SCIENTIFIC OPINION

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

EFSA Panels on Genetically Modified Organisms (GMO) and

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This document provides guidance for the risk assessment of food and feed containing, consisting of or produced from genetically modified (GM) animals, as well as for the health and welfare assessment of these animals, 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 assessment, which addresses the possible impact of biologically relevant change(s) in the GM animal and/or derived food and feed, the 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 food and feed derived from 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 in a given environment. The document does not cover the environmental risk assessment of GM animals, which will be addressed in stand-alone guidance under development by the EFSA GMO Panel.

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[2] GMO Panel members Hans Christer Andersson, Salvatore Arpaia, Detlef Bartsch, Josep Casacuberta, Howard Davies, Patrick du Jardin, Gerhard Flachowsky, Lieve Herman, Huw Jones, Sirpa Kärenlampi, Jozsef Kiss, Gijs Kleter, Harry Kuiper, Antoine Messéan, Kaare Magne Nielsen, Joe Perry, Annette Poeting, Jeremy Sweet, Christoph Tebbe, Atte Johannes von Wright, and Jean-Michel Wal. AHAW Panel members: Anette Bøtner, Donald Broom, Marcus G. Doherr, Mariano Domingo, Jörg Hartung, Linda Keeling, Frank Koenen, Simon More, David Morton, Pascal Oltenacu, Fulvio Salati, Mo Salman, Moez Sanaa, James M. Sharp, Jan A. Stegeman, Endre Szücs, Hans-H. Thulke, Philippe Vannier, John Webster and Martin Wierup. One member of the GMO Panel did not participate in the discussion on the subject referred to above because of potential conflict of interest identified in accordance with the EFSA policy on declarations of interests. Correspondence: GMO@efsa.europa.eu or AHAW@efsa.europa.eu

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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 (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 of or produced from genetically modified (GM) animals, within the framework of Regulation (EC) No. 1829/2003 on GM food and feed and guidance on the animal health and welfare of these animals. The document does not cover the environmental risk assessment of GM animals, which will be addressed in a stand-alone environmental risk assessment (ERA) guidance document developed by the EFSA GMO Panel.

The proposed strategy for the health and welfare assessment of the GM animals and the risk assessment of GM animal-derived food and feed seeks to deploy appropriate approaches to compare GM animals and derived food and feed with their respective comparators.

In relation to the food and feed risk assessment, the underlying assumption of this comparative approach is that traditionally-bred animals have a history of 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 and feed safety assessment of GM animals or their products and the welfare of the GM animals. The health status of a food and feed producing animal has traditionally been considered as an important indicator of the safety of derived food and feed and, 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 also addresses the details of the other components of risk assessment: 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 possible 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.

This guidance document also addresses the scientific requirements for the assessment of health and welfare of GM animals. The assessment is made in terms of the effective functioning of their body systems 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 is considered.

Post-Market Monitoring of the GM animals and their derived food and feed is also discussed.

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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 microorganisms. 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 this 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 from 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 related to GM animals.

At the beginning of 2009, EFSA implemented a Working Group (WG) of the EFSA Panel on Genetically Modified Organisms (GMO Panel) to consider the molecular characterisation and the food and feed safety assessment of products derived from GM animals. In 2010, EFSA implemented a WG of the EFSA Panel on Animal Health and Welfare (AHAW Panel) to deal with animal health and welfare aspects of GM animals. On the basis of deliberations in these WGs and the existing guidance documents on the subject (e.g. Codex Alimentarius Commission guidelines), as well as any other relevant background information available in the European Union and elsewhere, and comments received during the public consultation, the EFSA GMO and AHAW Panels prepared the present document that provides detailed guidance for the safety assessment of genetically modified animal-derived food and/or feed containing, consisting of or produced from these animals and guidance on their health and welfare assessment.

This guidance does not address issues related to risk management (traceability, labelling, coexistence). Ethical and socio-economic issues are also outside the scope of the document. 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.

To address the request of the European Commission for guidance on the environmental safety of GM animals, the EFSA GMO Panel considered background information provided by external contractors[4] and is currently developing the corresponding guidance document.

[4] http://www.efsa.europa.eu/en/supporting/pub/69e.htm
http://www.efsa.europa.eu/en/supporting/pub/71e.htm
http://www.efsa.europa.eu/en/supporting/pub/107e.htm

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 a guideline on the safety evaluation of GM animals, building on work carried out in the context of the Codex Alimentarius Commission, 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 would 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 EFSA to develop additional guidance on animal health and welfare aspects and, in parallel, to include relevant animal health and welfare aspects in the guidance on GM animal-derived food and feed safety assessment.

A. INTRODUCTION

This guidance document addresses the assessment of the safety of GM animal-derived food and feed for humans and animals, placed on the market within the framework of Regulation (EC) No 1829/2003[5]. It also addresses the health and welfare assessment of the GM animals. Guidance for the deliberate release of GM animals into the environment will be addressed separately in line with the Directive 2001/18/EC[6].

