Guidance document to facilitate notifiers in the preparation of plant GMO dossiers for consideration by the Scientific Committee on Plants (SCP/GMO/103-Final) - (Opinion expressed by SCP on 18 December 1998)

The Committee would welcome comments and observations from interested parties on this document. Such comments should be sent to the Committee Secretary in writing or using the e-mail address or fax number indicated on the site.

1. TITLE

Guidance document to facilitate notifiers in the preparation of plant GMO dossiers for consideration by the Scientific Committee on Plants.

2. BACKGROUND

The Scientific Committee on Plants has expressed a number of opinions on different genetically modified plants between February and October 1998 and these detailed scientific opinions have been published by the Commission on the Internet [1] **.** The opinions have primarily been given in the context of Directive 90/220/EEC which requires an assessment to be carried out before a product containing or consisting of genetically modified organisms (GMOs) can be placed on the market. The aim of each assessment is to evaluate risks to human health and the environment connected with the release of the GMO. For genetically modified plants, these must be based on the information outlined in Annex II B to Directive 90/220/EEC and take into account the proposed uses of the products.

Following the entry into force of the Regulation on Novel Foods and Novel Food Ingredients (EC No. 258/97) on 15 May 1997, the requirements of that Regulation must be satisfied in order that a GM plant and its derived products may be placed on the market for food purposes. However, as no equivalent regulation exists for the moment on Novel Feeds and Novel Feed Ingredients the Committee also evaluates the risks arising from these uses.

During the course of preparing its opinions, the Committee felt that it would be beneficial to draw notifiersÂ' attention to certain aspects relating to the format and content of dossiers. Since similar points tended to arise repeatedly in dossiers, it was decided that the most efficient and transparent approach would be for the Committee to prepare a document consisting of a compendium of its observations and suggestions. These could then be taken into account by notifiers when generating data and preparing future dossiers. The Committee recognised that additional points would become evident as it considers new dossiers submitted to it for opinion but it was, nevertheless, of the opinion that it was now timely to publish its observations and suggestions in the light of its experiences to date. Accordingly, it is the CommitteeÂ's intention to publish periodically similar guidance documents as the need arises.

This document does not have any regulatory status but seeks to assist notifiers in the preparation of dossiers to enable them to facilitate the review of their dossiers where the Committee is requested to deliver an opinion.

The Committee is of the opinion that the existence of clear guidelines for the preparation and submission of dossiers would be beneficial to both notifiers and evaluators and would contribute to transparency. The Committee invites the Commission services to prepare such guidelines. Pending the availability of these guidelines, the Committee is of the opinion that publication of the present recommendations would be beneficial.

3. OPINIONS OF THE COMMITTEE

3.1 General aspects relating to dossier format

Dossiers should be "stand-alone" and contain all information required for the Scientific Committee on Plants to prepare an opinion. The Committee should not be required to undertake any additional literature reviews or assemble or process data in the evaluation of the dossiers.

To facilitate easy access of information in dossiers, a detailed index should be prepared following the section headings as outlined in Annexes 2 and 3 of Directive 90/220.

Care should be taken to ensure that all parts of the dossier are fully legible. In this respect, particular attention is drawn to the presentation of experimental data including tables, physical maps and blots. Data presented in sections of the dossier should be clearly labelled whether in the format of tables, figures, photographs, experimental traces, analytical gels etc. In addition the appropriate controls or reference points included should be clearly labelled and referenced.

3.2 Information related to the genetic modification

3.2.1. Physical and genetic map of the plasmid used in transformation

This should include the position of all genes and promoters in the vector together with the notifierÂ'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 in the vector should accompany the map. The map/table should include information on any regions which have not been fully sequenced. The map/table should also indicate if there have been modifications which 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.

3.2.2. Information on the sequences actually inserted/deleted

The plasmid map and accompanying table must provide information on the predicted sizes of the genes to be incorporated into the GM plant. The GM plant should be analysed by Southern blotting to identify which components of the vector have been incorporated and whether or not they are of the predicted size. In the case of **Agrobacterium** mediated transformation this should include regions both within and outside the left and right borders of the T-DNA. Where a direct DNA uptake technique has been used for transformation then Southern blotting should be used to determine the extent of any DNA insertions or truncations. In all cases Southern blotting is required to provide information on the number of insertion events for each component of the DNA delivered.

