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Opinions of the Scientific Steering Committee

Preliminary Guidance document on the information needed for the risk assessment of genetically modified plants and derived food and feed

DRAFT

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I. INTRODUCTION

1. Scope of the document

This document is for the use of Notifiers[1] who intend to apply for the release of genetically modified plants and derived cultivars under existing Community legislation (Directive 2001/18/EC [Ref. 1]) and/or for the commercial authorisation of Novel (genetically modified, GM) food or feed. The document does not cover genetically modified animals, or micro-organisms (including micro-organisms intended for use under containment conditions which are regulated by Directive 90/219/EEC [Ref. 2], or medicinal products for human or animal use which are regulated by Regulation 93/2309/EEC [Ref. 3]). The environmental assessment of GM plants used to produce medicinal products is covered in this document.

2. Purpose of the document

This document does not have any regulatory status, but seeks to provide guidance to both notifiers and risk assessors. It also aims to assist notifiers in the preparation of their dossiers. Any guidance provided does not exclude the need for notifiers to adhere to the requirements laid down in the appropriate Directive or Regulation.

3. Legal background for the risk assessment of GMOs, GM food and GM feed at Community level

The principles regulating the deliberate release in the environment of genetically modified organisms (GMOs) are laid down in Council Directive 90/220/EEC [Ref. 4] which will be superseded by Directive 2001/18/EC [Ref. 1] on 17 October 2002.

Directive 2001/18/EC puts in place a step-by-step approval process made on a case-by-case assessment of the risk to human health, animal health and the environment before any GMOs or products containing GMOs could be released into the environment, or placed on the market. It introduces a time limit for the authorisation, which cannot be given for more than 10 years. Authorisations may be renewed on the basis of the result of a new assessment. Directive 2001/18/EC also introduces mandatory monitoring requirements of long term effects associated with GMOs and the environment.

Under Directive 2001/18/EC from October 2002, a Notifier who intends to market a GMO must submit an application to the competent authorities of the Member State where the product is to be placed on the market. The application must include a risk assessment. The principles for the environmental risk assessment are laid down in Annex II to Directive 2001/18/EC. Annex III of Directive 2001/18/EC has details of the required information on which to base the risk assessment (Annex IIIA for organisms other than higher plants, and Annex IIIB for higher plants). If the national authority gives a favourable opinion on the GMO, this Member State must inform the European Commission and other Member States. If no objections are raised either by the Commission or by any other Member State, the assessor Member State grants an

[1] The term Notifier is used hereafter as a generic reference to the official body submitting the notification.

authorisation and the product may then be marketed throughout the Community. If any objections are raised, a decision has to be taken at Community level. If an objection relates to human health or to the environment, the Commission must then consult the relevant Scientific Advisory Committee (Art. 28 of Directive 2001/18/EC [Ref. 1]).

Risk assessments carried out under Directive 2001/18/EC address human health related to exposure to the GMO concerned, including incidental consumption; it does not address the use of GMOs and their products as food. GM food is currently regulated by Regulation (EC) 258/97 on Novel Food and Novel Food Ingredients [Ref. 5]. At present, the authorisation decision must be in accordance with Regulation (EC) 258/97 [Ref. 5] and the authorisation procedure is as disclosed in the Directive 2001/18/EC [Ref. 1]. However, in July 2001, the European Commission adopted a proposal for a Regulation of the European Parliament and of the Council on genetically modified food and feed [Ref. 6]. This draft regulation lays down a Community procedure for the assessment, authorisation and supervision of GM food and feed. Based on a scientific risk assessment, this Regulation provides for a time limit of a maximum of 10 years for the community authorisation on GMOs for food and/or feed use, food and/or feed containing or consisting of GMOs, as well as food and/or feed produced from or by GMOs. The proposed regulation provides for such risk assessments to be carried out by the European Food Safety Authority established under the Regulation (EC)178/2002 laying down the general principles and requirements of food law and the procedures in matters of food safety [Ref. 7].

Regarding genetically modified seeds, the Community legislation (Directive 98/95/EC, [Ref. 8]) specifies that those national authorities that have agreed to the use of a GM variety on their territory must notify this acceptance to the European Commission. The Commission must examine the information supplied by the Member State concerned and its compliance with the provision of the Community seed legislation. If such is the case, the variety concerned is included in the "Common Catalogue of varieties of Agricultural Plant Species". The seed legislation requires that GM varieties must be authorised in accordance with Directive 2001/18/EC [Ref. 1]. Risk assessment for a given GM plant performed under Directive 2001/18/EC applies also for varieties derived by conventional breeding methods from the line concerned. If the varieties are intended for food crop production, it also must be authorised in accordance with the Novel Food Regulation prior to inclusion in the Common Catalogue. Specific conditions for the environmental risk assessments of GM plant varieties are currently being developed by the European Commission (White Paper on Food Safety (COM)1999, 719 final, 2 January 2000, [Ref. 9]).

