EXTERNAL SCIENTIFIC REPORT

Review of the strategies for the comprehensive food and feed safety and nutritional assessment of GM plants per se[1]

ADAS UK Ltd. & Rothamsted Research [2, 3]

ABSTRACT

This report reviews current scientific literature and risk assessment frameworks for assessing food and feed safety of GM plants, in cases where the comparative approach, as applied by EFSA, may not be fully applicable. This may apply to a range of GM traits coming onto the market which have received substantial modifications to the composition, metabolism and physiology of the plant (GM plants with 'novel' traits). A literature review was carried out using the principles of a systematic review to ensure objectivity in reviewing all relevant papers. Of the 983 relevant scientific papers which were initially identified, 92 passed further selection and were subsequently reviewed. The risk assessment approaches for GM plants of seven international bodies were also reviewed. Overall it was found that comparisons were always a cornerstone of food and feed risk assessment of GM plants, whether traits were 'novel' or otherwise. No evidence was found in the scientific literature or from international risk assessment bodies where GM plants were not being compared to an appropriate comparator, or a database. The comparative assessment as applied by EFSA is seen by the project team as the most reliable method to identify any unintended effects. However, given the range of GM plants that may require authorisation in the future, a flexible approach to the design of field trials will be required to account for situations where the receiving environment of the GM plant would be substantially different to the appropriate comparator. In addition, exposure assessment will be an important step for the risk assessment process, particularly for GM plants with 'novel' quality/output traits which provide nutritional benefits to the consumer. Post market monitoring is an area which has received little attention in the literature, and further work is needed to develop guidance on when this should be carried out.

KEY WORDS

GMO, risk assessment, trait, comparative assessment, substantial equivalence, novel

DISCLAIMER

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SUMMARY

The food and feed risk assessment strategy for genetically modified (GM) plants in Europe, as applied by the European Food Safety Authority (EFSA) GMO Panel compares GM plants and their derived food and feed with a conventional non-GM comparator, a plant with a history of safe use as food. This project was carried out to review the current scientific literature and risk assessment frameworks for food and feed safety of GM plants, in cases where the comparative approach as applied by EFSA may not be fully applicable. This may be the case for the range of GM traits coming onto the market which have received substantial modifications to the endogenous composition, metabolism and physiology of the plant (GM plants with 'novel' traits).

Definition of 'Novel' traits: Throughout the report, 'novel' traits are defined according to Appendix A. GM plants with 'novel' traits include plants which have undergone:

1. Alterations to concentration of storage compounds or nutritional content;
2. Introduction of 'foreign' storage compound(s);
3. Physiological/ morphological change to plant; and
4. Alterations in metabolite concentrations to enable the plant to tolerate stresses.

This is not an exhaustive list. It should be noted that 'novel' refers to the nature of the trait itself and not the methodology used to achieve the genetic modification.

Definition of 'comprehensive' risk assessment: 'Comprehensive' risk assessment is defined as any approach to food and feed risk assessment of GM plants which does not rely on the comparative approach alone to identify unintended and intended effects due to the lack of an appropriate comparator as defined in EFSA Guidance Documents.

The report contains: background information on the risk assessment of GM plants, results from a systematic literature review, risk assessment criteria, risk assessment approaches of international bodies', case studies and foreseeable scenarios. A literature review was carried out using the broad principles of a systematic review to ensure objectivity in reviewing all relevant papers. Of the 983 scientific papers identified as having relevance to the subject, 92 were subsequently reviewed. The risk assessment approaches for GM plants of seven international bodies were also reviewed. A database of GM plants deemed to have 'novel' traits were gathered from a publicly available database to determine the types of traits likely to be submitted for market authorisation in the future. Four case studies are used in the report. The project team used three in-depth case studies to demonstrate how international risk assessment bodies had assessed GM plants with 'novel' traits, to date. The fourth was a hypothetical case study. In addition the risk assessment methods used for novel foods were investigated. Given all the evidence presented, and based on the results of the literature, a foreseeable scenario is presented for risk assessment of GM plants with 'novel' traits.

Overall it was found that comparisons were always a cornerstone of food and feed risk assessment of GM crops, whether traits could be classed as 'novel' or otherwise. No evidence was found in the scientific literature or from international risk assessment bodies where GM plants were not being compared to an appropriate comparator, or a database. Current EFSA guidance stipulates the use of a comparator, and to use a comprehensive risk assessment in cases where a comparator cannot be used. Currently, risk assessments (*'comprehensive food and feed safety and nutritional assessment of GM plants, and derived food and feed')* which do not use a comparator are not being used by international risk assessment bodies. Furthermore, a comprehensive approach which does not require a comparator

has not been proposed in the scientific literature as a way of risk assessing GM plants. Hence, it can be concluded at the present time, procedures for risk assessing GM plants without using a comparator are not well understood and consequently, are not applied. Profiling techniques such as metabolomics and proteomics have received a lot of interest from the scientific community as an alternative, or an accompaniment to the comparative approach. However, large scale databases from which to conduct profiling do not currently exist, and there is no clear consensus on appropriate methodologies to be used. The majority of international risk assessment bodies base their risk assessment of GM plants on internationally recognised principles put forward by the OECD. As such, where these bodies have already risk assessed GM plants with 'novel' traits; they have used the same approaches as for other GM plants (those with agronomic/input traits such as herbicide tolerance). These principally rely on comparing to an appropriate plant with a history of safe use as food, as is applied in EFSA guidance.

Novel food risk assessments consider risks based on a product basis, i.e. taking a food in its processed form and basing the authorisation on a specific use or food application. Novel food assessments are carried out on a case by case basis, taking into account the risks associated with the product itself. In these assessments, appropriate methods to test for safety, largely based on substantial equivalence with other food products with a history of safe use, are carried out. The level of risk is quantified by the level of history of use as food of the product being assessed, and the likely dietary intake.

In the foreseeable scenario for risk assessment, the project team recommends an approach which takes its base in the current EFSA guidance for risk assessment of food and feed from GM plants. To account for the range of different GM plants with 'novel' traits that may require authorisation worldwide, a case by case, flexible approach to risk assessment will be required. In particular, hazard identification is important, and the approach advocated gives further weight to the extent to which changes to the plant (such as expressed proteins, or changes to metabolites) have a history of safe use as food or feed. This is largely to account for GM plants with 'novel' traits that may be new to the human diet, and thus present further items for testing (risk characterisation). The comparative assessment as applied by EFSA is seen as the most reliable method to identify any unintended effects. However, given the range of GM plants that may require authorisation in the future, a flexible approach to the design of field trials will be required to account for situations where the receiving environment of the GM plant would be substantially different to the appropriate comparator. Field trial design would be agreed on a case by case basis, and where required and simulate environments in which the GM plant may be grown in a field trial employing a fully factorial statistical design. An example is given of a salt tolerant GM plant where differing treatments of salinity would be administered to the GM plant and its conventional comparator.

In addition, exposure assessment will be an important step for the risk assessment process, particularly for GM plants with 'novel' quality/output traits which provide nutritional benefits to the consumer. Post market monitoring is an area which has received little attention in the literature, and further work is needed to develop guidance on when this should be carried out. It is proposed that a period of public consultation should be included in the risk assessment process, but that these should be time bound, with specific milestones for decision making. Particular consideration should be given to future revisions of Regulation (EC) 258/97 on novel foods, and how these may apply to the assessments of GM plants.

A number of recommendations for further work are given, including the need for a wider review of risk assessment strategies to inform the approach to risk assessment for 'novel' traits, further work to develop guidance on post market monitoring, guidance on cases where field trial design for 'novel' traits may need to be amended, further work on the concept of history of safe use and guidance on the management of the risk assessment process.

CONTENTS

BACKGROUND AS PROVIDED BY EFSA

For any genetically modified (GM) organism and derived food or feed to be authorised in the EU, a company must submit an application for authorisation on placing on the market in line with European legislation. In accordance with EU legislation an independent scientific risk assessment is to be carried out by the EFSA GMO Panel to evaluate the safety of the GMO and derived food or feed. The GMO Panels independent scientific advice is then used by risk managers from the European Commission and Member States when taking a decision on market approval.

The majority of the current GM plants applications for authorisation in the EU market concerns modifications to agronomic traits such as herbicide tolerance and/or insect resistance. A new generation of GM plants is currently being developed to express novel traits. Examples of novel traits include nutritionally enhanced crops (with qualitative and quantitative changes in proteins, amino acids, carbohydrates, fats, vitamins and/or minerals) or GM plants with complex phenotypes (e.g. providing adaptation to abiotic stresses such as drought or high salinity). These types of traits may induce major changes in the host plant which may result in substantial modifications to the endogenous metabolism and physiology of the plant. Therefore, the selection of appropriate comparators for the risk assessment of these GM plants as required by the EFSA Guidance for risk assessment of food and feed from GM plants may be difficult. When no appropriate comparator is available, the comparative approach, as traditionally applied to the food and feed risk assessment of GM plants, would not be fully applicable and the risk assessment should be based primarily on the evaluation of the characteristics of the GM plant and derived products per se.

Acknowledging this issue, the EFSA Guidance on the Selection of comparators for the risk assessment of GM plants (EFSA, 2011) lists the general requirements for the assessment of this new generation of GM plants, which is based to some extent on the approach defined by the Novel Foods and Novel Food Ingredients Regulation (EC No 258/97).