The 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, as described in Annex IA, part 1, of 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.

The scope of the guidance includes GM animals with new, heritable traits to be placed on the market. Food and feed derived from animals with introduced non-heritable traits are not covered. 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 C.2.4.1).

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. However, due to the variety of animal species covered, some aspects of this guidance may not be relevant for all species. 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 lines, and associated AHAW aspects, will have to be determined on a case-by-case basis.

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 in order to guarantee the quality of the resulting animal product, especially in terms of food safety aspects (hygiene for food of animal origin[7]). 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).

The aim of the safety assessment is to ascertain that the 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

[5] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (Text with EEA relevance). OJ L 268, 18.10.2003, p. 1–23

[6] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC - Commission Declaration. OJ L 106, 17.4.2001, p. 1–39.

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

be identified, a comparative safety assessment cannot be made and a comprehensive safety and nutritional assessment of the GM animal-derived food/feed 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 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) and 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

The risk assessment strategy for GMOs seeks to deploy appropriate methods and approaches to compare the GMO and derived products with their comparator(s). To this end, the concept of substantial equivalence was developed by WHO (WHO, 1991) and OECD (OECD, 1993) and subsequently 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 the Codex Alimentarius (Codex Alimentarius, 2008). The underlying assumption of this comparative assessment approach for GM animals is that traditionally-bred animals have a history of consumption as food and feed for the average consumer or animal to which the animal-derived products are fed. This equally applies to Animal Health and Welfare (AHAW) assessment. These traditionally-bred animals can serve as a baseline for the health and welfare assessment of the GM animals and the risk assessment of GM animal-derived food and feed.

In general, parallels can be drawn with the safety assessment of GM plants, for which guidance has already been developed (EFSA, 2011a). 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 useful). Application of this concept serves the purpose of identifying similarities and differences between the GM animal-derived food/feed and its 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 to GM plants. The health and welfare status of a food and feed producing animal has traditionally been considered as an important indicator of the safety of derived foods and feed. The practice of only allowing animals with known and acceptable health and welfare status to enter the human and animal food/feed supply is considered to be an essential step for ensuring safe food and feed. This approach will also be used for the safety assessment of GM animals.

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, feed intake, 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.

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 phenotypic 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 of any identified differences resulting from intended and 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 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 focused on the identification and characterisation of such effects with respect to a possible impact on animal health as such or following the consumption of GM animal-derived food or feed products by humans and animals, respectively.

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.

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.

C. 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 or group of foods (Codex Alimentarius, 2011). In the case of food and feed derived from GM animals, the hazard identification covers those hazards to which humans and animals, respectively, will be exposed through consumption. 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 may determine additional

information or 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 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. For chemical agents, a dose-response assessment should be performed. For biological or physical agents, a dose-response assessment should be performed if the data are obtainable (Codex Alimentarius, 2011)

This step focuses on the possible quantification of the potential toxicological and/or nutritional effects of the identified differences between the GM animal and derived food/feed and the comparator(s). Studies on laboratory animals and/or target animals may provide useful information for the hazard characterisation. An appropriate test model and suitable test material should be used in order to generate data identifying the onset of adverse effects, and possible dose-response relationships.

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 the food and feed derived from the GM animal, and the magnitude, frequency and duration of such exposure. It is necessary that every significant source and route of exposure is identified. In particular, it is of interest to establish whether the intake of the GM animal-derived products and their constituents is expected to differ from those derived from the conventional product. In this respect, specific attention should be paid to GM animal-derived food/feed with modified nutritional properties. 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 characterisation and exposure assessment' (Codex Alimentarius, 2011).

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 derived 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 analysis of key components 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-derived 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: i) family, ii) genus, iii) species, iv) subspecies, v) breeding line or strain, vi) 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 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 and vectors 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 the methods and criteria used for selection. When relevant this will include a description of the technologies used to remove part of the insert, to limit the chance of mobilisation of the insert, or to drive the trait through the population;

b. The cellular or tissue material to be transformed;

c. Nature and source of vector(s) used for transformation, including:

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

- 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.

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

e. The purity of the preparation containing the construct prior to introduction into recipient animals or cells.

2.1.2.1.2. Source and characterisation of nucleic acid 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 should be provided on the origin of the 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). Information regarding each donor organism should comprise its taxonomic classification and its history of use regarding food and feed safety.

In case of synthetic nucleotide sequences with no gene counterpart in existing organisms, information should be provided on the design and the functional elements of the synthetic nucleotide sequences introduced.

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

- History of 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 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);

- Available 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 introduced or modified

The introduced trait(s), its mode of action, and the resulting changes in the phenotype 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 animals, including the breeding strategy, information on whether the

initial GM animal was mosaic, whether the GM animals were to be marketed, are hemizygous or homozygous with regard to the sequence(s) actually inserted, and the ploidy of the GM animals to be marketed.