4. Presentation of Dossiers

Each dossier should be a complete document containing all of the information required for a full risk assessment of the product(s) in question. Assessors should not be required to undertake any additional literature reviews, or assemble, or process data to evaluate the dossiers.

To facilitate easy access of information in dossiers, a detailed index should be prepared. Continuous numbering of pages and appendices is required.

Care should be taken to ensure that all parts of the dossier are fully legible. Particular attention is drawn to the presentation of experimental data including tables, physical maps and blots. Statistical analysis of data should be provided and the statistical power tested whenever necessary. Data presented in sections of the dossier should be clearly labelled whether in the form of tables, figures, photographs, analytical gels etc. Such data can also be submitted electronically for clarity and to preserve the quality of the original data. In addition the appropriate controls or reference points included should be clearly labelled and referenced. Data provided for risk assessment should be restricted to what is necessary for a comprehensive risk evaluation.

Data provided in support of an application should be of at least the quality expected of data submitted to a high-ranking peer-review journal. Particular attention should be paid to the sensitivity and specificity of methods employed and to the adequacy and appropriateness of controls.

II. THE RISK ASSESSMENT STRATEGY

The risk assessment strategy for GM plants and products is based upon a consideration of:

- the characteristics of the donor and recipient organisms;
- the genes inserted and expressed;
- the potential toxicity and allergenicity of gene products and metabolites;
- the extent of equivalence (compositional, nutritional, safety and agronomic) with appropriate comparators;
- the potential environmental impact following a deliberate release;
- the potential for dietary impact;
- the influence of food processing on the properties of the food or feed.

The risk assessment strategy first seeks to deploy appropriate methodologies and approaches to identify the extent to which the GM crop or product is equivalent to its non-GM counterparts. The principle of establishing the extent of any equivalence provides a valuable starting point for the comparative process that underpins the many facets of risk assessment. It is obvious that the insertion of genes and other associated DNA from a donor organism into the host will result in a plant that is not completely identical to the parent. The risk assessment process therefore concentrates on the outcomes of the transformation process in their broadest sense using appropriate comparators. To this end the concept of substantial equivalence was developed by the OECD [Ref. 10] and further elaborated by WHO/FAO [Ref. 11]. The concept embodies the idea that existing crops, foods and feed stuffs can be used as the basis for a comparison when assessing the safety of crops, foods or feed that have been genetically modified. Such comparisons should be made with GM and non-GM counterparts grown under the same regimes and environments. Where substantial equivalence does not occur, this does not necessarily identify a hazard.

Different outcomes of a genetic transformation event can be envisaged:

Intended effects are those that are targeted to occur from the introduction of the gene(s) in question and which fulfil the original objectives of the genetic transformation process.

Unintended effects are considered to be consistent differences between the GM plant and its appropriate control lines, which go beyond the primary expected effect(s) of introducing the target gene(s) but which take the expected effect of the target gene into account. Such differences may be evident in the phenotype, response, or composition of the GM plant when grown under the same conditions as the controls. Such effects may be explicable in terms of our current knowledge of plant biology and metabolic pathway integration and interconnectivities, but may not always be immediately explicable under these terms of reference. Where the introduction of traits or multiple traits intended to modify composition significantly, do not allow the degree of equivalence to be considered as substantial, then the safety assessment of the whole plant becomes of greater importance. This applies to applications for deliberate releases into the environment and to the marketing of GM foods and feeds.
In cases when the degree of equivalence is established as substantial, a greater emphasis for safety assessment is placed on the newly introduced trait(s) and less emphasis is placed on the whole plant.

III. SPECIFIC INFORMATION REQUIRED FOR RISK ASSESSMENT

1. Molecular characterisation

The requirements for molecular data are the same for applications under Directive 2001/18/EC for commercial purpose (so called Part C releases) and for the assessment of GM food and GM feed. Some data necessary for traceability may not be relevant for risk assessment. Guidance is designed to highlight requirements for risk assessment.

1.1 *Information on the donor and recipient organisms*

Notifiers should provide information both on the organisms used as the DNA donor(s) for genetic modification and the recipient organism. This information should include the most recent taxonomic classification including the family, genus, species, subspecies, cultivar/breeding line or strain. Taxonomic information could be used to identify the need for specific analyses *i.e.* the known occurrence in the family of specific toxins which are typically expressed at low levels in the unmodified recipient species, but which may be unintentionally increased following the genetic modification process. Information should be provided on all issues of potential concern, such as the presence of natural toxins, allergens, virulence factors, marker genes and previous genetic modification.

