins; the molecular, compositional, and phenotypic characteristics of the GM animal; and, where applicable, the outcome of animal toxicity studies and feeding trials. Where animal feeding trials are not performed, an explanation should be provided as to why these were not considered necessary.

2.3.2.6. Exposure assessment

With respect to intake estimations, the methodologies used for intake estimations of GM animal-derived foods for humans should be evaluated with respect to uncertainties associated with the prediction of long-term intake. Specific attention should be paid to those GM foods which are aimed at modifying nutritional quality. Post-market monitoring requirements for foods with modified nutritional qualities should monitor the occurrence of changes of the overall dietary intake patterns, the magnitude of such changes, and whether or not the product induces known or unexpected side effects. If a post-market monitoring is deemed necessary, the reliability, sensitivity and specificity of the proposed methods should be discussed.

2.3.3. The result of risk characterisation

In accordance with Articles 4 and 16 of Regulation (EC) No 1829/2003, the applicant should ensure that the final risk characterisation clearly demonstrates that:

- Consumption of GM animal- derived foods/feed from is as safe for humans/animals as the consumption of comparator(s)
- the GM food/feed does not differ from the food/feed which it is intended to replace to such an extent that its normal consumption would be nutritionally disadvantageous for the consumer/animal;
- The health and welfare of the GM animals is the same or no worse that its comparators.

The applicant should clearly indicate what assumptions have been made during the risk assessment in order to predict the probability of occurrence and severity of adverse effect(s) in a given population, and the nature and magnitude of uncertainties associated with establishing these risks.

The applicant should also include detailed information justifying the inclusion or the non inclusion in the application of a proposal for labelling in accordance with Articles 5(3)(f) and 17(3)(f).

2.4. Other considerations

2.4.1. Risk assessment of genetically modified animals with non-heritable traits

In general, this guidance document will not be valid for the safety assessment of food and feed products derived from GM animals with non-heritable traits, although some aspects may also be applicable in these cases. The differences, however, prevail. In the case of GM animals with heritable new traits, the safety of the GM animals is assessed by analysing a number of the GM animals, and products derived thereof. It is then assumed that the characteristics of other animals derived from the same GM founder animal will have similar characteristics. If on the basis of the safety assessment it is concluded that the new GM animal, and derived products, are safe for consumption by humans and animals, it is assumed that this will also pertain to other GM animals with the same characteristics.

This is not a valid approach in the case of GM animals with a non-heritable trait. In the latter case, the genetic construct will either not be incorporated into the animal genome, or at different positions in

different animals, and indeed in different cells within the animal. As a result, important parts of the safety assessment procedure as described in this document will not be applicable to GM animals with non-heritable traits. For instance, the molecular characterisation assumes that the genetic construct is stably integrated into the animal genome. As this is not the case in GM animals with non-heritable traits, the molecular characterisation can confirm the identity of the construct to be inserted, but will provide little or no information on the potential for unintended effects in individual animals or tissues as a result of insertional mutagenesis, as there is not a single situation that is representative for other animals or tissues/cells.

In the same way the comparative compositional analysis of a limited number of GM animals with new, non-heritable traits will provide only limited information for other animals with the same new, non-heritable trait in terms of unintended effects of the insertional event. It may provide indications for secondary effects of the genetic modification, but here it needs to be taken into account that 1) the dynamic range of intended effects may be much larger in the case of non-heritable traits compared to stably integrated heritable traits, and 2) unintended effects may occur in individual (non-heritable) GM animals or animal-tissues and not in others. For the same reasons it may be less relevant to perform animal feeding trials with materials derived from GM animals with non-heritable traits.

As a result it may be necessary to analyse (many) more animals in the case of animals with non-heritable traits compared with animals with stably integrated heritable traits, to better assess the dynamic range of intended effects. For the unintended effects: the chance of the occurrence of unintended effects will be relatively small, comparable with the situation in GM animals with heritable traits, and the resulting effects are likely to be even smaller, because the effect will not occur in all cells/animals.