Additionally, the Guidance for risk assessment of food and feed from GM plants identifies the need to further develop the strategy for the assessment of these new GM plants where it says: *"In cases where a comparative assessment is not applicable, a comprehensive food and feed safety and nutritional assessment of the GM plant and derived food and feed should be performed. This should include, among others, a detailed compositional analysis and specific toxicological/nutritional analyses, selected according to the agronomic and compositional properties of the food and feed under assessment. Further development and detailing of this strategy is needed".*

TERMS OF REFERENCE AS PROVIDED BY EFSA

This report was awarded by EFSA to:

ADAS UK Ltd,

Review of the strategies for the comprehensive food and feed safety and nutritional assessment of GM plants *per se*.

Contract/grant number: CFT/EFSA/GMO/2012/04

This procurement aims at outsourcing a review of the current scientific literature and risk assessment frameworks relevant for the risk assessment of GM plants in cases where a comparative assessment may not be fully applicable.

The contractor should carry out comprehensive literature searches to identify and retrieve all related information/data published in relation to the specific objectives of this contract that should be critically

reviewed. Two technical reports should be produced that will be used as background information for further discussion within the EFSA GMO Panel.

LIST OF TERMS

ACRE	Advisory Committee on Releases to the Environment
ARMGs	Antibiotic resistance marker genes
AHTEG	Ad hoc technical experts group
Bt	*Bacillus thuringiensis*
CMV	Cucumber mosaic virus
CspB	Cold shock protein B
EFSA	European Food Safety Authority
EPA/ORD	Environmental Protection Agency/ Office of Research and Development
FAO	Food and Agriculture Organisation
FDA	Food and Drug Administration
FSANZ	Food Safety Australia New Zealand
GC-TOF	Gas chromatography – time of flight analysis
GIT	Gastro-intestinal tract
GMP	Genetically modified plant
GM	Genetically modified
GMO	Genetically modified organism
HGT	Horizontal gene transfer
ILSI	International Life Sciences Institute
IgE	Immunoglobulin E
LC	Liquid chromatography
LMO	Living modified organism
MALDI-TOFS	Matrix-assisted laser desorption/Ionisation – time of flight spectroscopy
MOA	Ministry of Agriculture
MS	Mass spectrometry
NPTII	neomycin phosphotransferase II
OECD	Organisation for Economic Co-operation and Development
PEPC	Phosphoenolpyruvate carboxylase
PMM	Post-market monitoring
PPO	Polyphenol oxidase
RA	Risk assessment
RNA	Ribonucleic acid
SDS-PAGE	Sodium dodecyl sulphate- polyacrylamide gel electrophoresis
SE	Substantial equivalence
WHO	World Health Organisation

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1. Introduction and Objectives

1.1. Introduction

For any genetically modified organism (GMO) and its derived food and feed to be authorised in the EU, an independent scientific risk assessment must be carried out in line with Regulation (EC) No 1829/2003. An important part of the risk assessment process is comparative assessment, which compares the GM plant and derived food or feed with appropriate comparators. The underlying assumption of the comparative approach is that traditionally cultivated crops have a history of safe use for consumers and/or domesticated animals. The comparative approach provides a baseline for identifying differences and equivalences in composition, agronomic performance and phenotypic characteristics between the GM plant and its comparator.

The majority of current GM plants authorised in the EU market[4] are those with modifications to agronomic traits such as herbicide tolerance or insect resistance. However a new generation of GM plants are in development with 'novel' modifications such as nutritionally enhanced qualities (such as changes to proteins or amino acids) or phenotypes (such as adaptation to abiotic stresses). To achieve these 'novel' GM traits, substantial modification is needed to the host plant, including modifications to its endogenous metabolism and physiology. Given the substantial modifications induced in the host plant to achieve these 'novel' GM traits, the selection of appropriate comparators for the risk assessment of these GM plants as required by the EFSA *Guidance for risk assessment of food and feed from GM plants* may be difficult.

1.2. Objectives

The project aimed to review current scientific literature, and approaches taken by international risk assessment bodies relevant to the risk assessment of GM plants in cases where a comparative assessment may not be fully applicable, or is not applied.

The specific objectives of the project, as outlined in the tender specifications were:

Review of the strategies for the risk assessment of GM plants expressing 'novel' traits; and

Review of the strategies for the comprehensive risk assessment of GM plants *per se* (i.e. approaches to risk assessments that are not based on the comparative approach).

As so many of the papers found in the literature review discussed elements of both of the objectives together, a decision was taken to combine objectives 1 and 2, rather than presenting separate literature reviews. Therefore the overall objective of the project team was to:

Review the strategies for the risk assessment of GM plants with 'novel' traits, and for 'comprehensive' risk assessment strategies.

1.3. Scope

The project results are based principally on a review of the available literature. Hence, the GM plants with 'novel' traits which are discussed in the report, only include those which are publicly known and have published literature associated with them. Any 'novel' traits which have little information

[4] See http://ec.europa.eu/food/dyna/gm_register/index_en.cfm for the EU register of authorised GMOs

available (as they are in the early stages of development) are therefore not considered in this assignment in terms of risk assessment approaches.

2. Materials and Methods

2.1. Literature review

2.1.1. Background and objectives

The literature review of risk assessment strategies proposed in the scientific literature, and from international risk assessment bodies followed the basic principles of a systematic review[5]. It was recognised at the outset of the project that due to the broadness of the question asked (i.e. to review strategies), a full systematic review according to EFSA guidelines (see EFSA, 2010) would not be appropriate. It also became apparent from preliminary searches of the literature that the types of records sourced would not contain extensive amounts of numerical data, rather dialogue, and to some extent opinion from the author or risk assessment body regarding strategies for risk assessment. There were two principal objectives of the literature review:

Objective 1: Review strategies from the scientific literature, and strategies from international risk assessment bodies for GM plants expressing 'novel' traits (food and feed risk assessment); and

Objective 2: Review strategies from the scientific literature, and strategies from international risk assessment bodies for comprehensive risk assessment strategies (i.e. not based on the comparative approach) for GM plants *per se.*

2.1.2. Search terms

Search terms were developed and agreed by the project team for sourcing scientific literature for each objective (Table 1 & Table 2). For sourcing risk assessment approaches from international risk assessment bodies it was not deemed necessary to agree search terms.

[5] A systematic review (SR) is an overview of existing evidence pertinent to a clearly formulated question, which uses pre-specified and standardised methods to identify and critically appraise relevant research, and to collect, report and analyse data from the studies that are included in the review (EFSA, 2010).

Table 1: Search terms for objective 1

Search Group		Search Terms
Risk assessment		"Safety assess*" OR "risk assess*" OR "comprehensive assess*" OR "comparative assess*" OR "food safety" OR "feed safety"
Genetic Modification	AND	Transgen* OR novel OR event OR trait OR "complex trait" OR "output trait" OR "2nd generation" OR "second generation" OR "genetic* modif*" OR "genetic* engineer*" OR recombin* OR transform*OR GM OR GMO OR GE OR GEO
Trait	AND OR	Food OR feed OR "Amino acid" OR lysine OR protein OR "fatty acid" OR lipid OR vitamin OR mineral OR carbohydrate OR starch OR degreening OR nutr* OR allergenicity OR toxicity
		Ripening OR senescence OR "vegetative growth"
		"Stress toleran*" OR drought OR salinity OR heat
		"Fungal resistan" OR "virus resistan" or "bacterial resistan"
		Secondary effects… "gene take up" OR "gene transfer" OR "miRNA" OR" bioactives" OR "nutritional value"
	AND NOT	"herbicide toleran*" OR Bt OR "insect resistan*"
	AND NOT	"environmental risk assessment"

Table 2: Search terms for objective 2

Search Group		Search Terms
Risk assessment		"Safety assess*" OR "risk assess*" OR "comprehensive assess*" OR "food safety" OR "feed safety"
Genetic Modification	AND	Transgen* OR novel OR event OR trait OR "complex trait" OR "output trait" OR "2nd generation" OR "second generation" OR "genetic* modif*" OR "genetic* engineer*" OR recombin* OR transform*OR GM OR GMO OR GE OR GEO
"Non-Novel"	AND/OR	"herbicide toleran*" OR Bt OR "insect resistan*"
"Novel"	AND/OR	New OR novel OR alternative
"Food"	AND/OR	Food OR diet OR nutrition OR consumption OR feed

As the scientific literature was searched it became apparent that papers discussing risk assessment strategies for GM plants with 'novel' traits, and those discussing 'comprehensive' risk assessment approaches were often similar. For many papers where risk assessment strategies for 'novel' GM traits are discussed, so are 'comprehensive' risk assessment strategies. In many cases the terms 'novel' and 'comprehensive' discussed in the scientific literature, do not bear any relation to the way in which they were described in the original brief for the present study. As so many of the papers discussed elements of both of the objectives together with no clear distinction, a decision was taken by the project team to combine objectives 1 and 2, rather than presenting separate literature reviews.