1.2 *Physical and genetic map of the DNA used in transformation*

This should include the position of all genes and promoters together with the notifier's selected restriction sites for the generation of probes, and the position and nucleotide sequence of primers used in PCR analysis**.** A table identifying each component, its size, its origin and its role should accompany the map. The map/table should include information on any regions that have not been fully sequenced. Information is required on the extent of sequences flanking the genes and promoters used to prepare the constructs used for transformation of the recipient organism. The map/table should also indicate if there have been modifications that affect the amino acid sequence of the product of the introduced gene. Supporting documentation should be provided to allow adequate risk assessment of the changes made. If carrier DNA is used in a transformation event, its source must be stated and a risk assessment provided.

1.3 *Information on the sequences actually inserted/deleted*

Notifiers should provide:

- the copy number of all inserts;
- the full nucleotide sequences of all inserts, including flanking regions of host DNA. Sufficient sequence is required to assess the possibility that DNA insertion has created new open reading frames, or perturbed existing ones or known regulatory elements, within the wild-type DNA;

– a copy of all sequence information, preferably in electronic format, including the location of primers used for detection.

1.4 *Information on the expression of the insert*

Notifiers should be aware that the information on the expression in the plant of genetic elements from any part of the inserted DNA may be required if a potential risk is identified. Such requests may be made even where the gene is under the control of a bacterial promoter. Where tissue-specific promoters have been used, information may be requested on expression patterns of target genes in other tissues. In the absence of appropriate controls, lack of a distinct signal in a northern blot need not necessarily indicate that the corresponding protein is not accumulated. In such cases the highly sensitive RT-PCR method may be used to confirm lack of expression. As with all PCR reactions, the RT-PCR product requires independent characterisation and appropriate controls must be employed. Microarrays may be used to provide comprehensive datasets on gene expression. Where this is the case, it is again imperative that all appropriate controls and statistical treatments are applied.

Immunochemical determination by ELISA has proven adequate for determining the levels of novel gene products in the genetically modified plants, while Western blotting provides additional information on the molecular weight of the gene product. Where ELISA tests are routinely used to quantify the expression level of the target protein, it is imperative that the specificity of the antibodies developed are validated. For example, where crude plant extracts are used as test material there will be non-specific cross-reaction in Western blots with protein other than the target protein.

1.5 *Risk assessment related to the genes inserted*

Notifiers are encouraged to develop "clean vector" technologies [Ref. 12] in which genes extraneous to the successful deployment of the target transformation event are removed. Whenever possible, notifiers are encouraged to develop, for commercial release, those transgenic lines in which only DNA essential to the modification of the trait in question is transferred to the plant.

The choice of a particular marker gene should be given careful consideration in view of the amount of information required for risk assessment. At an early stage in the development of GM plants some strategies are available which can be considered best practice to reduce the potential identified risks and to avoid some unidentified risks in the environment [Ref. 12]. The overall aim is to reduce environmental exposure and the potential risks from the transgenes and their products. Three principle ways can achieve this:

– Avoid or minimise the inclusion of superfluous transgenes or sequences;
– Avoid or minimise superfluous expression of the transgene;
– Avoid or minimise the dispersal of transgenes in the environment.

Risk assessment should take into account any potential impact of horizontal DNA transfer between plant or plant components and micro-organisms in relevant

environments. Genes integrated in the GM plant should also be subjected to risk assessment with respect to the possible effects of ingestion of the protein expressed in plant parts.

2. Comparative analysis

2.1 *Choice of the comparator*

In the case of vegetatively propagated crops, comparative analyses should include the parental variety used to generate the transgenic lines. In the case of crops that reproduce sexually, comparators would include appropriate isogenic lines. Since many crops used to produce food and feed are developed using back-crossing, it is important that in such cases, substantial equivalence testing uses the most appropriate controls and does not simply rely on comparisons with original parental material. For example, specific male pollinator lines may be used in the generation of the final product. In all cases, evaluation of the extent of equivalence will be greatly enhanced by additional, valid comparisons, with published data on the performance and composition of commercial varieties of the crop species in question and which have a known history of safe use. Such data could indicate that the GM lines fall within the variation reported for the species in question.

2.2 *Field trials*

Protocols of field trials performed with genetically modified and control crops must be specified and documented with respect to:

Number of locations, growing seasons, geographical spreading and replicates.

The basic set of data should be obtained from a comparison of the GM plant and an appropriate control line grown in the same field under identical conditions. This comparison should cover at least two growing seasons and multiple geographical locations representative of the various environments in which the GM plants will be cultivated. The number of replicates at each location should reflect the plant's inherent variability.

Statistical models for analysis, confidence intervals.

Experimental design should be rigorous and analysis of data should be presented in a clear format. Field trial data should be analysed statistically, using an appropriate analysis of variance. A completely randomised design, for example, could indicate whether the experimental factors (location, year, climatic conditions, plant variety) interact with one another. The confidence intervals used for statistical analysis should be specified (normally 95%, with possible adjustment according to the potential hazard of the constituent to be compared). If conclusions are drawn from non-significant effects, then they should be supported where appropriate with a power analysis.