The above is required as the DNA used in transformation can include genes encoding for selectable markers(s) and for bacterial plasmid replication. Whilst these may or may not be relevant to the risk associated with the plant itself (they are likely to be under the control of bacterial rather than plant promoters) such knowledge is important to risk assessment when considering the potential for gene transfer from plant material to microbe(s) in the human or animal digestive tract and to soil micro-organisms. A full risk assessment can only be carried out therefore if it is known that the intact genes or open reading frames are incorporated into the plant genome.

3.2.3. Information on the expression of the insert

This is related to section 3.2.2 above. Notifiers should be aware that the Scientific Committee on Plants may require information on the expression, in the plant, of genetic elements from any part of the vector 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 non-target tissues. Ideally expression should be determined at the level of both transcription and translation. 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. For this reason it is important that notifiers have the relevant tools (specific antibodies etc) for such studies.

3.2.4. Risk assessment related to the genes inserted

Notifiers are encouraged to develop "clean vector" technologies 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.

Risk assessment should take into account any potential impact of horizontal DNA transfer between plant or plant components and bacteria in relevant environments, for example, within the gastro-intestinal tract or soil environment. 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.

The choice of a particular marker gene should be given careful consideration in view of the amount of information required for risk assessment. For example, in the case of antibiotic resistance genes, and particularly where bacterial promoters are involved, the data requirements may be high, particularly where the antibiotic in question has an important clinical or veterinary use.

3.3 Toxicology and Residues

3.3.1. Toxicology and residues of plant protection products as expressed or applied to GM plants

The Committee differentiates between the risk assessment to be carried out for substances expressed in the GM plants which fall under the scope of Directive 90/220/EEC and those which arise from the application of a plant protection product which fall under Community legislation on plant protection products. However, the Committee in preparing its opinions

has taken into account the transitional measures provided for in Directive 91/414/EEC concerning the placing of plant protection products on the market and the wide scope of the questions referred to the Committee.

Data should be provided on the toxicity, metabolism and residue levels of compounds with plant protective properties as expressed in GM plants compared to those of externally applied compounds e.g. Bt toxins. Similar data should be presented for GM herbicide tolerant plants. In particular, information on the kinetics of the formation and degradation of known and possibly newly formed metabolites should be generated. In the case of new metabolites appropriate toxicity studies should be carried out with respect to the assessment of animal and human safety as laid down in the Directive 91/414. Information should be provided on residue levels of parent compounds and metabolites in edible parts of GM plants according to existing data requirements in the European Community. Studies with livestock animals should be performed in order to investigate the occurrence of residues in animal based products destined for human consumption. When GM plants are herbicide tolerant it is also important to use plants normally treated with herbicide in toxicological studies.

3.4 Food and Feed - safety aspects

3.4.1. Product description and intended use

A description of the protocols used in the processing of GM plants or plant parts should be provided and target animal species should be identified. The notifiers should indicate the extent to which DNA incorporated in the GM plant is destroyed by the processing procedures. Information on levels of expression products (proteins, metabolites) remaining in processed feed is also required.

3.4.2. Origin of gene products used for safety assessment

The low level of expression of many introduced genes in the GM plants has led notifiers to clone the gene into a prokaryotic system for ease of product isolation. Where this is the case it is important to demonstrate that production in such an alternative host does not result in differences in post- translational modifications which alter behaviour in subsequent experiments designed to establish safety.

3.4.3. Target animals

It is desirable that feeding studies with GM plants or by-products derived from GM sources should be performed, wherever possible, in the target animal. When GM plants are herbicide tolerant it is also important to use plants normally treated with herbicide in toxicological studies. Where data are to be extrapolated to humans, studies using the rat and one other laboratory species should be included. More extensive studies may be appropriate for GM plants when there is reason to suppose that the introduced trait(s) may alter the composition of the plant. These may encompass the assessment of bio-availability of macro or micro-nutrients, the nutritional or toxicological consequences of any modification of major nutrients such as starch and/or the presence or increase in level of a toxic substance, and the confirmation of the absence of any anti-nutritional effects.