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 the 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. The analysis should span the entire insert 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 the nuclear or mitochondrial genome, or maintained in a non-integrated form) and methods for ascertaining those sub-cellular location(s) of the insert(s);

e. Sequence information for both 5' and 3' flanking regions at each insertion site, with the aim of identifying interruptions of known genes and functional elements, 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)[8] 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. 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 metabolite(s) may be the most informative. The assessment of risk associated with a change of protein and metabolite level(s) is covered in Section C.2.1.4.

Data should be derived from animals bred, fed and reared under representative conditions. 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 should be provided. The requirement for information on developmental expression should be

[8] Open Reading Frames 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.

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.

In 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). If the genetic modification results in newly expressed protein(s) and where the analytical method has been shown to be specific, the comparative approach is not applicable.

The applicant should provide the following information:

a. Description of the method(s), including specificity and sensitivity, used for expression analyses;

b. The mean and range of concentrations of newly produced proteins or expression levels of 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 related 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 on the expression of the inserted/modified sequence may also be required for the assessment of impacts on other organisms (see ERA GD under preparation). In such cases, information on expression in various parts of the animal during development is required. Data should be related to the conditions in which the animals are bred, fed and reared in Europe.

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

Information should be provided to demonstrate the inheritance and 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).

The applicant should provide data on the inheritance pattern and the stability of the introduced/modified nucleotide sequences and associated phenotypes in the offspring across multiple sexual generations, dependent on the animal species. The source of the material, the sampling design, the number of animals used for the analysis and the number of generations should be specified and clearly indicated on the breeding diagram.

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 issues.

- It should be specifically indicated whether the molecular characterisation of the genetic modification(s), raises safety issues with regard to production of unintended proteins/products, including new toxins or allergens, as well as the potential mobilisation of the insert.

- 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 of the GM animals and derived products, and their non-GM counterparts, 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 and physiology; and

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

GM animals and their derived products should be representative of those which will enter the market. 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 should be carried out. This would be the case where the GM animal is not closely related to an animal with a history of 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. Criteria for the selection of the comparator(s)

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 which has a history of consumption as food and feed. The term "comparator" should be used in all other cases (i.e. cases in which the comparative assessment includes genotypes which do not fit the definition of a conventional counterpart, as provided above).

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). 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.

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, 2010a). Certain genetic modifications may result in husbandry conditions that are appropriate for the GM animals but are 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 of typical commercial practice as possible, with conditions that approximate as closely as possible to those that produce a food/feed product with a history of 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 this 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 animal and its comparator, but such that both can be reared without unacceptable risk of mortality or adverse health and welfare issues. Both the GM animal and its comparator must be reared under these selected conditions.

2.1.3.2. Animal trials for comparative analysis - experimental design and 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, 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 already described by EFSA (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 consumption as food and feed. Identified differences should be placed into a 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 recognised 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 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 are 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 be 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 the 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 in order 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 computerised 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, in order to assess whether the effects of the genetic modification are influenced by such practices. Similarly, and also 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 minimised. 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.

All test materials, the GM animal and any comparator(s), should be fully and properly randomised 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, to avoid a loss of statistical power.

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 undertaken at the planning stage of the study. The power analysis should use only information verifiable as available prior to the study, and 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 carried out, 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, if feasible, 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 undertaken for biological reasons, and the reason why they are categorised so should be explained. 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 trials, should be comprehensively described and fully justified. The use of all veterinary drugs and the reason for their administration should be described fully.

The applicant should list explicitly, in writing, all the questions that each trial is designed to address. 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), focusing on the biological relevance of these differences within the context of hazard 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 consumption as food and feed employed to test equivalence and/or any extra comparators included within the design. When an equivalence test is carried out 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 (see Sections D.1.2.1 and D.1.2.2) 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, for example, the results of physical inspection, clinical and physiological examination, the records of illnesses and therapies, feed intake, performance parameters such as growth and development, feed efficiency, abnormal behaviour 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 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 comparator(s). 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 material 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 C.2.1.3.4.3). The

preparation of the test material and the analyses must be carried out according to appropriate quality standards. Examples of materials to be used for analysis on a case-by-case basis are given in Table 1.

Table 1: Examples of materials to be used 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 use	Tissues: - Meat, muscle (M.long.dorsi; M.bic.femoris) - Body fat (site or organ specific fat) - Blood - Some organs (liver, kidney, spleen, brain, etc.) - Residue body (e.g. meat and bone meal as feed)	Tissues: - Meat, muscle (breast, thigh) - Abdominal fat - Blood - Some organs (liver, kidney, spleen, etc.) - Residue body (e.g. animal body meal as feed)	Tissues: - 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 C.2.1.4.3), the compositional analysis should be carried out for an appropriate range of constituents. In each case, key measures should include macro- and micro-nutrients, as well as bioactive compounds (if identified as important, for example, hormones and growth factors), and key allergens (EFSA, 2010b) (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 C.2.1.5).

Key nutrients present in a specific food and feed are those components that have a major impact on human/animal health (i.e. proteins, carbohydrates, lipids/fats, vitamins and minerals). 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 analysed taking into consideration 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.