The baseline used for consideration of natural variations

Data demonstrating the natural range in component concentrations found in non-GM counterparts should be provided to enable additional comparisons with the GM plant in question. Data may be generated by the notifier or compiled from literature. The databases that were used for comparison have to be specified. Special attention has to be paid to the comparability of the analytical methods used to create the data. Ranges as well as mean values should be reported and considered.

Statistically significant differences in composition between the modified crop and its traditional counterpart grown under the same conditions should trigger further investigations as to the relationship with the genetic modification process. Modifications that are within normal ranges of variation might require less extensive evaluation than those outside normal ranges.

2.3 *Selection of compounds for analysis*

Analysis of the composition of the GM plant/food/feed is crucial to establish the extent to which the product is equivalent to its non-GM counterparts. Analysis should include all parts of the plant for which the gene product is relevant. Data should also be included on by-products and co-products and conserved material when appropriate to the intended use.

In each case, key macro- and micro-nutrients, toxicants, anti-nutritional compounds, and other constituents (including moisture and total ash) should be determined. Examples of the key nutrients, anti-nutrients and toxicants characteristic for plant species and information on the extent of natural variation are provided in OECD consensus documents which may provide further guidance for compositional analysis to establish the extent of compositional equivalence. Such documents [Refs. 13 & 14] have been prepared for soybean, oilseed rape maize, and potato, and others on sunflower, sugar beet, wheat, barley and rice are in preparation.

Key nutrients are those components that have a major impact on the diet, *i.e.* proteins, carbohydrates, lipids/fats, fibre, vitamins and minerals. The specific analyses required will depend on the plant species examined, but should include a detailed assessment appropriate to the intention of the genetic modification, the considered nutritional value and use of the plant. For example, a fatty acid profile should be included for oil-rich plants (main individual saturated, mono-unsaturated and poly-unsaturated fatty acids) and an amino acid profile (individual protein amino acids and main non-protein amino acids) for plants used as an important protein source. Measures of plant cell wall components (*i.e.* acid - and neutral - detergent fibre, and acid-detergent lignin) are also required for the vegetative parts of plants used for feed purposes.

Key toxicants are those compounds, inherently present, whose toxic potency and levels may harm human/animal health. The concentrations of such compounds should be assessed according to plant species and the proposed use of the food/feed product [Ref. 15]. Examples would include digestive enzyme inhibitors and those anti-nutritional, potentially toxic, or allergenic compounds recognised as being normally present, or newly introduced as a result of the genetic modification.

Knowledge of the introduced trait may suggest the possibility of effects beyond that specifically intended. For example, if the introduction of a gene that confers herbicide tolerance is functionally equivalent to an existing gene involved in aromatic amino acid synthesis, analysis of the protein content and amino acid composition would be prudent. If changes relative to the parent line are found, then any downstream metabolic and toxicological consequences should be examined.

2.4 *Methods of analysis*

Established and preferably validated protocols should be used and the data analysed using appropriate statistical techniques. Significant differences that occur between the GM product and appropriate controls may indicate unintended effects that require further investigation.

To increase the probability of detecting unintended effects, non-targeted metabolic profiling techniques may provide an important addition to traditional chemical analysis. Profiling methods are capable of separating and quantifying several hundred compounds simultaneously, broadening significantly the range of metabolites that could be incorporated into a risk assessment. Metabolic profiling techniques, together with the potential of assessing unintended effects at the levels of transcription and translation *i.e.* using DNA microarrays and proteomic based approaches, offer a powerful combination of risk assessment tools. Should data of this nature be provided, then the expectation is that all approaches are properly validated and that statistical analyses have been performed to the highest standard [Ref. 16].

2.5 *Agronomic traits*

Compositional analysis represents a key component of the risk assessment process. However, unintended effects may also manifest themselves through, for example, changes in susceptibility to important pests and diseases, through morphological and developmental changes or through modified responses to agronomic and crop management regimes. A holistic approach towards substantial equivalence testing should take these issues into account.

3. Environmental risk assessment

Environmental risk assessments are carried out on a case-by-case basis taking into account the biology of the recipient plant, the characteristics of the donor organism from which the transgene was derived, the properties of the genetic modification, the scale of release and the evaluation of any risk to the receiving environment that might arise from the release of the GMO.

Examples of possible interactions between the GM plant and its environment including potential impact on other organisms are:

- Selective advantage conferred to a wild relative through the transfer of genetic material to sexually compatible plants;
- Expression of pollen-mediated toxicity;
- Effects on microbially-mediated biogeochemical cycles and soil bio-transformation and decomposition processes on ecosystem function;

- Increased persistence in the environment through increased survival, establishment and invasiveness of GM plants;
- Instability of the genetic modification;
- Adverse effects on non-target organisms;
- Wider biodiversity implications as a consequence of specific agronomic practices to manage GM plants;
- Changes in pesticide residues as a result of changed crop protection practices on/or metabolic changes in GM plants.