In the case of GM animals with non-heritable traits, it may be prudent to confirm the non-heritability in subsequent generations.

In general, it will be difficult to develop a structured food and feed safety strategy and therefore a case-by-case approach should be followed with respect to the food and feed safety assessment of products derived from GM animals with non-heritable traits.

2.4.2. Potential risk for horizontal gene transfer

The applicant should assess any potential risk associated with horizontal gene transfer from the processed product to humans, animals and micro-organisms, if intact and functional nucleotide sequences remain after the processing events. The issue of horizontal gene transfer will be addressed in the guidance on environmental risk assessment of GM animals under preparation.

ASSESSMENT OF ANIMAL HEALTH AND WELFARE

1. GENERAL PRINCIPLES

The following subsections deal with the general principles of assessment of health and welfare of genetically modified animals. The concept of substantial equivalence and comparative approach as outlined above for the comparison of compositional and phenotypic characteristics also applies to the health and welfare assessment.

1.1 Potential effects of genetic modification on animal health and welfare

Previous studies on GM animals have indicated that genetic modification can result in either (i) better health and welfare, including in some cases better resistance to disease (e.g. after genetic modification with genes conferring disease resistant traits), (ii) no change from the average for unmodified animals (e.g. animals producing a pharmaceutical protein through their milk), or (iii) poorer health and welfare (e.g. additional increase in growth rate in a strain in which current fast growth rate has been shown to impair welfare).

The questions that must be considered are not only whether there is a change per se, but whether the change affects the animal's health and welfare.

The intention of the applicant producing a GMO will be to promote some form of potential benefit e.g. increased productivity, disease resistance. However, while that benefit might occur and be verified, there may be side effects i.e. unintended effects, that result. It is this latter point that is a cause for concern for animal health and welfare.

A wide range of assessment measures may be necessary because the actual effects on the individual will seldom be known and because species and individuals vary, both in the methods by which they try to cope with adversity and in the measurable signs of failure to cope. A simple indicator could show that welfare was not poor but absence of an effect on one indicator of poor welfare does not mean that the welfare is good and so it is good practice to use several indicators and a multi-disciplinary approach. For example, if the unintended side effect of a genetic modification was a behavioural abnormality or an increase in disease susceptibility but only growth rate was measured, an erroneous conclusion would be reached. The choice of measurements should include the main methods of assessing poor welfare (Broom and Johnson 2000, Broom and Fraser 2007, EFSA 2011c) but often it will be obvious from a preliminary study of phenotype, or a clinical examination, which measures will be most relevant.

Experience gained from conventional breeding and previous welfare assessments in EFSA opinions (EFSA 2009, 2010c), can also be useful for the assessment of health and welfare of GM animals. Multigenerational studies need to be carried out to see if there is any impact on health and welfare for successive generations. Decisions by the applicant will have to be made on the degree of poor welfare or health as well as its incidence before a GMO strain of animal is released into the commercial environment (field trials).

1.2 Assessment strategy

At some point, successfully developed GM animals are to be released from the laboratory environment for field trial. These environmental changes include exposure to a range of different stimuli and stressors related e.g. to:

- climatic conditions
- housing, husbandry and management conditions
- exposure to infectious agents

There are many differences between commercial farming systems within the EU, which may impact on the animal according to its genotype and the purpose for which the GM animal was made. Furthermore, the health and welfare of the animals may vary during different situations and time periods. There should be a comparator group, whenever possible, so that the specific effects of the genetic modification on the animals can be assessed.

Information gathered at all stages of developing GM animals should be considered during the assessment of health and welfare. Health and welfare consequences also need to be followed during its commercial lifetime (post-marketing monitoring). While the burden of proof is on the manufacturer to show that the health and welfare of the GM animal is acceptable, i.e. comparable to those of similar breeds/lines, a three-stage approach (A to C, see below) is suggested with the overall objective to identify the incidence of any adverse effect and its impact on the animal health and welfare.