2.1.3. Data sources

To source scientific literature the team used SciVerse Scopus, and Google Scholar. For international risk assessments the websites of individual country risk assessment bodies, and research institutes

were searched, as well as using databases available publicly such as the Biosafety Clearing House[6], International Service for the Acquisition of Agri-biotech Applications (ISAAA)[7] and the Centre for Environmental Risk Assessment (CERA)[8].

[6] Established by the Cartagena Protocol on Biosafety, see http://bch.cbd.int/about/ for more details.
[7] A not for profit international organisation sharing knowledge on crop biotechnology, see http://www.isaaa.org
[8] Established by the International Life Sciences Institute to develop environmental risk assessment of agricultural biotechnology, see http://cera-gmc.org/

2.1.4. Study selection criteria

All literature generated from the above search terms were gathered and screened for their applicability to the two principle objectives of the literature review. The screening took place in two phases, a 'gate 1' and 'gate 2' assessment. The gate 1 assessment reviewed the titles, and if available the abstract of the paper for relevance to the objective, whilst at gate 2 (if the paper passed gate 1) the full text article was reviewed for its applicability to the study.

Table 3: Gate 1 literature criteria

Question	**Criteria**
What is the format of the record?	Records will be accepted at Gate 1 for review if they are one of the following: Scientific peer reviewed literature; Competent authority guidance; Competent authority published risk assessment; Scientific opinion; or Book chapter.
Does the record refer to 'novel' GM plants?	Record must refer to a 'novel' GM plants as defined in Appendix A.
OR	
Does record refer to 'comprehensive' risk assessment GM plants?	Record must refer to strategies for comprehensive risk assessment of all GM plants (strategies not based on the comparative approach), in a format as: Risk assessment methodologies; or Scientific assessment to test safety of plants. Criteria for safety assessment of plants.
AND	
Does the record refer to risk assessment approaches?	Record must be focussed on one of the following: Risk assessment methodologies; Scientific assessment to test safety of plants; or Criteria for safety assessment of plants.
Does the record refer to risk assessment of food and feed safety and NOT environmental safety?	
Does the record refer to risk assessment as primary focus?	Record must be based on one of the following: Food safety assessment; Feed safety assessment; or Nutritional safety assessment.
If record is an approach taken by an appropriate risk assessment body	Record must: Be a risk assessment document (not a country decision or similar); and Record must be in English.

The full text articles of all scientific literature which passed the gate 1 assessment for each objective were then obtained, to be put through a further screening for inclusion in the study. If a decision could not be made on the piece of literature at gate 1 from the title or abstract then the paper was passed to gate 2 assessments and the full text was obtained. Gate 2 assessment was used to confirm the subject and content of the piece of literature to ensure it was in line with objectives. It was not deemed necessary to put in country risk assessment strategies through a further assessment as the remit was to review the individual approaches taken by the said country.

Table 4: Gate 2 scientific literature criteria

Question	Criteria
Does record refer to 'novel' GM plants within the body of the paper?	Record must refer to a 'novel' GM plants as defined in Appendix A.
OR	
Does record refer to 'comprehensive' strategies for risk assessment of GM plants within the body of the paper?	Record must refer to strategies for comprehensive risk assessment of all GM plants, in a format as following: Risk assessment methodologies; Scientific assessment to test safety of plants; or Criteria for safety assessment of plants.
AND	
If record is an approach taken by an appropriate risk assessment body	Record must: Be a risk assessment document (not a country decision or similar); and Record must be in English.

Both gate 1 and gate 2 assessments for all studies were carried out by two members of the project team who agreed together which studies passed the assessment criteria. Where these two members could not agree on whether a study should be included, it was then sent to a third member of the project team to make the decision.

Using the search terms, 983 papers were identified as having relevance to the two objectives.

The gate 1 assessment reduced the number of applicable papers to 231 papers.

The gate 2 assessment reduced the total number of papers reviewed to 92 papers.

2.1.5. Presentation of results

Due to the nature of the questions asked, much of the information in included studies tended to be descriptive and qualitative, describing approaches rather than producing quantitative or original data. The project team took the approach of reporting findings from each paper in turn, grouped as much as possible by their relation to specific elements of risk assessment. The results of the literature review were then discussed in a subsequent section.

2.2. Other tasks

2.2.1. Risk assessment criteria

The risk assessment criteria for GM plants with 'novel' traits was presented, and based on findings of the literature review. The section described the overall approach to food and feed risk assessment of GM plants with 'novel' traits according to the results of the literature review.

2.2.2. Case studies- risk assessment of GM plants with 'novel' traits

There are four case studies in the report. Three of these case studies were used to demonstrate the approaches used by different international risk assessment bodies to risk assess GM plants with 'novel' traits. They aimed to represent a range of traits defined as 'novel' by the project team in Appendix A. The case studies are all plants that had previously undergone food and feed risk assessment by various international risk assessment bodies around the world. The analysis gave a

background to the GM plant, and described an overview food and feed risk assessment approach taken by different international risk assessment bodies.

2.2.3. Novel food risk assessment frameworks

Approaches used to risk assess novel foods were evaluated to assess the similarities and differences between approaches to food and feed risk assessment. The UK's Advisory Committee on Novel Foods and Processes (ACNFP), on behalf of the UK Food Standards Agency (FSA) process for assuring food and feed safety of novel foods were reviewed. This process is under the Novel foods regulation (EC) 258/97. In addition the approach used by Health Canada, for novel food assessment in Canada is assessed. These two approaches were chosen given the amount of publicly available information on the process, and the differing ways in which the two bodies carry out risk assessments. Information used was sourced mainly from publicly available literature, however for the ACNFP data was also gathered from a face to face interview with members of the FSA secretariat for the ACNFP.

2.2.4. Foreseeable scenarios for risk assessment

The foreseeable scenarios for risk assessment represents the project teams overall discussion and conclusions provided as information which may help to guide the development of risk assessment approaches for 'novel' traits. The foreseeable scenarios were based on findings from all the information gathered for this project, and by discussion with the entire project team representing experts in GMOs, human nutrition, food and feed safety and risk assessment. A project team, face to face meeting was convened, where evidence gathered for the project was presented. The team then brainstormed different scenarios for risk assessment, finally coming to a mutually agreeable approach that was seen to be appropriate to ensure food and feed safety of GM plants with 'novel' traits. The foreseeable scenarios section also includes a hypothetical case study, of a GM plant with a 'novel' trait which may require market authorisation at some stage.

3. Discussion of literature review results

This section represents the discussion of literature review results which are included in Appendices B & C.

3.1. Background note: substantial equivalence

In the 1993 Organisation for Economic Co-operation and Development (OECD) Green Book *'safety evaluation of foods derived from modern biotechnology: concepts and principles'* it is advocated that the most practical method to determine safety of food, and food components from genetically modified organisms is to consider whether they are **substantially equivalent** (SE) to analogous food product(s) (OECD, 1993). The concept of SE states that existing organisms used as food, or as a source of food can be used as a basis of comparison when assessing the safety of the new food that is modified (GM plants). If the new food that is modified can be demonstrated to be substantially equivalent to an existing food then further safety or nutritional concerns are expected to be insignificant (OECD, 1993). Substantial equivalence (SE) is not the safety assessment itself, but represents the starting point to assess a new food, based on a comparison with its conventional counterpart. However, since its creation, it became clear that the principle of SE left scope for different interpretation by various national bodies (Kok and Kuiper, 2003).

The concept of SE is applied by using the **comparative approach** in European Food Safety Authority (EFSA) guidelines. As with the OECD guidelines, food and feed derived from GMOs is compared to an appropriate comparator, defined by Regulation (EC) No 1829/2003 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'.* Under this legal requirement the EFSA GMO Panel gives details on the appropriate selection of comparators, under different scenarios (see EFSA, 2011):

For GM plants containing single events the plant should be compared to a) the non-GM isogenic variety, in the case of vegetatively propagated crops or b) a genotype with a genetic background as close as possible to the GM plant for crops that are propagated sexually. In this case the plant that the crop is compared to is called a '**conventional counterpart'**; and

For other cases, for example where the GM plant is compared to genotypes that do not fit with the above definition, the term **'comparator'** is used.

Other risk assessment bodies may state that their risk assessment approach is based on the principles of SE, however individual approaches to demonstrate substantial equivalence may differ (i.e. the selection of appropriate comparators). Given these differing approaches, within this report the terms 'Substantial Equivalence' and 'Comparative Approach' are used throughout and when stated refer to the definitions provided above. Within the literature review results, authors may use these terms in their original papers, however in some cases it is not clear whether their definition of the terms substantial equivalence or comparative approach are the same as those presented above. As such, in the results section, the term used by the author is used when presenting individual results, but this may not necessarily confirm to the definitions above.

3.2. Comparative approaches to safety assessment

SE is a cornerstone concept of the current OCED/EFSA guidelines to risk assessing food and feed safety of GM foods. Despite this, there are some differences in the way that groups define SE in the literature. For example it can be defined either as a comparison with a single 'conventional counterpart' or with a non-GM 'comparator'. The difference between conventional counterpart and

comparator is noted in EFSA guidelines and described in section 3.1. However, despite these definitions it is apparent from the literature review that these terms are used interchangeably, and in many cases no context is given. Since the original definition of SE given by the OECD in 1993, the concept has left much scope for interpretation by the scientific community and risk assessment bodies. Very few (if any) scientific papers use the terms 'conventional comparator' and 'comparator' as is defined by EFSA.