3.1 *Geographical relevance of data*

Wherever possible, data should be provided from field experiments in those geographical regions where the GM plant will be grown commercially in order to reflect relevant meteorological, soil and agronomic conditions. Where supporting data from field studies on other continents are supplied, the notifier should submit a reasoned argument that the data is applicable to European conditions.

3.2 *Impact on wild plants*

The potential for genetic exchange with wild relatives of the cultivated GM crop should be evaluated and a risk assessment made of the likelihood of the establishment of the modified trait outside the crop. This will depend on sexually compatible plants being present and available outside the crop to receive pollen and produce fertile hybrids. Selection pressure in non-crop habitats that is required to maintain the selective advantage of any transferred trait should be identified. For example, transferred herbicide tolerance may not be an advantageous trait in habitats where the herbicide is not applied.

3.3 *Impact on non-modified crops*

The potential for out-crossing to other crop cultivars should be considered and assessed. This will vary with crop. For example, the release of GM oilseed rape raises the issue of gene transfer, since this crop will readily cross pollinate with nearby oilseed rape crops and may spontaneously hybridise with some wild relatives. In cases where gene transfer cannot be prevented between certain adjacent crops of, for example, oilseed rape or maize, the risk assessment should focus on the consequences of cross pollination even at very low frequency. If isolation is proposed it should be considered in relation to distances accepted for conventional seed production [Ref. 17]. If out-crossing is to be avoided, appropriate risk management practices should be identified to separate crops adequately both in time and space.

3.4 *Impact on non-target organisms and ecological processes*

Clear and well-defined risk assessments should be carried out for each of the different functional environmental compartments that are exposed to the GM plant. Whether any parts of it will remain in the environment after harvest, will depend on the specific crop and its management regime or agronomic practices. For example, exposure should be estimated to soil organisms and decomposition function (*i.e.* earthworms, micro-organisms, leaf litter breakdown) in relation to potential transfer to soil microfauna and impact on degradation. The assessment should also address the

fate of any (newly) expressed substance(s) in those environmental compartments where they are introduced and which result in exposure of non-target organisms (*i.e.* in soil after the incorporation of plant material).

Potential impact should be assessed on non-target arthropods (including pollinators, beneficial and predatory arthropods), grazing birds and mammals or, if appropriate, the aquatic environment. Such studies may include laboratory exposures at levels likely to be experienced in the field. This risk assessment should take account of where in the plant and to what degree the inserted genes are expressed and therefore the extent to which non-target organisms are exposed either directly or indirectly.

Data on the comparative susceptibility of the GM plant to pests and diseases compared with that of the non-modified plants are useful indicators of effects together with observations on agronomic performance during greenhouse and experimental field trials.

An assessment of the potential impact of growing GM crops on wider biodiversity in the crop ecosystem requires the combination of several different approaches [Ref. 18]. The notifier should describe the appropriate commercial management regime for the crop including changes in pesticide applications, rotations and other crop protection measures. The risk assessment should consider the management of the GM crop compared with the equivalent non-GM crop under the full range of intensities of crop production within which the GM is likely to be grown. It should also consider impact across the potential geographical range in Europe. The notifier should aim to assess the direct and indirect, immediate and delayed effects, of the management of the GM crop on all affected habitats. This should include the biodiversity within the GM crop and adjacent non-crop habitats. Such risk assessment may need to be supported by comparative experimental studies demonstrating the management of the GM crop compared with the range of management practices for the appropriate non-GM crop type. The necessary scale of such studies will depend on the level of risk associated with a particular GM crop and on the quality and extent of the available literature that is relevant to the particular risk assessment.

3.5 *Resistance management*

Where the risk assessment identifies a general requirement for management *i.e.* to prevent the establishment of insect or herbicide tolerance, the management regime should be capable of detecting resistance at an early stage. Thus, in order to delay the onset of any resistance in the target pest *i.e.* associated with a Bt-protected plant, a clear resistance management strategy should be developed for implementation by growers [Ref. 19]. The notifier should be involved in providing guidance, technical support and advice to the growers on best practice for growing the crop. The same principles should be applied in the case of crop plants that have been modified for herbicide tolerance. Additionally, in the event of gene stacking (the accumulation of different GM traits) further crop protection measures may be required to control volunteers and weeds.

4. Food/feed safety assessment

In addition to the molecular characterisation of the genetically modified plants and the necessary compositional data to assess the extent of equivalence, further information is needed for the safety assessment of material intended for use as human food or animal feed.