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), pasteurisation/sterilisation, or fermentation. Processed products may be assessed together with 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 products from the non-GM counterpart. 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:

- Whether biologically relevant differences have been identified in phenotypic characteristics between the GM animal and its comparator(s), except for the introduced trait(s);
- In case an equivalence test was performed, whether phenotypic characteristics of the GM animal are, except for the introduced trait(s), equivalent or not to the reference breeds, taking into account natural variation;
- Whether biologically relevant differences have been identified in compositional characteristics between the GM animal and its comparator(s), except for the introduced trait(s);
- In case an equivalence test was performed, whether compositional and phenotypic characteristics of the GM animal-derived food/feed are, except for the introduced trait(s), are 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 on both 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 ensure 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/target 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 (Smith, 2002; Renwick et al., 2003).

Toxicological assessment should be performed:

a. To demonstrate that the intended effect(s) of the genetic modification has no adverse effects on human and animal health upon consumption of the GM animal and derived food or feed. The 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 are assumed to have occurred based on the preceding molecular analyses and comparative phenotypic and compositional analyses, have no adverse effects on human and animal health upon consumption of the GM animal and/or derived food or feed.

It is emphasised that, in general, toxicological testing of most GM animal-derived food and feed and specific food and feed constituents will not be necessary.

The requirement for toxicological testing should 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 comparator(s), including intended as well as unintended changes). In principle, the assessment should consider: i) the presence of newly expressed proteins, ii) the potential presence of other new constituents, iii) the possible changes in the levels of natural constituents beyond normal variation, and/or iv) the impact of other changes in composition due to the genetic modification. The specific information requirements and testing strategies are outlined in the following sections.

GM animals should be evaluated for potential effects of newly/differently expressed bioactive substances with, for example, endocrine, pharmacological or immunological activity, as part of the overall animal health evaluation. It is possible that such substances (e.g. hormones) may be active in humans. Consideration should therefore be given to potential dietary exposure to the newly expressed substances or to altered levels of naturally occurring compounds and 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 section, 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, when required, internationally agreed test methods described by the OECD[9] or by the European Commission (EC, 2002). The most up-to-date version of any test guideline should be followed. Test protocols may need to be adapted for the toxicological testing of GM animal-derived products. Any 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

[9] 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

substances[10]. If such tests are carried out they should follow the OECD Principles of Good Laboratory Practice[11] (GLP). With regard to studies other than toxicological studies, they should be conducted under ISO or GLP standards or other appropriate quality assurance.

Selection of test protocols depends on the type of GM animal-derived food/feed, type of the genetic modification and resulting intended and unintended alterations, intended use and exposure/intake, and the available knowledge, and should be scientifically justified and documented.

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 C.2.1.4.3 to C.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

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 the GM animal's health 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 expressed proteins have a proper use and consumption as food and feed, and where no negative impact of the genetic modification on the GM animal's health or welfare status (see Section D.1.4.1.) 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 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, for example, microorganisms, is used, the structural, biochemical and functional equivalence of this substitute to the newly 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 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

[10] Directive 2004/10/EC of the European Parliament and of the 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. OJ, L50, 44-59.
[11] OECD Principles of Good Laboratory Practice. Available from http://www.oecd.org/document/63/0,3746,en_2649_37465_2346175_1_1_1_37465,00.html

between the newly expressed proteins and other animal constituents should be evaluated with respect to the 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 expressed protein to proteolytic enzymes (e.g. pepsin), for example, 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. Repeated dose toxicity studies using laboratory animals may be required, 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[12]. 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 other than proteins

Identified new constituents other than proteins, for instance, bioactive compounds with, for example, endocrine, pharmacological or immunological activity, should be evaluated. Here, the health status of the GM animal physiologically producing these compounds should also be carefully observed as a basis for the subsequent food and feed safety assessment strategy. The 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. To establish the safety of new constituents having no history of consumption as food and feed, information analogous to that described in the EFSA "Draft guidance on submission for food additive evaluations"[13] and 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[14], should be provided.

[12] OECD Guideline for the Testing of Chemicals. Adopted by the Council on 27th July 1995. Repeated Dose 28-day Oral Toxicity Study in Rodents. Available from http://www.oecd.org/dataoecd/50/41/37477972.pdf

[13] Public consultation on the draft guidance on submission for food additive evaluations. Available from http://www.efsa.europa.eu/en/consultations/call/111117.htm

[14] Commission Regulation (EC) No. 429/2008 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. OJ L 133, 22.5.2008, p.1.

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 constituents in the GM animal-derived food and feed is altered beyond their natural variation.

Natural food and feed constituents comprise a large variety of substances (e.g macro- and micronutrients, and anti-nutrients, as well as other animal metabolites including bioactive compounds). 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, the health status of the GM animal physiologically producing these compounds should also 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 analysis of the health status and phenotypic characteristics and a 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 with specific tissues and/or organs of the GM animal may be considered or, in specific cases, also other toxicological studies. 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 toxicity study with whole food and feed derived from a GM animal should be performed according to the principles of OECD guideline 408[15] following the guidance for a repeated dose 90-day toxicity study as provided by EFSA (EFSA, 2011a, b).