In most cases the studies assessed in this literature review (including Faust, 2002, Llorente *et al.*, 2011, Chassy *et al.*, 2007) have either used or supported the use of comparative approaches to assess the safety of GM crops with 'novel' traits as outlined in EFSA Guidelines. The studies all suggested the use of an appropriate comparator, which did in some cases included a number of crop varieties of the host species. The comparative approach is used to identify intended/unintended differences and compares these to the range of natural variation (estimated from a set of non-GM reference varieties with a history of safe use).

For a 'novel' GM plant, there might be substantial differences between it, and both a 'conventional counterpart' or a 'comparator'. Despite this, there is a lot of evidence that SE is still adequate when the GM crop is compared to the natural variation within its species, especially when environmentally-induced phenotypic variation is considered. It is the case that the compositional variation resulting from environmental variation, is more commonly greater than that of the unintended effects caused by the introduction of 'novel' traits (Llorente *et al.*, 2011). Using comparative approaches, a test of difference and a test of equivalence of agronomic, phenotypic and compositional characteristics can be carried out, comparing a GM crop to the natural variation within its species population.

EFSA guidelines state that material used in a compositional analysis should take into account the end use of the GM product and that unless justified, analysis should occur on the raw agricultural commodity. Additional analysis of the processed product is carried out on a case-by-case basis. The use of a case-by-case approach, as proposed by Talas-Oğraş (2011); Heinemann *et al.* (2001); and Chassy *et al.* (2007), would aid the analysis of food and feed safety and also nutritional assessment in the context of the form the crop is likely to be consumed in. Furthermore, it has been suggested by a number of groups that the process of food and feed safety evaluation should be standardised to avoid repeated safety assessments, and to bring about the formalisation of risk assessment protocols (Heinemann *et al.*, 2011). To allow the use of a case-by-case approach which is formalised for all risk assessment bodies, it may be that a structured procedure for assessing the form of the 'novel' trait would provide the most appropriate method of risk-based food and feed safety and also nutritional assessment.

A number of authors have noted that safety assessments for GM crops are more stringent than risk assessments for conventionally bred crop varieties. Some methods have been proposed to address these discrepancies, such as introducing a general screening framework for all new plant varieties (GM or non-GM) (Kok *et al.*, 2008), a consideration of the intended use of the GM variety, or through the development of a risk-sensitive safety and nutritional assessment protocol.

The literature review has shown that many differing views exist on approaches to risk assessment of GM plants with 'novel' traits. Whilst some of the scientific literature states that the current comparative approach recommended in EFSA guidelines may not be suitable to assess these plants, all the international risk assessment bodies reviewed used the same approach for these GM plants, as for non-novel traits. Given the conclusions of the literature review, this section suggests what additional questions (on top of the current EFSA guidance on risk assessment of food and feed safety) may need to be taken on board to adequately assess safety of GM plants with 'novel' traits. In addition, risk assessment strategies are proposed to accurately identify hazards for an assessment of overall risk. It is proposed that the risk assessment process for GM plants with 'novel' traits should be assessed/evaluated on a case by case basis, depending on the modifications of the plant and as such,

the likely risks. The case by case approach takes into account the differences between GM plants with 'novel' traits, such as those with altered nutritional properties, and those with complex input traits. A particular focus is paid in this section to the hazard identification stage as 'novel' GM traits may not have an appropriate comparator.

3.3. Profiling Techniques

This literature review shows that there is a level of discussion around the identification of unintended effects (changes beyond the intended change), especially for newer novel traits (Xue *et al.*, 2012, Glenn, 2007, Heinemann *et al.*, 2011, Talas-Oğraş, 2011, Deng *et al.*, 2008). Disagreement is widespread as to whether non-targeted broad compositional analysis such as the -omics approaches should replace targeted analysis of specific compounds of known nutritional, allergenic or toxicological risk (Kuiper *et al.*, 2002, Chassy, 2010, Heinemann *et al.*, 2011) to ensure unintended effects are assessed. Current EFSA Guidelines stipulate that intended and unintended effects should be assessed with respect to their safety, allergenicity and nutritional impact. A number of papers suggest that a compromise for safety assessment is to use these strategies on a case-by-case basis when unintended effects cannot be predicted with any certainty (Glenn, 2007 and Davies, 2010). It is also suggested that these methods should be validated before incorporation into standard safety assessments (Talas-Oğraş, 2011).

It has been shown that these non-targeted techniques can be used to facilitate the risk assessment using a comparative methodology for 'novel' traits. This is substantiated by Catchpole *et al.* (2005), who discuss the use of hierarchical metabolomics to demonstrate substantial compositional similarity between 'novel' GM plants and conventional potato crops. In this case the context is demonstrating compositional 'similarity', rather than 'equivalence'. Rapid metabolome fingerprinting, and other non-targeted methods can be used to identify metabolites responsible for differences between crop genotypes which will be risk assessed subsequently for safety (Kusano *et al.*, 2011). This could include key components which are likely to have a possible health risk including: nutrients, anti-nutrients, toxins, allergens and secondary plant metabolites (Seskieran *et al.*, 2008). Despite claims that these will improve evaluation of unintended effects, there is no evidence in the literature that non-targeted techniques have identified unintended effects of any health risk. Additionally, these techniques may be of questionable importance because compositional variables will vary greatly depending on the developmental stage at which the crop is sampled.

This review has uncovered discussion about the relative usefulness of different -omics approaches to address unintended changes within transgenic crops. Metabolomics is widely supported as the most suitable method of assessing unintended compositional changes as a result of the genetic insertion (Llorente *et al.*, 2011). On the other hand, proteomics has been identified as a strong candidate for recognising protein changes which lie at the route of downstream alterations to metabolism/ development/ stress tolerance. Xue *et al.* (2012) supported the use of an integrated risk assessment using comparative proteomics for transgenic rice (*Oryza sativa*) expressing Bt and Phosphoenolpyruvate carboxylase. Additionally D'Alessandro and Zolla (2012) suggested that proteomics might provide insight into changes that do not necessarily present themselves through metabolic biological parameters, and that this method could be used to assess SE at multiple levels. It can be said that difficulties may arise when extrapolating differences in protein expression to significant changes in, for example, storage compound concentration for those GM adaptations which hope to increase the level of a non-protein.

Compositional analysis is a major part of nutritional assessment (Varzakas *et al.* 2007), and therefore validated, rigorous and functional methods to verify compositional safety must be developed. This is particularly true for 'novel' GM traits which have often undergone specific alterations to crop composition, or plant metabolic pathways. GM lines with 'novel' traits may not have a suitable conventional counterpart to assess unintended compositional effects as developed in the GM food and

feed safety risk assessment proposed in OECD guidelines (OECD Green Book, 1993). This review has revealed links proposed in the literature between compositional analysis to identify unintended effects and the production of a crop-specific database for use as a comparator to which GM crops can be compared (Varzakas *et al.*, 2007; Chassy *et al.*, 2007). This database is proposed to provide detailed compositional variables for the major structural, storage and enzymatic substances (Sesikeran *et al.*, 2008 and Novak and Haslberger, 2000). This could be used to address the compositional safety of both 'novel' and 'non-novel' crops. This database could be peer-reviewed and could be developed using compositional information such as the Crop Composition database compiled by ILSI.

A number of papers have identified that for most compositional variables, GM crops would fit within the natural variation of current conventionally bred and consumed crops of the same species (Barber *et al.*, 2008 and Chassy, 2010), especially when environmental variation is considered (Davies, 2010). The database would therefore act as an appropriate comparator, by assessing whether each compositional value would fit within the tolerance levels as set by the natural variation for that substance in that crop. This would be a powerful tool that would assess whether the GM line is substantially similar to a broad crop population, rather than a conventional counterpart, or more specifically the isogenic line. This could also facilitate the use of baselines and thresholds for each variable, assigned due to the natural variability of crop populations, (Schmidt *et al.*, 2011) and act as a desirable benchmark for safety assessment (Davies, 2010 and Novak and Haslberger, 2000). Although there are current databases describing compositional data, it is questionable as to how more detailed datasets might be compiled, and how the current holes in compositional information might be filled.

It is discussed that for those variables which are shown to lie outside the limits of current natural variation, further detailed investigations would be carried out, with respect to: allergenicity, toxicity, abundance of the compound and chemical structure (Barber *et al.*, 2008). A further conclusion of this literature review is that this database could include reference intervals to describe safe intake levels for a healthy heterogeneous population for each compositional variable, as suggested by Herman *et al.* (2010). This may be less suitable because reference levels may not be defined. It should be noted that all approaches (proteomics, metabolomics and metabolite profiling) have detection limits which could be set.

3.4. Comparing to a food product

Codex guidelines discuss the eventuality of a modification resulting in a food product, like vegetable oil, with a significantly different composition from its conventional counterpart. It is stated that in such cases it may be suitable to use conventional foods/ food components whose nutritional composition is closer to the food derived from the GM plant as appropriate comparators for assessing the nutritional impact of the food. A number of the 'novel' GM traits that will request authorisation in the future are nutritionally enhanced crops that contain a compound that is currently eaten within the diet but from another source (e.g. particular fatty acids from fish oils). One proposal for safety assessment of these compounds is to compare them with the current similar compounds consumed within the diet. For example this might be a commonly consumed food oil or protein from another food product (Constable *et al.*, 2007). Varzakas *et al.* (2007) suggest GM material should be compared with the parent plant and material from the parent plant genetically modified to express an empty construct. This is useful in plant genetics research but comparison with parent plant may be more relevant to risk assessment.