4.1 *Product Specification*

Specification of the origin and the composition of the GM plant and GM food/feed is needed to ensure the identity between the product tested/evaluated and the product to be marketed. In the design of the specification, parameters most relevant for the characterisation of the product from a safety and nutritional point of view should be considered. Information on the availability of specified reference material should be submitted.

4.2 *Effect of the production process*

For processed foods/feeds derived from GM sources a description of the production process should be provided which should comprise a general outline of the processing steps and a detailed description of the conditions applied (description of physical, chemical and biochemical parameters). It is important to determine if, and to what extent, the processing steps lead to the concentration or to the elimination, denaturation and/or degradation of DNA and the novel protein(s) in the final product.

4.3 *Characterisation and previous use of the donor and recipient of the genetic modification*

Information on the most recent taxonomic classification including the family, genus, species, subspecies variety or strain should be provided (see 1.1 above). Information on the family/genus indicates the need to consider up-regulation, as a result of genetic modification, of an undesirable trait(s) characteristic of the family/genus, but which is typically expressed at low levels by the unmodified recipient species.

4.4 *Anticipated intake/extent of use*

An estimate of the expected intake is necessary for the safety evaluation of GM food/feed and to evaluate nutritional significance. Information should be provided on the intended function, the dietary role of the product, and the expected level of use. On the basis of the available consumption data, the anticipated average and maximum intake of exposure of the GM food should be estimated. If possible, particular sections of the population with an expected high exposure should be identified.

Any assumptions made in the exposure assessment should be described.

The concentrations of the new gene products and constituents produced, or modified by the intended genetic modification (*i.e.* due to changes in metabolic pathways) in those parts of the GM product intended for food or feed use, should be determined by appropriate methods. Expected exposure to these constituents should be estimated

taking into account the influences of processing, storage and expected treatment of the food/feed in question.

5. Toxicology

Toxicological studies should be conducted using internationally agreed protocols. Test methods described by the OECD [Ref. 20] or in the most up to date European Commission Directives on dangerous substances are recommended [Ref. 21]. Use of any methods that differ from such protocols should be justified. Studies should be carried out according to the principles of Good Laboratory Practice (GLP) described in Council Directive 87/18/EEC and accompanied by a statement of GLP-compliance [Ref. 22].

There may be circumstances, when the notifier considers that a decision on safety can be taken without conducting some of these tests and/or that other tests are more appropriate. In such cases the notifier must state the reasons for not submitting the required studies or for carrying out studies other than those mentioned below.

The toxicological requirements for food and feed derived from GM plants must be considered on a case-by-case basis and will be determined by the degree of equivalence with a conventional counterpart. In principle, the safety assessment must consider the presence of novel proteins expressed as result of the genetic modification, the potential presence of other novel constituents and/or possible changes in the level of natural constituents beyond normal variation. These potential deviations from the conventional counterparts require different toxicological approaches.

Testing of novel proteins: To demonstrate the safety of novel proteins the following information is needed:

A thorough molecular, biochemical and functional characterisation of the novel protein including the determination of the primary sequence and the molecular weight, studies on post-translational modifications and a description of the function are needed. In the case of novel enzymes, information on the principal and subsidiary enzyme activities is needed including the temperature and pH range for optimum activity, substrate specificity, and possible reaction products.

A search for homology to proteins known to cause adverse effects, *i.e.* protein toxins, should be conducted. A search for homology to proteins exerting a normal metabolic or structural function can also contribute valuable information. The database(s) used to carry out the search should be specified.

The protein's stability under conditions of processing, storage and expected treatment of the food/feed in which it is present should be studied. The influences of temperature and pH changes should normally be examined and potential modification(s) of the proteins (*i.e.* denaturation) and/or stable protein fragments generated through such treatments should be characterised.

Data concerning the resistance of the novel protein to proteolytic enzymes (*i.e.* pepsin) should be obtained, *i.e.* by *in vitro* investigations using appropriate and

validated tests. Stable breakdown products should be characterized and evaluated with regards to the potential hazards linked to their biological activity.

The toxicity of the novel protein should be examined by appropriate feeding studies. These studies should be performed over a period of at least 28 days with laboratory animals (according to OECD guidelines [Ref. 20]) able to react rapidly and actively to physiological or metabolic disturbances, such as young animals undergoing rapid growth. Targeted investigations should be conducted if the protein is suspected to act on specific organs or tissues including interactions with receptors of the endocrine, reproduction or nervous system.

It is essential that the tested protein is equivalent to the novel protein as it is expressed in the GM plant. If, due to the lack of sufficient amount of test materials (*i.e.* plant proteins), a protein is used which was produced by micro-organisms, the structural and functional equivalence of the microbial substitute to the novel plant protein has to be demonstrated. For example, comparisons of the molecular weight, the isoelectric point, amino acid sequence**,** post-translational modification, immunological reactivity and, in the case of enzymes, the enzymatic activity are needed to provide evidence for the equivalence.