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. 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. The applicant should justify both the selected lowest and highest dose level. 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 will need to be assessed on a case-by-case basis. 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. The statistical analysis should focus on the detection of possible differences between animals fed the test material and animals fed 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

[15] OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents. Available from http://www.oecd-ilibrary.org/environment/test-no-408-repeated-dose-90-day-oral-toxicity-study-in-rodents_9789264070707-en

feeding trial further studies may be considered (e.g. studies on reproductive/developmental effects, chronic toxicity).

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 an animal model for non-ruminants; lambs for ruminants; or other rapidly growing species). 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 C.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 assessed as 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 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 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 assessment should be evaluated in the light of anticipated intake of the GM animal-derived food/feed (see Section C.2.2).

2.1.5. Allergenicity assessment

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 is the only form causing acute 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: i) *sensitisation* where no symptoms occur while the capacity of the immune system to react increases dramatically, and subsequently, ii) *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 with: i) 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[16]); 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 having 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 *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

[16] Transgene: a gene or genetic material that will be transferred from one organism to another.

amino acids is considered a minimal requirement (EFSA, 2010b). All sequence alignment parameters used in the analysis should be provided, including calculation of percentage 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 optimise 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 a 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 standardised conditions (Thomas et al., 2004), at low pH values and high pepsin:protein ratios. It is recognised 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 under 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 and products from the GM animal

In general, foods of animal origin are a source of common food allergens[17]. In addition, it is noted that the most important ones (e.g. milk, eggs) mainly affect young children for whom these food allergies are the first way of sensitisation.

There are different allergenic proteins present in allergenic food of animal origin. Additionally, several isoforms that may differ by small changes in the amino acid sequence or post-translational modifications may be identified for each of these allergenic proteins. The allergen profiles, qualitatively and quantitatively, may vary between the breeds, the animals, and even for each individual animal, depending on the age/physiological status and environment. Furthermore, animal products are complex food matrices in which interactions between proteins and other constituents may occur and which 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 *versus* non-GM animals.

It can be noticed that, in this particular situation, the hazard is clearly identified; however, in most cases hazard and risk characterisation might not be feasible due to the lack of detailed information on exposure of at risk groups of the population and, essentially, also because no data on dose-response relationships (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 to be common food allergens (e.g. milk, eggs, fish, etc.) 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 based on, for example, the compositional analysis that the allergenicity of the whole GM animal-derived food might be substantially, 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 investigated.

To assess any possible increase of the 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 E.2) on Post-Market Monitoring).

Should the genetic modification be aimed at reducing the allergenicity of the animal products, evidence should then be given using 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.

[17] Directive 2007/68/EC of the European Parliament and of the Council of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients. OJ, L310, 11-14.

2.1.5.3. Adjuvanticity

Adjuvants are substances that, when co-administered with an antigen, increase the immune response to the antigen and therefore might also increase the allergic response. In cases when known functional aspects of the newly expressed protein or structural similarity to known strong adjuvants may indicate possible adjuvant activity, the possible role of these proteins as adjuvants should be considered. As for allergens, interactions with other constituents of the food matrix and/or processing may alter the structure and bioavailability of an adjuvant and thus modify its biological activity (EFSA, 2010b).

2.1.5.4. 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 substantially, 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 assess any possible increase of allergenic risk under 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. Where applicable, 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 nutritionally important endogenous constituents in the GM animal-derived food and feed; and 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 C.2.1.2. and C.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 with respect to their nutritional impact. 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 a comparator (see Section C.2.1.3) and the introduced trait(s) have no nutritional impact, no further studies to demonstrate nutritional equivalence are required. If, on the basis of the comparative assessment, it is not possible to conclude anything about the nutritional equivalence, further studies should be carried out (see Sections C.2.1.6.1 and C.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 in relation to the non-GM counterpart should consider:

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

b. The nutritional quality of the GM animal-derived food (after required transport, storage and expected treatment of the foods);

c. The anticipated dietary intake of the GM animal-derived food (see Section C.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. pigs), whereas additional nutritional data relative to food may require studies with the target species. Additional information may also be available where a 90-day oral toxicity study in rodents has been carried out as part of the toxicology assessment (see Section C.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 and its composition has not been significantly altered, it may not be necessary to perform additional nutritional studies in animals. However, if there are specific reasons, 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 which 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 *in vitro* and/or *in vivo* for digestibility and/or bioavailability should be selected on a case-by-case basis depending on the food 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.