3.5. Allergenicity Assessment

IgE-mediated food allergy has been the focus of RA of allergenicity in GM plants. The literature compiled using this systematic review has identified key areas of debate as to the structure and validity of safety assessment methods. Allergenicity is a major example of where discussion within the scientific community has lead to widespread disagreements as to the strength of the current

allergenicity evaluation system. Goodman *et al.* (2008) in particular question the validity of a number of the more standard allergencity risk assessment procedures, including bioinformatic searches, digestibility studies and animal studies. Despite this, all of the major studies (proposed by EFSA and Codex) are supported by a number of authors (Snell *et al.* 2012; Lack, 2002; Llorente *et al.* 2011). There are calls for the identification of allergenic potential of GM plant products to be validated, ensuring it is robust, and reproducible (Talas-Oğraş, 2011), and that it should continue with Codex Guidelines (2009) to provide a weight of evidence approach (Snell *et al.*, 2012; Goodman *et al.*, 2005 and Singh *et al.*, 2009). A number of groups suggested the implementation of a decision tree approach to assess allergenicity (Meredith, 2005).

A number of themes, aside from the specific protocols used to assess allergenicity, have stemmed from literature searches. For allergenicity assessment of newly expressed proteins and of purified proteins prepared by expression in bacteria it needs to be considered that plant proteins may undergo alternative glycosylation patterns compared to those in bacteria (Lin *et al.*, 2010). Such modifications may alter the immunogenicity of the allergens structure. Secondly, there are questions as to the assessment of both intrinsic allergenicity and also cross reactivity with pre-conditioned allergenic reactions, the second of which is suggested to pose the most unpredictable risk (Lack, 2002).

Improvements to specific allergenicity assessment methods include substantiating serum banks (Barber *et al.*, 2008), to take into account the geographical location, the intensity and nature of the environmental allergens in the area and the potential cross-reactivity among allergenic molecules when selecting patient sera. EFSA Opinion on allergenicity (2010) recommends that when there is sequence homology/structure similarity to known allergens, individual sera from allergic individuals should be used rather than pooled sera. This study also suggests guaranteeing the integrity of in vitro methodologies. There are also comments about the lack of validated *in vivo* models.

Furthermore, bioinformatic search algorithms and scoring criteria for potential cross-reactivity could be developed (Goodman *et al.*, 2005). The proposed merits and issues of each of the allergenicity risk assessments will not be fully examined as part of this discussion. Subjects discussed in the literature review are also examined in the EFSA Opinion including: clinical and structural aspects, *in silico* approaches, IgE binding studies, cell-based methods, profiling techniques and animal models.

Goodman *et al* (2008) highlighted that great stringency in allergenicity assessment (including non-validated tests) could lead to the rejection of safe and beneficial products, excessive costs and, in the extreme, a negative impact on trade with no significant reduction of risk. These authors also reported that there is a low likelihood associated with generating neo-allergens. It is possible that including an assessment of the probability of hazards and an estimation of at-risk groups/ exposure assessment could strengthen the current system (Goodman and Tetteh, 2011).

Reactions can occur between food proteins and antibodies to contact and respiratory allergens: the latex fruit syndrome and pollen fruit syndrome. Hence it is necessary to include relationships to all allergens (not just food allergens) in the risk assessment. The most appropriate approach for assessing allergenicity of GM food and feed is considered by EFSA Scientific Opinion on allergenicity to be the weight-of-evidence, case-by-case approach (EFSA, 2010). The authors discuss the ways of improving the strength and accuracy of the allergenicity assessments including: clinical aspects, structural aspects, *in silico* approaches, IgE binding studies, cell-based methods, profiling techniques and animal models. These are discussed as summarised above.

3.6. Toxicity Assessment, Nutritional Assessment and Animal Feeding Trials

As shown above, the different components of the risk assessment in the EFSA Guidelines (EFSA, 2011) include: Molecular characterisation, Toxicity assessment, Allergenicity and Nutritional assessment. Safety assessment for toxicology can be carried out using animal models or *in vitro*

systems to characterise hazards. Standardised guidelines for toxicity tests exist and are internationally agreed by the OECD. There is little discussion in the literature about the potential coordination of these two types of toxicological assessment, whereas the merits of each assessment method are touched on by Domingo *et al.* (2011), Atherton, (2002), Zhou *et al.* (2012), Flachowsky and Wenk, (2010), and Momma *et al.* (2000). *In vitro* models suggested to support toxicity assessments include an *in vitro* ruminal epithelial cells system developed to study the effects of the insect specific Bt toxin, gastrointestinal cell culture models from digestive tract (Talas-Oğraş, 2011) and an *in vitro* system simulating the transport of substances form the gut into the blood that detects risk of incorporation in humans or animals (Schmidt *et al.*, 2011). There is significant debate as to the requirement of longer term feeding trials in toxicology risk assessment. EFSA guidelines currently state that depending on the quality of available data, animal (laboratory/targeted) feeding trials with whole food/feed may be considered on a case-by-case basis. It should noted that new Regulation from the EC taking effect this year will require 90 toxicity studies of whole food as mandatory, rather than on a case by case basis. If the composition is substantially modified (as in 'novel' GM), not only the new constituents but also the whole food must be tested. Test protocols are described (in the 'opinion of the EFSA Scientific Committee') for 90-day feeding trials. There has been support for 90 day rodent toxicology studies to be used where appropriate (Chassy *et al.*, 2007), or to comprise a larger part of the risk assessment.

Snell *et al.* (2012) are opposed to longer studies which might also be carried out on a case-by-case basis. However, many say that longer studies would (Flachowsky *et al.*, 2005, Momma *et al.*, 2002) allow for investigating malformations, reproductive disorders, mutatagenicity or carcinogenicity caused by consumption of any GM product.

Toxicity trials can involve feeding of the concentrated novel compound, or feeding of the whole food, and as with allergenicity assessments, the literature calls for a standardisation and validation of toxicology trials. To contribute to the development of this standard system, there is discussion as to relevance of adding whole foods to toxicity risk assessment in the literature. Studies with laboratory animals can backup observations from other parts of the safety assessment (Chassy *et al.*, 2007, Llorente *et al.*, 2011), although they may not be sensitive enough to show unintended minor changes (Atherton, 2002 and Flachowsky *et al.*, 2007). Despite this, the scientific validity of laboratory animal models has been called into question, especially as most studies have not shown a negative effect of the transgenic breeding process (Chassy, 2010). The use of whole foods may still be appropriate because the correct processing of the product can be facilitated, and major unintended effects might be identified. EFSA (2010) recommend future work focuses on acquiring data on the modifications induced from processing and the impacts of this on release, stability and allergenicity potential of the protein. The influence of processing and storage on the characteristics of the derived products is currently an element of the risk assessment of GM plants and derived food and feed. Animal trials also allow the GM product to be compared to other food products naturally in the food chain. Additionally, feeding trials with target animals might provide a more realistic estimation of the nutritional benefits of nutritionally enhanced feed crops and also the safety of these foods (Flachowsky *et al.*, 2007; Flachowsky *et al.*, 2012; Flachowsky and Âhme, 2005; Flachowsky and Wenk, 2010).

The nutritional assessment is in place to ensure the GM plant is not nutritionally disadvantageous. It has been proposed that nutritional evaluation should be used to assess whether the GM product will affect the nutritional balance of the whole diet, i.e. that it will not replace a nutritionally important part of the diet. The nutritional assessment should assess: the nutritional relevance of any new constituents, changed levels of endogenous constituents and potential alterations in the total diet for the consumers/animals. The food and feed safety assessment is therefore separate from the nutritional assessment. New experimental designs should be developed for novel crops to consider the area to be grown in, growing seasons, geographical spread of consumption, the number of replicates in feeding trials and the statistical analysis used to verify results (Flachowsky *et al.*, 2012). Additionally, it might be that pre-market studies in humans become of importance to assess safety (Chassy *et al.*, 2007). Momma *et al.* (2000) suggested a safety assessment carried out using a cultured human cell system.

However, this was the only paper in the literature review which suggested this: there may be problems extrapolating from cell models to animal/human systems.

The nutritional assessment of 'novel' GM foods should also consider the biological efficacy of the nutrient components and an assessment of dietary intake and resultant nutritional impact (Varzakas *et al.* 2007). To do so, there is a need for an exposure assessment alongside nutritional evaluation, to address saturation in food chains.

3.7. DNA Transfer

The results of this literature review have shown that on the whole, there is limited perceived risk of transfer of DNA from the crop product to mammalian cells through the GIT, although some DNA (transgenic or otherwise) may not be fully degraded by gut enzymes (Rizzi *et al*., 2012 and El-Sanhoty *et al*., 2006). Despite this Rizzi *et al*. (2012) discuss the use of a novel, highly sensitive in vitro system to assess the possibility of DNA-transfer. On the other hand the prediction of the fate of dietary DNA is currently unachievable in the majority of studies Rizzi *et al*. (2012) and therefore should not form the basis of risk assessment for novel GM food products. It seems of limited relevance to safety assessment. It is also reasonable to suggest that the risks of transfer of transgenic DNA into human cells are equal to that for non-transgenic DNA.