Testing of novel constituents other than proteins: Other identified novel constituents should be evaluated according to the traditional toxicological approach on a case-by-case basis. For establishing their safety, information analogous to that described in the "Guidance on submissions for food additive evaluations by the Scientific Committee on Foods" [Ref. 23] is needed. This implies the submission of information on a core set of studies and the consideration of whether any other type of study might also be appropriate. Normally, the core set includes information on metabolism/ toxicokinetics, subchronic toxicity, genotoxicity, chronic toxicity/carcinogenicity and reproduction and developmental toxicity.

Information on natural food constituents: Natural food constituents comprise a large variety of substances: macro- and micronutrients, secondary plant metabolites as well as natural toxicants and antinutritional factors. If the content of such natural food constituents is increased beyond the natural variation, a detailed safety assessment based on the knowledge of the physiological function and/or toxic properties of these constituents should be submitted. The result of this assessment would determine if and to what extent toxicological tests are required.

Testing of the whole GM food/feed: If the composition is modified substantially, or if there are any uncertainties on the equivalence to a traditional counterpart, not only novel constituents, but also the whole GM food/feed should be tested.

For foods, the testing programme should include at least a 90-day feeding study in rodents. Special attention must be paid to the selection of doses and the avoidance of problems of nutritional imbalance. Additional toxicological studies may also be necessary, depending on the potential exposure, the nature and extent of deviation from traditional counterparts and the findings of the feeding study.

For feeds, it is recommended that comparative growth studies are conducted with a fast growing livestock species such as the broiler chick. Because of their rapid weight

gain, broilers are particularly sensitive to any change in nutrient supply or the presence of toxic elements in their feed. However, studies of this type are limited to those materials suitable for inclusion in broiler diets and which can be nutritionally matched to a suitable control diet.

For feedstuffs intended for aquaculture, extrapolating results from a growth study made with a fish species such as the catfish, may be preferable to an extrapolation from results obtained with broilers. Similarly, in the presence of a known toxicant, feeding studies may be restricted only to those livestock known to tolerate the compound. For example, gossypol prevents the use of cottonseed meal in animals other than ruminants. In this case milk production parameters, also recognised as relatively sensitive indicators of body condition, might substitute for growth rate.

Where the modification is expected to substantially change bioavailability as well as composition then a suitable comparator for feeding studies is unlikely to be available. In such cases feeding trials with all major target species without comparison to a non-GM feed to should be made to demonstrate wholesomeness for the animal and that the intended modification produces any nutritional benefits claimed.

6. Allergenicity [2]

The potential allergenicity of a protein is not a completely predictable parameter and will depend upon the genetic diversity and variability of specific IgE response in atopic humans. Given this lack of complete predictability it is necessary to obtain, from several steps in the risk assessment process, a cumulative body of evidence which minimises any uncertainly with regard to the protein in question.

The intrinsic allergenicity of the foreign protein(s) encoded by the introduced gene(s) must clearly be considered. Moreover, the consequences of any possible unintended effects of the genetic modification on the allergenic potential of the plant or plant product should also be considered on a case-by-case basis. For example, unintended qualitative or quantitative changes could occur in the pattern of allergenic proteins naturally present in the conventional plant or product.

6.1 *Assessment of allergenicity of the newly expressed protein*

In line with the recommendations of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology [Ref. 24] and the Codex *ad hoc* Intergovernmental Task Force on Foods Derived from Biotechnology [Ref. 25], an integrated, stepwise, case-by-case approach, as described below, should be used in the assessment of possible allergenicity of newly expressed proteins.

1. In every case a search for sequence homologies and/or structural similarities between the expressed protein and known allergens should be made as the first step in the assessment. Identification of potential linear IgE binding epitopes

[2] This assessment strategy is not applicable for assessing whether newly expressed proteins are capable of inducing gluten-sensitive or other enteropathies. See Codex documentation, Ref. 25.

should be conducted by a search for homologous peptidic fragments in the amino acid sequence of the protein. The size of the contiguous identical or chemically similar amino acid search should be based on a scientifically justified rationale in order to minimise the potential for false negative or false positive results.

2. If the source of the introduced gene is considered allergenic, but no sequence homology to a known allergen is demonstrated, specific serum screening of the expressed protein should then be undertaken with appropriate sera from patients allergic to the source material using relevant validated immunochemical tests.

   If the source is not known to be allergenic but if a sequence homology to a known allergen is demonstrated, the specific serum screening should be conducted with sera from patients sensitised to this allergen.

3. If the source of the gene/protein is not known to be commonly allergenic and no sequence homology to a known allergen is demonstrated, or if the result of the specific serum screening of a newly expressed protein from a source known to be allergenic is equivocal, additional tests should be performed. These include pepsin resistance tests or targeted serum screening.