Serious and frequently occurring, unexpected and unintended effects are likely to be detected during the laboratory stage, Stage A. During this stage measurement of the intended effect(s) of the genetic modification should also be performed. The detection of less frequently occurring unintended effects

is likely to require larger population size, and they will more likely be seen during field trials when the animals are exposed to field or normal production conditions. Post-marketing monitoring and surveillance should pick up unintended effects that occur at low frequency.

It is likely that for some species (cattle, sheep, goats, pigs) only a small number of animals will be available for health and welfare assessment but numbers will increase from Stage A to Stage C. For some species, however, large numbers will likely be available for assessment as early as Stage A e.g. fish, poultry and bees. Reference should be made to a comparator group and the differences between the GM animal line and the control group will be assessed. All animals showing adverse effects at Stages A and B are unlikely to meet the necessary criteria to be worth progressing further.

The species being studied will determine in detail what should be done in terms of the actual clinical observations, the tests to be carried out, and the times at which they are to be determined. The following concepts are applied:

- The animal is considered as the most sensitive indicator and measurements of observations of the animal and of its functioning (welfare outcome indicators[12]) are the most appropriate to use.

- It is important that those making welfare assessments consider the biological functioning of that species of animal and the environment in which it is placed. Those carrying out these assessments should have the appropriate knowledge and skills in assessing both the health and the welfare of the appropriate animal species, and should integrate welfare with health assessments.

The following general approach will allow the overall assessment of health and welfare.

- Clinical observations and examinations to detect deviations from normal behaviour conducted and recorded by acknowledged experts.

- Details of health and welfare monitoring and inspections conducted on a regular basis are given.

The evidence provided should demonstrate that the health and welfare of the GM animals is not significantly impaired. Normally, a comparison will be made on the health and welfare of the comparator(s) and the GM animal for their commercial lifetimes.

During the different stages of assessment (A through C), which comprise a tiered approach, observations and records should be devised so that they detect negative effects. Negative effects are interpreted as deviations from the comparator group, or in some cases from historical published data. The criteria used should be selected on a case-by-case basis, appropriate for the species and enable sound statistical analysis with the appropriate power. Furthermore, there may be a situation where the intended effect is so beneficial that it outweighs any small or possible negative effect but this decision is a management issue.

1.2.1 Monitoring at laboratory level (Stage A)

Objective: to define in laboratory conditions the intended effects and to determine the consequences of any possible unintended effect on the health and welfare of the animals. This is to be carried out in the laboratory at all generations and development stages, to assess individual GM animals for their health and welfare. This will include:

[12] A welfare outcome indicator is an observation, a record or a measurement used to obtain information on an animal's welfare that can be reliably used in practice by trained people. It may be the outcome of genetic selection or modification or of a period of housing, management, handling, transport, stunning or other treatment.

a. Genetics

- At the outset the choice of the line to modify is important e.g. the genetic profile of the parent stock (e.g. MHC diversity, freedom from genetic disorders, mitochondrial DNA disorders, etc) in order to minimise the chances of incurring poor health and welfare. Thereafter, with the objective of establishing a stable heritable line, technical failures and details such as copy number, viral vectors, targeted genome insertion sites, and promoters should be determined. In addition there is a need to include a standard set of measurements that are appropriately tailored to the genetic modification and the reproducibility of its effects whether intended or unintended.

b. Health status

This comprises several aspects and measurements as advised by formal or acknowledged experts in the relevant clinical specialities. For example, inter alia:

- Clinical examination and associated laboratory tests
- Zootechnical data e.g. normal development, growth rate, fertility, feed conversion rate.
- Other measurements of physiological aspects such as immune function e.g. immunological challenge with antigens producing a B-cell or T-cell response, heat stress tests to see how animals could cope with varying climatic conditions.
- Health records of disease (i.e. free of major infectious diseases) of parent stock from which the GM animals are derived or produced (e.g. surrogate dams) e.g. evidence of the results of post mortem and associated laboratory examination, of all animals found sick or dead to identify the cause of death, post mortem examination of all animals slaughtered or euthanised at the end of the experiments, incidence of body injuries, body malfunctions and disease should be measured.