3.8. Post-Market Monitoring, Exposure Assessment and Vulnerable Groups Assessment

Post-market monitoring (PMM) is not a routine absolute requirement: it is on a case-by-case basis for foods such as those with altered nutritional composition and modified nutritional value, or for those with specific health claims (see EFSA, 2011). Under EFSA guidelines an estimate of expected intake (exposure assessment) is an essential part of the risk assessment process (EFSA, 2011).

Considerations of PMM have been addressed with papers selected by this literature review. It is suggested that this process be used where appropriate to validate predictions of exposure levels within different populations (Hlywka *et al.*, 2003). This process is appropriate to use on a case-by-case basis, under certain conditions where a better estimate of dietary exposure and/or nutritional consequence of a food is required. This would be especially relevant when the levels of a compositional variable are within reach of maximum daily allowance levels, or where the food could have significant health ramifications if consumed to high levels, or by particular at-risk groups.

An exposure assessment should be required as part of a food safety assessment process (Hammond and Jez, 2011; Araya-Quesada *et al.*, 2010 and Lack, 2002). This should include addressing the implications of food processing on the exposure of different populations to the compositional units found in the GM food. This is because exposure to functionally active proteins will be dependent on how they are affected during processing of the food, as many thermal and mechanical practices will alter the structure of components of the food product. A degree of exposure assessment and processing assessment should also be used to assess the level of risk involved with allergenic and toxic compounds that may be found within the unprocessed food.

A degree of exposure assessment is particularly important when considering safety of nutritionally improved 'novel' crops (Chassy *et al.*, 2007), as introduction into the consumer market may result in over-consumption and/or replacement of another nutritionally valuable substance. For those crops that might be distilled to form highly purified ingredients, a risk assessment of the exposure to the processed component may be carried out (Chassy, 2010). For those crops that will be consumed at very low levels, it might be that changes to nutritional value may pose less of a risk to consumer nutrition.

Exposure assessment should also consider population differences that may result in segregated risks. This applies also to vulnerable subsets/ at-risk groups of a population, including diabetics, nursing mothers, pregnant women, children, and the elderly, which should be separately evaluated for exposure, to determine whether the GM food crop may pose a separate risk to them.

3.9. Stacked Traits

Current guidelines (EFSA, 2011) discuss the requirements for GM plants with a combination of transformation events (stacked events) including:

- Risk assessing the plants with the traits independently (single events) if not previously carried out;
- Ensuring the combination is stable;
- Ensuring no interactions (synergistic or antagonistic) between events occur which may raise safety concerns;
- Looking at the expression of introduced genes and their products; and
- Further toxicological/nutritional assessments, depending on the results of the above points.

Results from the literature review discussing stacked traits suggest that risk assessments should consider the risk assessments from each of the single trait crops. Varieties with stacked input traits have often been produced by crossing varieties with separate individual traits that have already been on the market and therefore have a record of safe use (albeit a short one in some cases). This may not be the case with novel traits because they may never be marketed on their own without a stacked input trait. It should be noted that EFSA do not consider GMO products authorised in the market as having a history of safe use.

A case-by-case basis should be used to identify whether interaction between the trait compounds/proteins, is or is not likely as a result of their individual metabolic pathway interactions and synergistic activity (De Schrijver *et al.*, 2007 and Kok *et al.*, 2008). It is possible that breeders could produce sufficient information to suggest that no interaction occurs between the stacked traits. Where traits such as insect resistance have already been approved by SE then only the new ('novel' GM trait) trait need be assessed if there is no interaction. For those stacked crops where interactions are identified, further toxicity testing may be considered relevant (De Schrijver *et al.*, 2007).

Further evidence could be use to verify the safety of stacked-trait crops from extrapolation of single-trait risk assessments, including evidence of the presence and copy number of the parental inserts, evidence that expression levels are similar in parental and stacked lines, and proof that the insert is conserved during the breeding process (De Schrijver *et al.*, 2007). A basic agronomic, morphological and compositional analysis may also serve to address potential adverse effects that might result from interbreeding of GM cultivars and justify substantial similarity to the comparators. Novel traits are likely to be stacked with herbicide tolerant or insect resistance traits by the time they reach the market.

3.10. Risk Proportionality: Graded approach/tiered approach

In May 2008 the EFSA GMO Panel endorsed for public consultation a draft updated guidance document. This demonstrated the importance of transparency in the risk assessment process. A tiered approach may be suitable for risk assessing GM plants, as supported by (Chassy *et al.*, 2007 and Chao & Krewski, 2008;). Risk assessment would in this case be weighted against the potential risks with a particular GM plant, thereby making testing more efficient by focusing on higher-risk GM plants. The

risks could be defined by a preliminary investigation of type of substance, estimated exposure level, prior knowledge of toxicity and nature of concern related to unintended changes. A tiered risk assessment could be modelled on the US chemical regulatory system, which decides on the level of risk assessment on the basis of submitted evidence from the producer (Goodman *et al.*, 2008). Therefore regulation is applied differentially depending on the perceived risk on review of this information.

It is possible that assessment of the hazards involved with a given GM crop before risk assessment may allow the potential risks and benefits involved with its adoption to give a risk-benefit analysis. There are a number of papers which highlight the issues involved with too high a level of stringency involved with the risk assessment of GM, including the rejection of safe and beneficial crops which would serve a public need if cultivated (Durham *et al.*, 2011). The tiered approach to risk assessment would combat this problem, without preventing the detailed assessment of crops which could pose a severe risk. It is recommended that GM crop information could be put through a period of public consultation at some point during this tiered assessment process before the decision is publicised.

3.11. Statistical Analysis

Field trial experimental design and statistical analysis are discussed in the EFSA guidelines (EFSA, 2011). Field trials must be adequately described and environmental variability controlled and replicated. Statistical analysis also has specific guidance: the test of difference and test of equivalence where a linear mixed model calculates confidence limits (for both tests) and a different mixed model estimates the equivalence limits in the equivalent test. However, a number of papers have criticised current methods of statistical analysis for differences between a GM crop and conventional counterpart, arguing that further developments should be made to be secure in the results of a risk assessment of GM by the comparative approach (Hothorn *et al.*, 2006 and Kuiper *et al.*, 2002). This criticism is targeted at the use of a non-significant p-value for compositional variables as a measure of safety. Even a slim probability that a compositional variable is significantly different might be a reason to doubt a risk assessment; an example of a false-negative result. It is suggested that safety thresholds could be used to define an absolute or scale-variant to test the differences between GM and a conventional variety (Hothorn *et al.*, 2006). It is possible that this could be expressed as a percentage change from the control.

The statistical analysis of the proposed approach to compare 'novel' trait GM crops to the natural variation within a species, taking into account variation in response to the environment and population differences, has also been criticised (Ward *et al.*, 2011). It is put forward that equivalence limits are not appropriate when dealing with variation in a compositional variable, and that distribution-free tolerance intervals for compositional data would be more appropriate when determining unintended effects (Hermann *et al.*, 2010).

3.12. Risk assessment approaches taken by international risk assessment bodies

Broadly all risk assessment bodies analysed in this review risk assess GM foods using the standard concept of SE as proposed the OECD. Although all risk assessment bodies have adopted this principle to determine safety, each risk assessment structure is varied depending on the framework in which it is developed (Paoletti *et al.*, 2008). For example, the US Food & Drug Administration (FDA) use history of safe use as a primary risk characterisation method for proteins added to host species, and decisions about risk assessment protocols will be made as a result of the level of concern raised about the safety of the introduced protein. This will result in the inclusion or removal of assessments such as animal feeding trials dependent on the level of concern raised. Despite this, risk assessment by the FDA does also focus on a comparison of composition and characteristics of the bioengineered food, and food derived from the parental variety or another commonly consumed variety. This comparison has an

emphasis on the important nutrients, and toxins that naturally occur in a given food type (Paoletti *et al.*, 2008).

There are minor variations in the protocols used to risk assess GM plants, especially those used to nutritionally characterise the crop. The US provide a decision tree to specify the information and protocol requirements for GM plants with 'novel' and 'non-novel' traits to be risk assessed (Faust, 2002). Risk assessment strategies applied differ, from those that are more rigid, with specific data requirements (such as that used by the EFSA), to those requiring data on a case by case basis depending on the specific GM trait under analysis (such as that used by Australia and New Zealand and Canada).

The issue of stacked traits is addressed differently by the various country bodies. EFSA Guidelines state in order to risk assess GM plants with stacked events a risk assessment must be carried out for the plants with the traits independently (single events), the combination must be shown to be stable, there should be no interactions (synergistic or antagonistic) between events and the expression of introduced genes and their products must be looked at. Further toxicology/nutritional assessments may then be needed depending on the results. However, it is rare for a stacked trait to be revaluated by the FDA if each individual single trait has passed the risk assessment process already. The Philippines also assume the safety of GM crops with stacked traits in which each individual trait has been separately risk assessed. The only further tests that are carried out are those to verify the efficacy of each trait when stacked. On the other hand, Japan and Canada apply a range of additional tests to confirm the safety of the GM with more than one trait added, similar to in European countries. Further evaluation of stacked traits is proposed by the Ad Hoc Technical Expert Group (AHTEG) group, which suggest that a number of characteristics should be re-assessed, including the insertion site, presence of the full sequence, and a number of phenotypic characteristics, such as expression levels and combinatorial effects.