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 i) the Regulation (EC) No 1069/2009 on health rules animal by-products not intended for human consumption[18] replacing Reg (EC) No 1774/2002 ii) Reg (EC)

[18] Regulation (EC) No 1069/2009 of the European Parliament and the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation) OJ, L300/1

142/2011[19] on the implementing rules of Reg (EC) No 1069/2009 and iii) Regulation (EC) No 999/2001 (TSE Regulation)[20].
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 the current legal restrictions mentioned above, on a case-by-case basis to assess the impact of the GM feed. Where applicable, a control diet containing its comparator(s) may be formulated by supplementing it with a 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, also considering 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 target animals to test the nutritional value of GM animal-derived feeds modified for enhanced nutritional characteristics will depend on a number of factors, including 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 (ILSI, 2007; EFSA, 2008).

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 to its conventional counterpart, taking natural variations into account.

The results of the nutritional assessment should be evaluated in the light of the 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 is 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 up to date appropriate consumption data should be used. Data on import and production quantities may provide additional information for the intake assessment.

[19] Commission Regulation (EU) No 142/2011 of 25 February 2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive OJ, L 54/1

[20] Regulation (EC) no 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies. OJ, L 147/1

The concentrations of the newly expressed proteins, other new constituents and natural constituents occurring at altered levels in consumed parts of the GM animal as a result of the genetic modification (e.g. due to changes in metabolic pathways) should be determined using appropriate methods. The exposure assessment of food constituents should consider the contribution from the total diet and not only the fraction from the GM animal-derived food and feed products. Thus, taking into account that processing, storage and expected treatment of food/feed may alter the levels of food/feed constituents, the expected intake of these constituents from the total diet should be estimated based on the average and maximum data on content, and mean and high (e.g. 97.5th percentile) food/feed intakes.

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 the case where the GM animal-derived foods are expected to provide 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 analyses, including molecular analysis, comparative analysis of phenotypic characteristics (including health status) and compositional analysis, toxicity and allergenicity testing and nutritional assessment. 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 only be 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 the husbandry practices 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 the characterisation and exposure assessments. However, 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 for identifying the need for specific analyses (e.g. occurrence of specific toxins or allergens in the unmodified recipient animal which may be unintentionally increased as a 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 genetic modifications and expression of nucleotide sequences.

Where molecular characterisation has identified potential hazards, additional molecular analysis, compositional and phenotypic analysis, or other analyses should be used to show that there is no safety issue.

2.3.2.2. Comparative assessment

The goal of the comparative safety assessment is to identify possible differences between the GM animal and its comparator(s). These differences in the composition of the GM animal compared to its comparator(s) should be assessed with respect to their possible impact on food and feed safety or nutrition. The estimated risk and associated uncertainties should be as measured as possible and taken into account.

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. The choice of the comparator(s) is the key and its selection should be justified, in particular with respect to its history of consumption as food and feed and the evidence 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.

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

If statistically significant differences are observed, using the methodology as described under Section C.2.1.3.2, the following background data may be considered in order to put them into context with respect to their potential relevance for 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 analysed, 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 for 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, also taking 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 uncertainty 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 animals 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 intake levels, 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, for instance, 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

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. Post-market monitoring requirements for foods with modified nutritional qualities should monitor the occurrence of changes in 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

The applicant should ensure that the final risk characterisation clearly demonstrates that:

- Consumption of GM animal-derived foods/feed is as safe for humans/animals as the consumption of comparator(s);
- The GM animal-derived 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 than 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) of Reg. (EC) No 1829/2003.

2.4. Other considerations

2.4.1. Risk assessment of animals with introduced non-heritable traits

In general, this guidance document is not applicable for the safety assessment of food and feed products derived from animals with introduced non-heritable traits, such as, for example, immunisation of animals with naked DNA in order to improve the health characteristics of the product or animals expressing growth hormone for productive improvement, 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 these 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, however, 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 animals with introduced non-heritable traits. In the latter case, the genetic construct will either not be incorporated into the animal genome, or in 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 these animals. For instance, the molecular characterisation assumes that the genetic construct is stably integrated into the animal genome. As this is not the case in animals with introduced 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 no single situation that is representative for other animals or tissues/cells.

In the same way, the comparative compositional analysis of a limited number of animals with new, non-heritable traits will provide only limited information for other animals with the same 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: i) the dynamic range of intended effects may be much larger in the case of non-heritable traits compared to stably integrated heritable traits, and ii) unintended effects may occur in individual (non-heritable) 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 animals with introduced non-heritable traits.

As a result, it may be necessary to analyse (many) more animals in the case of animals with new, non-heritable traits compared with animals with stably integrated heritable traits, in order to assess better the dynamic range of intended 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 animals with introduced 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 animals with introduced non-heritable traits.

2.4.2. Assessment of the potential risk associated with horizontal gene transfer

The applicant should assess any potential risk associated with horizontal gene transfer from the GM animal and its products to humans, animals and microorganisms. The issue of horizontal gene transfer will be addressed in the guidance on environmental risk assessment of GM animals which is under preparation.

D. 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 a comparative approach, as outlined in section B, 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 or productivity, 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 those changes affect an animal's health and welfare, for better or for worse, and whether those changes might lead to other requirements for housing, nutrition or in management.