c. Welfare status

Any measure used to assess welfare should be outcome based and have been validated, as well as being reliable and robust. There is a large scientific literature detailing measures used in animal welfare research. A comprehensive range of these measures should be used and amongst those to consider are the welfare outcome indicators described in the Welfare Quality reports (Welfare Quality®, 2009) and EFSA report on animal based welfare indicators (EFSA, 2011d). One group of measures concerns the ability of GM animals to carry out normal behaviour and physiological functions and to develop normally. Other direct indicators of good and poor welfare are of normal and abnormal physiology, behaviour, brain function, immune system function and ability to grow and breed. Abnormal behaviours may relate to feeding, locomotion, social behaviour (e.g. aggression), other responsiveness measures such as flight distances and the occurrence of stereotypies including the extent to which strongly preferred behaviours can be shown and the extent of any behavioural aversions.

d. Pre-birth

Any possible effects on the surrogate dam during pregnancy e.g. due to fetal size, endocrinological effects, including analysis of offspring aborted and born dead.

The information gathered above would be used to identify the most relevant measures that should be followed in Stage B.

1.2.2. Experimental field trial assessment (STAGE B)

Objective: to determine the health and welfare consequences on the animal of the intended and any unintended effects of the genetic modification under controlled farming conditions.

It will be necessary to assess the impact of the genetic modification on a larger number (e.g. 10s) of animals exposed to normal environmental challenges under semi-commercial conditions on specified, registered farms e.g. experimental farms. They would have a higher standard of stockmanship than most commercial farms in that the GM animals could be monitored more carefully than normal. In addition, specific extra tests may need to be carried out to determine detailed aspects of a GM animal's health and welfare. These tests may be more invasive than the simple observations required at later stages e.g. require blood sampling, some specific testing such as flight distances, heat stress.

In general the tests are likely to be similar to those in Stage A but it is likely that any common intended and common unintended effects will have been identified during Stage A. Thus the emphasis at this stage should be directed to subtler changes in health and especially the behaviour of the GM animal in relation to other animals. It might also be possible to discontinue some laboratory tests that proved negative during Stage A. All findings will be compared with in-contact non-GM animals of the same species.

Surveillance of breeding fitness and tests on the offspring of the GM animals should commence at this stage. Potential specific genetic environmental interactions in future husbandry systems are likely to be seen at this stage.

The information gathered above would be used to assess the most relevant measures that should be followed in Stage C.

1.2.3. Field trial assessment (STAGE C)

Objective: to determine any unintended effects of the GM animal during its use in commercial farm settings.

Based on the findings in Stages A and B, GM animals would have to be released from the control of the animal research legislation before being further tested for their health and welfare. At that point it would be possible to apply for authorisation for the use of GM animals in field trials. These trials would involve the surveillance of 100s numbers of animals on several farms/holdings operating under normal commercial conditions. The trials should be on GM animals and, where possible, comparator non-GM animals, on commercial farms with varying types of husbandry and housing conditions, and varying environmental conditions.

It will be important to monitor the physical, biological and social environments on these farms so that any changes identified for the first time at this stage can be related to hazards/factors identified on the farms where changes were observed. These trials should incorporate the principles of blinded Randomised Control Trials. Where possible, the stockmen and observers would be blinded to the identity of the GM animals and comparisons should be made with animals of the same breed and strain but without the genetic modification. In some circumstances, the phenotypic colour typing or size of the GM strain may cause a practical problem.

The animals should be examined in a similar way and with the same procedures as for Stage B. However the examinations and tests can be modified depending on the outcome from Stages A and B and the intervals between tests could be extended, subject to epidemiological advice concerning the numbers of animals and time needed to establish the evidence for change or substantial equivalence as appropriate. This stage should cover several production cycles.

The information gathered above would be used to assess the most relevant measures that should be followed in post-marketing monitoring.