4. Case studies- risk assessment of GM plants with 'novel' traits

The purpose of the case studies is to discuss approaches which have been used to risk assess 'novel' GM plants by international risk assessment bodies. For this reason, the traits in the case studies are those which are either near commercialisation or commercialised already. The traits aim to cover a broad range of different types of 'novel' trait discussed in this report. Four case studies are discussed in this report. The three case studies in this section are: MON87460 Maize with drought tolerance, LY038 Mavera™ Maize with increased production of lysine and CGN-89564-2 FLAVR SAVR™ Tomato with delayed ripening/softening of the fruit. A fourth 'hypothetical' case study (soybean with long chain polyunsaturated fatty acids) is documented in the 'foreseeable scenarios' (Section 8) as an example of the project teams approach to changes to the risk assessment.

4.1. MON87460 Maize with drought tolerance

4.1.1. Trait information

MON87460 Maize with drought tolerance (Genuity Drought Guard) was developed by Monsanto via agrobacterium-mediated plant transformation. This trait allows the plant to maintain normal cellular function under stress (water-limited conditions) by preserving Ribonucleic acid (RNA) stability and translation. The cold shock protein B (CspB) and neomycin phosphotransferase II (NPTII) are expressed. CspB is an RNA chaperone which interacts with RNA secondary structures, limiting misfolding under stress conditions. This GM trait aims to reduce yield loss caused by drought stress. This is an agricultural-input trait which is specified as 'novel' under the project teams definition (see Annex A because it alters metabolite concentrations to enable the plant to tolerate stresses such as frost, cold or salt.

4.1.2. Global Status of Approval

MON87460 has been approved for domestic use/import in Columbia and in the Republic of Korea, for use as food and in processing. Additionally, Canada has approved the GM crop for commercial environmental release. The GM plant is approved for cultivation in the USA, Canada and Japan, and is pending authorisation for food and feed use in a number of countries. This crop was approved in July 2010 in Australia and New Zealand by Food Security Australia New Zealand (FSANZ).

4.1.3. Risk assessment in the EU

The EFSA GMO Panel compared the composition and phenotypic and agronomic characteristics of maize MON87460 with those of its conventional counterpart. All statistically significant differences identified were assessed for their safety and nutritional implications. The EFSA GMO Panel found that maize MON87460 is as safe as and as nutritionally valuable as its conventional counterpart and commercial varieties, and concluded that this maize, and its derived products, is unlikely to have adverse effects on human and animal health, in the context of their intended uses.

In the hazard identification stage of risk assessment differences were identified between the forage and grain from of maize MON87460 and the conventional counterpart, under non-water-limited conditions. The expression of the introduced protein and selectable marker were different (as expected), but MON87460 only contains single copies of the CspB and NPTII expression cassettes and the inserted DNA is stable over multiple generations. Other differences were perceived to be low enough to not raise safety concerns for human/animal consumption. In water-limited conditions, maize MON87460 exhibited enhanced agronomic performance characteristics (e.g. yield) as expected. There

were also some expected differences in chemical composition, compared to its conventional counterpart; these also raised no safety concerns.

The safety assessment of the introduced proteins and the crop as a whole, involved an allergenicity and toxicity assessment, using analytical and bioinformatics studies, as well as in-vitro pepsin and pancreatin resistance tests, and a sub-chronic 90-day rat feeding study. Previous history of safe use of the NPTII protein (the selectable marker) meant that it was deemed to not raise safety concerns. There was no evidence that the introduced protein significantly altered the allergenicity of the crop. A feeding study in chickens showed that the GM crop was nutritionally equivalent to its conventional counterpart. The implications of processing on the safety and nutritional value of the drought tolerant maize were also considered.

See EFSA (2012) for more information.

4.1.4. Risk assessment in Canada

MON87460 was risk assessed in Canada by the Canadian Food Inspection Agency for use as livestock feed. A standardised process was used to formally identify any risks that the GM crop posed to livestock nutrition, as well as tests of the safety of the transgenic protein to humans to assess the risk of inclusion of lysine maize into the human diet. The risk assessment process involved the investigation of the safety issues derived from the introduced protein, in particular the potential mammalian toxicity and allergenicity of CSPB protein (shock protein) including glycosylation tests, acute oral toxicity studies and in vitro digestive fate studies. Furthermore, the toxicity and allergenicity of the selectable marker was assessed.

Two criteria for assessing livestock feed are routinely used in Canada. In this case, the first was the examination of potential impacts of the maize event MON87460 on livestock nutrition. Nutritional studies confirmed compositional equivalence to both the isogenic, unmodified counterpart and commercial maize varieties, through analysis of the major compositional variables and anti-nutrients/secondary metabolites associated with drought stress, under water limited conditions. For any statistically significant differences, it was determined that the mean lay within normal variation for commercial maize and the range of literature values. Nutritional studies in broiler hens also showed substantial equivalence to commercial maize varieties in bird performance variables.

The second criterion routinely investigated is the potential impact of the maize event MON87460 on livestock and workers/bystanders. This was assessed through the characterisation of the safety of the donor organism, the toxicity and allergenicity issues associated with the transgene and the selectable marker (history of safe use and acute oral toxicity studies in mice), and the nutritional/health effects associated with the whole food (as studied using broiler feeding trials).

As opposed to proving nutritional equivalence and the safety of both intended and unintended effects as carried out by EFSA, the Canadian risk assessment process seeks to address; the potential for introducing new toxins or allergens into the food or feed supply, the implications of a food/feed's nutritional characteristics for the human or animal population; and, for feed specifically, the safety of the feed to humans via consumption/exposure to GM-fed animal products. Although comparative techniques are still used to verify safety and nutritional value, the emphasis of the Canadian risk assessment lies more in proving safety than proving equivalence. The risk assessment procedure in Canada verifies compositional equivalence through multiple field trial sites of the GM crop and isogenic counterpart, so that the effects of varying environmental conditions are considered in the assessment. Furthermore, those compositional variables which were significantly different between the lines were deemed safe if they fit in the range of natural variation for commercial varieties and literature published values. Means of predicted anti-nutrients and toxins were also deemed safe if they were within natural variation of commercial varieties.

See CFIA (2012) for more information.

4.1.5. Risk assessment in Australia & New Zealand

Food derived from drought tolerant maize has been risk assessed by FSANZ (application A1029) as a result of an application from Monsanto Australia Limited, June 2009. The application requested the approval for sale and use of food derived from this GM variety of maize. The safety assessment considered the genetic modification, the potential toxicity and allergenicity of the novel proteins and the compositions of MON87460 maize compared with that of conventional varieties. This found that there are no safety issues involved with the consumption of this GM food, and that it is as wholesome as food derived from commercial varieties. This risk assessment process involves a reduced emphasis on the predicted intake of GM crop by populations on market approval, as compared to the assessment process carried out by EFSA. MON87460 was approved by FSANZ in 2010.

See FSANZ (2010) for more information.

4.1.6. Conclusions for risk assessment of drought tolerant maize (MON87460)

All risk assessment bodies that have assessed MON87460 have principally done so using a comparative approach to safety assessment and any unintended effects have been assessed using a targeted approach.

4.2. LY038 Maize with increased production of lysine

4.2.1. Trait information

LY038 Maize was developed by Renessen LLC and has increased levels of the amino acid, lysine. This GM plant has also been stacked with MON810 insect resistant maize (in partnership with Monsanto). The trait was achieved using microparticle bombardment; a recombinant DNA technique. The higher lysine content improves nutritional value for use as a feed ingredient. This is a quality-output trait, and is described as 'novel' as it involves the introduction of a 'foreign' storage compound.

4.2.2. Global status of approval

LY038 Maize is currently approved for domestic use/import for food in Columbia, Japan, Mexico, the Philippines, the USA, and Japan. Mexico and the Philippines also have approval for processing and Canada has commercial approval for environmental release.

4.2.3. Risk assessment in Canada

The Canadian Food Inspection Agency have risk assessed LY038 Maize (high lysine) for use as livestock feed. A standardised process is used to formally identify any risks that the GM crop may pose to livestock nutrition, alongside tests of the safety of the transgenic protein to humans to assess the risk of inclusion of lysine maize into the human diet.

The potential impacts of maize event LY038 on livestock nutrition were tested using the principle of compositional equivalence between maize event LY038 and 20 commercial maize varieties. Compositional equivalence was verified if either the levels of particular compounds were not statistically different to the commercial lines, or if they were within the range of the commercial maize varieties. This showed few statistically significant differences between forage nutrients (fibre, amino acids, fatty acids, minerals, vitamins and secondary metabolites) in the LY038 and commercial maize lines, the intended change in total and free lysine. The values of some lysine catabolites were outside the tolerance interval of the reference maize varieties but within the literature range reported for field maize. All statistically significant differences, except lysine catabolites, were found to be within the range of all conventional varieties.