The intention of producing a GMO will be to promote some form of potential benefit (e.g. increased productivity, increased feed conversion ratio, disease resistance, reduced emissions). However, while the intended effects might occur and be verified, unintended side effects could occur. For example, a change in disease resistance may lead to more silent carriers. It needs to be shown that both the intended and unintended effects do not jeopardise the health and welfare of the GM animal.

A wide range of assessment measures may be necessary and it is good practice to use several indicators and a multi-disciplinary approach. A single 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. 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 health and welfare but often it will be obvious from a preliminary study of phenotype, or a clinical examination, which measures will be most relevant. Decisions by the applicant should be made regarding expected welfare and health consequences, based on preliminary health and welfare assessments conducted during the laboratory phase, on the degree of poor welfare or health, as well as its incidence.

Assessment strategy

The assessment methodology described below is broad and general, as it would not be possible to anticipate the large number of potential genetic modifications, the number of different species involved and the different methods of production (or categories). EFSA will consider the applications on a case-by-case basis depending on the genetic modification and the information provided by the applicant. The evidence provided should demonstrate that the health and welfare of the GM animals are not significantly impaired. A comparison of the health and welfare between the comparator(s) and the GM animal for their commercial lifetimes should be made.

The assessment strategy is divided into three stages A, B and C.

During the different stages of assessment (A through C), which comprise a sequential approach, observations and records should be designed so that they are most likely to detect negative and positive effects. These effects are interpreted by the degree of deviation from the comparator group, or in some cases from historical published data (i.e. known benchmarks). The criteria used should be selected on a case-by-case basis, appropriate for the species and category, and enable sound statistical analysis with the appropriate power.

The health and welfare of the GM animals themselves will be assessed at Stages A and B in a research setting, and any serious health and welfare effects seen in a GM line will be unlikely to be developed further.

The next stage (Stage C) is when animals enter into field trials. At the end of Stage C, a GM animal should have no obvious or serious negative health and welfare.

Animals should be exposed to a range of farming conditions, which means that they will be exposed naturally to a range of different, and sometimes novel, stimuli and stressors relating to:

- Climatic conditions;
- Housing, husbandry, nutrition and other management conditions;
- Infectious agents.

Applicants should show that the GM animals' health and welfare is resilient to a range of different commercial farming systems likely to be encountered within the EU. They should be prepared for release (e.g. by vaccination, and also other preventive measures to cope with any microbiological challenges). This might include a strategy of introduction (quarantine and adaptation) to herds of different health statuses, but they should not be introduced into herds in which the health status differs considerably from their own. Furthermore, the health and welfare of the animals has to be maintained under different physiological periods (e.g. sexual maturity, pregnancy).

Information gathered during the assessment of health and welfare at all stages of developing a GM animal line should be considered as relevant information for a dossier to be submitted to EFSA.

It is likely that for some species (cattle, sheep, goats, pigs) only a small number of animals will initially be available for health and welfare assessment. The maximum use of animals should therefore be made (i.e. it may be possible to carry out more than one test on each animal). Nevertheless, numbers will need to be increased as the development progresses from Stage A to C. For some species (e.g. fish, poultry), large numbers are likely to be available for assessment as early as Stage A.

Significant differences at Stages A and B should be set at 1 in 20 (a 5 % probability that there are no differences between the GM animal and its comparator). The detection of less frequently occurring unintended and unexpected effects (e.g. 1:100 or less) will require larger population sizes (i.e. during field trials at Stage C). Any scientific studies evaluating whether or not there are negative effects of the genetic modification may fail to do so if the expression of the effect is not evident in all individuals or if the study sample size is too small. Studies should be powered sufficiently to provide a robust enough analysis to detect differences, based on known variation.

Animals showing serious adverse effects at Stages A and B are unlikely to meet the necessary criteria to be worth progressing further. Animals showing changes that do not appear to have an effect on animal health and welfare (e.g. coat colour) should be evaluated. Animals that show a positive welfare effect, or a reduction in negative effects, should be evaluated. There may be a situation where the

intended effect is so beneficial that it outweighs any small or possible negative effect and a decision will have to be made of how to proceed in these cases.

The species and production category (broilers, layers, fattening, dairy, etc.) being studied should 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 should be measured. The following considerations should be applied:

- Measurements or observations of the animal and its functioning (animal-based welfare measures[21]) provide the most sensitive indications of welfare;

- Those making welfare assessments should consider the biological functioning of the species of animal in the environment in which it is placed. Those carrying out these assessments should have demonstrated the appropriate knowledge, experience 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 should allow an overall assessment of health and welfare:

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

- Health and welfare monitoring, including production indices and inspections, should be conducted on a regular basis.

At the outset, the choice of the line to modify is important, particularly when several genetically different lines (e.g. broiler and pig strains) are available. The chosen line will be influenced by a variety of factors, but the genetic line to be modified should have a high health status (i.e. free of major infectious diseases) and have few deleterious genes (genetic load), and should have a high health status (i.e. free of major infectious diseases). A rationale for line selection should be given.