POST MARKET MONITORING (PMM)

A. POST MARKET MONITORING AND SURVEILLANCE OF HEALTH AND WELFARE OF GM ANIMALS

The objective of post market monitoring and surveillance is to determine unintended effects of the genetic modification on large numbers of animals and in more varied commercial conditions.

At the conclusion of the three-stage evaluation process, there should be a post-marketing programme of monitoring and surveillance to look for long-term unexpected effects with low incidence. Traceability of the GM animals will be an essential prerequisite for such a monitoring.

Monitoring of health and welfare will also be dependent on reporting any adverse effects by veterinarians, farmers and others (e.g. yellow card system). The adverse events reports will have to be labelled as urgent or routine, in line with EU legislation on the reporting of adverse health effects of GM animals after their release on to the open market.

As for the previous stage, any scientific studies evaluating whether or not there are negative effects of a GM procedure may fail to do this if the expression of the effect is not evident in all individuals and the study sample size is too small.

B. POST MARKET MONITORING (PMM) OF GM ANIMALS-DERIVED FOOD AND FEED

Where appropriate a Post Market Monitoring (PMM) programme should be performed for GM animal-derived food/feed. The appropriateness of performing a PMM is indicated by findings in the pre-market safety assessment. A PMM does not substitute for a thorough pre-marketing toxicological and nutritional testing programme but complements it in order to confirm the pre-market risk assessment. It may increase the probability of detecting rare unintended effects. Therefore the PMM for GM food/feed should be designed to generate a reliable and validated flow of information between the different stakeholders in order to potentially relate GM food/feed consumption to any (adverse) effect on health.

As pre-market risk assessment studies cannot fully reproduce the diversity of the populations who will consume the marketed product, the possibility therefore remains that unpredicted side effects may occur in some individuals of the population, such as those with certain disease and susceptibility states (i.e. allergic consumers, see Section 2.1.5) or people with impaired digestive functions (see Section 2.1.6.1), those with particular genetic/physiological characteristics or those who consume the products at high levels. Indeed, risk assessment also relies on an estimate of exposure to the food/feed, which is variable and subject to uncertainty before the food/feed is marketed. A PMM should therefore address the following questions: i) is the product use as predicted/recommended (refer to the corresponding paragraph), ii) are known effects and side-effects as detected during the pre-market risk assessment as predicted and iii) does the product induce unexpected side effects (Wal et al., 2003). However, it needs to be realized that a PMM may not always have the sensitivity to estimate individual intake of a specific food item or intakes of particular age groups. Given the practical difficulties in performing a PMM, it should be required only in specific cases. Those cases could include GM (functional) food/feed with altered nutritional composition and nutritional value and/or food/feed altered to achieve specific health benefits. Because of its specific properties, the intake of this type of GM food/feed might be increased compared to the intake of the conventional counterpart, which could result in a significant impact on the long-term nutritional and health status of some individuals of the population.

RECOMMENDATIONS

There is a very limited amount of background data available on, for instance, the composition of the broad variety of different animal-derived food and feed products, which could be used to characterize the safety and nutritional status of the products. This may hamper the interpretation of potentially observed differences in a comparison of GM animals and derived food and feed products with

conventional comparators. It is recommended to establish informative databases on the composition of the broad range of animal-derived food and feed products, including the natural variation in the different constituents in similar products derived from different breeds or breeds reared under different housing and feeding conditions.

There is a need for further development of databases that compile omics profiles of animal-derived food and feed materials for risk assessment. The development and use of *in vitro/situ/silico* methodologies in concert and analytical techniques is highly encouraged in order to reduce/replace testing with animals. Omics technologies have in recent years demonstrated their potency in biological sciences. Omics data may provide valuable information regarding possible alterations in composition of the GM animal-derived food and feed indicating the presence of possible unintended effects.

Finally, it is important to develop consensus documents on the composition of food and feed derived from different animal species. These documents will provide information that is important, on a consensus basis, for the risk assessment of these foods/ feeds. To this end, the documents should compile information on the major nutrients, toxicants, anti-toxicants and allergens of specific animal derived food and feed.

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