   Resistance to pepsin digestion; has been observed in several food allergens thus a certain correlation exists between resistance to digestion by pepsin and allergenic potential. Therefore, in the case of resistance of a protein to degradation in the presence of pepsin under appropriate conditions, further analysis should be conducted to determine the likelihood of the newly expressed protein being allergenic. The use of a well-validated pepsin degradation protocol is recommended, however alternative protocols may be used where adequate justification is provided.

   Targeted serum screening; assesses the capacity of the newly expressed protein to bind to IgE in sera of individuals with clinically-validated allergic responses to categories of foods broadly related to the gene source. If no relevant serum is available the expressed protein should be analysed for evidence of cross-reactivity and/or sensitising potential using other tests such as (appropriate) animal models or search for T-cell epitopes, structural motifs, etc. Complementary data on the biological origin and function and structural features of the newly expressed protein may also be provided in order to increase the body of facts to support a conclusion.

6.2 *Assessment of allergenicity of the whole GM plant or crop*

If the host of the introduced gene is known to be allergenic, any potential change in the allergenicity of the whole GM food/feed should be tested by comparison of the allergen repertoire with that of the conventional non-GM variety**.**

It should be pointed out that these approaches should be applied on a case-by-case basis depending on the available information on the allergenic potential of the source and/or the host.

Data on the prevalence of occupational allergy in workers or in farmers who have significant exposure to GM plant and crops or to the airborne allergens they may contain will provide useful information for the risk assessment process.

7. Nutritional assessment of GM food

The development of GM-foods has the potential to improve the nutritional status of individuals and populations and provide products with enhanced functionality for populations in developed and developing countries. However, and unlike animal feeding studies in which intake and nutrient composition is strictly controlled, novel foods also have the potential to introduce nutritional imbalances as a result of both expected and unexpected alterations in nutrients and other food components.

The nutritional evaluation of GM-foods should consider:
a) nutrient composition (see compositional studies as described in section 2),
b) biological efficacy of nutrient components in the foods
c) assessment of nutritional impact

When substantial equivalence to an existing food is demonstrated, the only further nutritional assessment will deal with the impact of the introduction of the novel food on general human dietary intake patterns. Information on the anticipated intake/extent of use of the novel food will be required and the nutritional consequences should be assessed at average and at upper levels of daily intake. The influences of non-nutrient components of the novel food should also be considered.

Specific additional requirements should be applied to those GM foods aimed at modifying nutritional quality and also to those in which unexpected nutrition-related modifications occur. In these cases additional detailed studies on specific metabolites and/or processes, tailored according to the introduced genetic(s) modification(s), would be required.

The introduction of a significant nutritional change in a food may require post-market assessment to determine if the overall diet has been altered and to what degree. As many implications for human nutrition cannot be accurately predicted, a proposal for a surveillance program should accompany the marketing of a GM food.

8. Animal products

The safety of products derived from animals fed a diet containing GM ingredient(s) and consumed by humans should be considered. However, it is considered highly unlikely that any introduced protein will become directly incorporated into animal products. Consequently, it is not considered necessary to test routinely for the presence of introduced genes or their products unless their characteristics suggest cause for concern.

Proteins introduced into the GM plant and known to modify plant metabolism may alter the nature or concentration of metabolites whose residues may have toxicological implications for the animal and/or consumers of animal products. In such cases further studies should be performed with respect to the toxicological

implications for the animal and/or consumers of animal products. Guidance on these issues is provided in the relevant sections of this document.

9. Post-marketing monitoring

Directive 2001/18/EC (Article 20) introduces an obligation for notifiers to implement monitoring plans in order to trace and identify any direct or indirect, immediate, delayed or unforeseen effects on human health or the environment of GMOs as or in products after they have been placed on the market. Monitoring plans will need to be developed on a case by case basis taking account of the environmental risk assessment, the modified characteristics specific to the GMO in question, its intended use and the environment into which it is released.

Monitoring of potential cumulative long-term effects should be considered as a compulsory part of the monitoring plan. Monitoring should primarily focus on potential effects arising from the placing on the market of a GMO that have been highlighted as a result of the environmental risk assessment and should be carried out for a sufficient time period to detect not only immediate and direct potential effects, where appropriate, but also delayed or indirect effects. Plans for monitoring should be based on three key sections: 1) monitoring strategy, 2) monitoring methodology and analysis, 3) reporting and review. Particular attention should be given to the possible long-term impact of intake of foods derived from GM plants on the occurrence of new allergies or increased prevalence of allergies to traditional foods that have been genetically modified, in at risk groups of the general population. Dietary intake should be monitored and a monitoring plan designed. In case adverse effects are reported, the data should be validated with respect to the clinical outcome and causality between the reported adverse effects and the exposure to the specific GM food should be established.

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