1.1.1. Monitoring at the laboratory level (Stage A)

Objective: to define under laboratory conditions the intended effects and to determine the consequences of any possible unintended effect on the health and welfare of the animals on an individual basis. This is to be carried out in the laboratory during the animal's development and possibly over several generations and physiological stages depending on the species. This will include:

a. Genetics

   One objective should be to establish a stable heritable line. Details such as viral vectors, gene construct, copy number and genome insertion sites, should be determined and recorded. In addition, there is a need to include a standard set of health and welfare measurements that are specifically tailored to the genetic modification and any adverse or beneficial 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;

[21] An animal-based measure 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.

- Zootechnical data (e.g. normal development, feed intake, growth rate, fertility, feed conversion rate, productivity indices);

- Measurements of relevant aspects of physiology, such as immune function (e.g. immunological challenge with antigens producing B-cell or T-cell responses, temperature tolerance);

- Health records, for example, evidence of the results of post-mortem and associated laboratory examinations of all animals found sick or dead in order to identify the cause of death; post-mortem examination of all animals slaughtered or euthanised at the end of the experiments; and incidence of body injuries, body malfunctions and disease (infectious and non-infectious), and medicine use, should all be taken into account.

c. Welfare status

Any measure used to assess welfare should be reliable and robust. A comprehensive range of these measures should be used and amongst those to consider are the welfare measures described in the Welfare Quality® reports (Welfare Quality®, 2009) and the EFSA Opinions for various species and categories (e.g. EFSA, 2011c, in preparation). One group of measures should address the ability of the GM animals to carry out normal behaviour, physiological functions and to develop normally. Abnormal behaviours may relate to feeding, locomotion, social behaviour (e.g. aggression), and other responsiveness measures, such as flight distances. The occurrence of stereotypies, the extent to which strongly preferred behaviours can be shown, and the extent of any avoidance behaviours should be recorded (e.g. filming animals) and could be carried out at Stage A, with subsequently more detailed studies at Stage B and C, if necessary.

d. Pre-birth

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

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

1.1.2. Experimental farm 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 of animals exposed to normal environmental challenges under normal farming conditions (e.g. transport, new groupings, varying climatic conditions) on specified, registered farms (e.g. experimental farms). These would have a higher standard of stockmanship than most commercial farms in that the GM animals should be monitored more carefully than normal. In addition, specific extra tests should be carried out to determine detailed aspects of a GM animal's health and welfare in response to different environments. These tests may require blood sampling, and some specific testing, such as flight distances, and heat stress.

Studies should be carried out to see if there is any impact on health and welfare for successive generations.

The emphasis at Stage B should be directed to subtle changes in health and especially the behaviour of the GM animal in relation to other animals. It may also be possible to discontinue some tests in Stage

B that proved negative in Stage A, but the applicant should provide justification. All findings should 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 variations in genetic – environmental interactions that may occur in commercial husbandry systems should be investigated at this stage (e.g. heat stress tests to see how animals could cope with varying climatic conditions).

The information gathered in Stages A and B should be used to assess the most relevant measures that should be followed in Stage C.

1.1.3. Field trial assessment (Stage C)

Objective: to determine any low frequency unintended effects in the GM animal during its use under farm settings.

The types of farm settings should reflect the range of systems and managements that are common within the EU.

Following the findings in Stages A and B, it should have been shown that there are not likely to be adverse consequences for the GM animals when they are exposed to a wide range of commercial farming environments. Stage C trials would involve the surveillance of animals on several farms/holdings operating under normal farming practices and conditions, and should compare GM animals with comparator non-GM animals.

The physical, biological and social environments on these farms should be monitored so that any changes identified for the first time at this stage can be related to hazards/factors identified on the farms where the changes were observed. These trials should incorporate the principles of blind Randomised Control Trials. Where possible, the stockmen and observers should be blind 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 for blind studies.

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 and statistical 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 in the dossier to submit to EFSA and may indicate the most relevant measures that should be followed in post-marketing monitoring.

E. POST-MARKET MONITORING (PMM)

1. 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.

Monitoring of health and welfare should be dependent on reporting any adverse effects by veterinarians, farmers and others (e.g. yellow card[22] system). The adverse events reports will have to be labelled as urgent or routine. At present there is no harmonised system for measuring health and welfare of food animals for the implementation of PMM surveillance in the EU, although some

[22] Yellow card system is a notification for any adverse effects seen in the field.

member states are developing such systems. This would provide an ideal baseline for benchmarking health and welfare issues.

2. 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 relate GM food/feed consumption potentially to any (adverse) effect on health.

As pre-market risk assessment studies cannot fully reproduce the diversity of the populations that 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 C.2.1.5) or people with impaired digestive functions (see Section C.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 realised that a PMM may not always have the sensitivity to estimate the individual intake of a specific food item or the 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. Due to 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.

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