3.4.4. Degradation in the digestive tract

Evidence of degradation of the introduced gene products should be based on data obtained **in vivo** by feeding the GM plant material or its derived products to the intended target animal. It is also important that separate evidence of the extent of degradation in ruminants is obtained **in vivo** for all GM products or parts of products designed for use as rumen feeds. The use of **in vitro** simulation of gastric and intestinal digestion of the gene product should be considered supplementary to **in vivo** experiments designed to measure the survival of the gene products when fed to animals as an integral part of the GM plant. Isolated proteins are known which are fully degraded in the simulated gastric system but survive gut passage intact when fed as part of a normal diet.

3.4.5. Use of intact plants or plant parts or by-products

Data on substantial equivalence and safety is usually obtained from studies with the whole plant or appropriate plant part (seed, fruit). However, in cases where by-products are the usual source of animal feed, and particularly where the production of such by-products may lead to a concentration of introduced gene products, safety evaluations should include the appropriate by-product(s).

3.4.6. Substantial equivalence

Data should be obtained from a valid comparison of GM plants and a conventional line which is preferably isogenic, based on samples from at least two seasons, grown at a number and variety of geographical locations, and should be accompanied by an appropriate statistical treatment. The specific analyses expected will depend on the plant species examined but should include a detailed assessment appropriate to the considered value of the plant. For example, a full fatty acid profile for oil-rich plants and carbohydrate and/or amino acid profile for plants in which the carbohydrate and/or protein content makes a recognised contribution to nutrition. Assessments should always consider those anti-nutritional, potentially toxic or allergenic compounds recognised as being normally present, or newly introduced as a result of the genetic modification. Key toxicants to be assessed may be determined by knowledge of the function and expression product of the inserted gene. Notifiers are also encouraged to provide data which demonstrate the range in component concentrations found in non-GM counterparts and thereby to make comparisons with the GM plant in question.

3.5 Environmental Aspects

3.5.1. Geographical relevance of data

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

3.5.2. Impact on non-target organisms

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; this will depend on the specific crop and if any parts of it will remain in the environment after harvest. For example, exposure should be estimated to soil organisms and function (e.g. earthworms,

micro-organisms, leaf litter breakdown), non-target arthropods (including pollinators, beneficial arthropods), grazing birds and mammals or, if appropriate, the aquatic environment. This risk assessment should take account of where in the plant the inserted genes are expressed and the consequent exposure of non-target organisms. The assessment should also address the fate of the expressed substance in those environmental compartments where they are introduced and may cause exposure of non-target organisms (e.g. in soil after the incorporation of plant material). Data on the comparative susceptibility of the GM plant to pests and diseases compared to the non-modified plants are useful together with observations on agronomic performance during greenhouse and experimental release trials.

3.5.3. Impact on non-modified crops

The potential for out-crossing to other crop cultivars should be considered and assessed. If isolation is proposed it should be considered in relation to distances accepted for seed production. Claims of zero out-crossing should be supported by evidence from measurements in the field. If out-crossing is to be avoided, appropriate crop management practices should be identified.

3.5.4. Impact on wild plants

The potential for genetic exchange with wild relatives of the cultivated crop should be evaluated and a risk assessment made of the likelihood of the establishment of the modified trait outside the crop. Selection pressure in non-crop habitats, required to maintain the selective advantage of any transferred trait, should be identified.

3.5.5. Resistance management

Where the risk assessment identifies a general requirement for risk management related to insect or herbicide tolerance for example, the subsequent management programme should be given in detail. For example, in order to delay the onset of any resistance in the target pest e.g. associated with a Bt-protected plant, a clear resistance management strategy should be developed for implementation by growers. This should include the active involvement of the notifier and provide guidance, technical support and advice to the grower on best practice to grow the crop. The same principles apply in the case of crop plants modified for herbicide tolerance where gene stacking may lead to additional crop protection measures to control volunteers and weeds.

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[1] http://ec.europa.eu/food/sci-com/scp_en