The introduced protein was investigated for safety in livestock diets, and evidence showed that the catabolites produced to levels outside the range of commercial varieties are rapidly degraded by the animal's liver to enter the tricarboxylic acid cycle as acetoacetl-CoA. A broiler study also verified that the amounts consumed by birds were degraded with no negative effects on bird performance or health. Suspected anti-nutrients in maize were investigated to show that the levels of phytic acid, raffinose, ferulic acid and p-coumaric acid were comparable to those in conventional maize. The bioefficacy and nutritional composition of lysine maize and five commercial varieties were shown to be equivalent as assessed using a 42 day study in chickens. Carcass characteristics and meat composition were similar in those birds treated with lysine maize and the dietary treatments which included supplemental lysine. Chick and adult mortality were low and not related to LY038.

To assess the potential impact of LY038 maize on livestock and workers/bystanders, tests were carried out to confirm no allergenicity and toxicity issues were present. An equivalent bacterially-produced transgenic protein (LY038 event) was used in an acute oral toxicity study and simulated gastric fluid digestion study. Bioinformatic analysis of the biologically relevant structural or immunological similarities between the transgenic protein and known allergens, toxins or pharmacologically active

proteins was carried out. Furthermore, the history of safe use of the transgenic protein (the lyase subfamily are present in many foods) meant lysine maize was not expected to be toxic or allergenic.

See CFIA (2011) for more information.

4.2.4. Risk assessment in Japan

The risk assessment process in Japan for LY038 maize gave information on:

- The donor nucleic acid;
- Information concerning the vector;
- Method of preparing the GM plant;
- The stability and inheritance of the trait caused by the nucleic acid;
- Method of detection and identification of the GM plant and the sensitivity and reliability of methods; and
- The difference from the recipient organism or the species to which the recipient organism belongs.

See BCH (2008) for more details.

4.2.5. Risk assessment in the Philippines

Risk assessment of the high lysine trait in the Philippines by the Department of Agriculture (2002) was carried out through the identification and description of novel traits, stable integration into a plant's genome, and subsequent nutritional and safety evaluation of the GM plant. These phases involved demonstrating the safety of donor organisms (Corynebacterium glutamicum) by indicating their common distribution as a soil bacterium, and their wide use in commercial fermentation. Furthermore, it was exhibited that the transgenic protein has a history of safe use, as it is naturally present in feed and food in maize, rice, soy and wheat. The previous assessments and approval for this trait in the USA was taken into consideration.

The nutritional composition was evaluated using a comparison between the transgenic and a conventional counterpart. All differences were within a tolerance interval, apart from the intended differences in lysine, saccharopine and alpha-aminoadipic acid. Additionally, anti-nutrition characteristics were considered; the levels of anti-nutrients such as phytic acid and raffinose were not statistically different between the transgenic and a conventional counterpart, and were within the range of 20 commercial varieties and scientific literature values.

See BCH (2008) for more information.

4.2.6. Risk assessment in the USA

The composition of forage and grain from LY038 maize was evaluated in comparison to forage and grain from a negative segregant as a control material. The variation in nutrient composition was assessed by comparing the maize to 20 conventional maize hybrids. Forage and grain samples were collected from all plots and analyzed for nutritional components, anti-nutrients and secondary metabolites. The assessment noted statistically significant differences in composition between LY038

and the negative segregant reflecting the increase in levels of lysine in LY038. Other compositional differences were noted to be within tolerance levels based on historical ranges for maize.

See FDA (2005) for more information.

4.2.7. Risk assessment approaches discussed in the scientific literature

Glenn *et al*. (2007) include a recommendation for the risk assessment of high lysine maize. The authors suggest that the current comparative methodology for risk assessing GM nutritionally enhanced crops is still appropriate, and that based on available data, lysine maize is as safe as conventional maize whilst being nutritionally enhanced for animal feed diets. The safety assessment of a nutritionally improved food or feed was proposed to include a comparative assessment with an appropriate comparator crop and a safety and nutritional impact study on a case-by-case basis in the context of the proposed use of the product in the diet and consequent dietary exposure. The production and processing of GM lysine maize was deemed not to be expected to differ from conventional maize. It was understood to pose no risk to humans if accidentally incorporated into human food; it was thought to be unlikely that adventitious amount of lysine maize grain would enter the food chain. The safety assessment of newly introduced protein was proposed to follow a tiered approach, so that the protein introduced to lysine maize would have a lower level safety assessment since the exposure to the protein was expected to be low due to its expression pattern.

A compositional analysis of crops with known toxicants or anti-nutrient compounds was suggested, including analysis of specific analytes. It was also proposed that, if warranted, an evaluation of the targeted metabolic pathway should be conducted. However, since the lysine metabolic pathway is well understood there would be no need to perform untargeted compositional analysis. Instead, compositional analyses were conducted on samples from multisite field trials in various environmental conditions as compared to a near-isogenic counterpart and conventional maize.

It was deemed that studies in laboratory animals may serve a useful role in confirming observations from other components of the safety assessment and that nutritional animal feeding studies should be performed with a suitable species, which should include a target species. High lysine maize was risk assessed using a 90-day feeding trial, which compared responses of rats at 11 to 33% of diet with a near-isogenic control and traditional maize hybrid diets. Additionally, broiler chicken experiments were used to look for any effects on growth rate, feed efficiency and carcass characteristics.

This risk assessment procedure deviates from that used by EFSA because it suggests the development of safety and nutritional evaluation on a case-by-case basis, with reference to the production and processing, expected exposure and level of understanding of the metabolic pathway in which the introduced substance is involved. The implications of this development would be a less intensive risk assessment process for GM crops of a lower perceived risk, with a consideration of the level of characterisation/appreciation of the pathways involved with the intended change to the crop.

Glenn *et al*. (2007) stated that for the traits which modify poorly understood metabolic pathways, untargeted compositional analysis might only be more informative when standardisation of the reporting structure for ‘omics’ data has been facilitated and databases of baseline metabolomes that are validated and monitored have been generated. The authors also recommend that different environmental conditions are represented within crop-profiling databases to enable baseline assessments, which GM crop variables can be compared against.

4.2.8. Conclusions for risk assessment of LY038 Maize

For all risk assessment bodies that have evaluated LY038 maize a comparative approach has been used. Apart from the increased levels of lysine in the plant, none of the assessments found statistically significant differences in the composition of LY038 maize and its comparators. The levels of lysine expressed in the plant were shown to be equivalent to that added to feed currently as a supplement.

4.3. Tomato with delayed ripening/softening of the fruit

4.3.1. Trait information

Various breeders have developed tomato plants with delayed ripening/softening of the fruit to aid shelf life. This has been achieved through mediated plant transformation which suppresses the transcription of the endogenous polygalacturonase gene responsible for the breakdown of pectin molecules in the cell wall. This is a quality-output trait, and is 'novel' because it involves a, 'physiological/morphological change to plant, e.g. changes to protein or metabolite abundance to alter plant processes.

4.3.2. Global status of approval

In the USA, Calgene produced the FLAVR SAVRTM tomato (CGN-89564-2) which was the first GM plant to be commercially grown, and granted a licence for human consumption in 1994. Production of the tomato was carried out until 1997 until Calgene ceased production and was acquired by Monsanto. It was authorised in the USA by the FDA for commercial cultivation and approved for use as food, Mexico also authorised the tomato for import as food. In the UK, Zeneca produced a GM tomato that used technology similar to the FLAVR SAVRTM which was used chiefly for processing to a tomato paste. It was sold between 1996 and 1998 in the UK. However, production of the tomato paste ceased in the UK after supermarkets withdrew the product due to changing consumer perceptions over the technology.

4.3.3. Risk assessment in Canada

The FLAVR SAVR^{TM} tomato was risk assessed in Canada by the Health Canada Department in October 1999. Health Canada notified the producer, Calgene Inc., that they had no objection to the food use of the GM tomato after a food safety assessment of the FLAVR SAVR^{TM} tomato according to its Guidelines for the Safety Assessment of Novel Foods (September 1994). The GM tomato was compared to other commercial varieties and no difference in composition or nutritional characteristics. The assessment of the GM tomato investigated dietary exposure, the nutritional value of the GM tomato, and the safety implications in terms of toxicity and allergenicity of the product to the Canadian food supply. It was found that the GM tomato would have no significant impact on the nutritional quality of the Canadian food supply, and the trait was not judged to have any potential for additional human toxicity or allergenicity. Therefore the GM tomato was deemed to be as safe and nutritious as current commercial tomato cultivars.

See Health Canada (1997) for more information.

4.3.4. Risk assessment in the UK

Within the UK, Zeneca Group plc produced a GM tomato using technology similar to the Calgene 'FLAVR SAVR^{TM}', which was sold on major supermarket shelves in its processed form (a concentrated tomato paste). The GM tomato itself was risk assessed by the Advisory Committee on Novel Foods & Processes (ACNFP) prior to the introduction of EC Regulation 258/97 on novel foods, and novel food ingredients. This specific risk assessment considered the GM tomato in its processed form (tomato paste). A comparative approach was used to assess safety, and aimed to establish if tomato products from the GM tomato hybrids were as safe for human consumption as similar products from non-GM tomatoes. The detailed information provided by the company on the GM procedure satisfied the ACNFP that no unintentional changes had occurred at the molecular level. Compositional

analyses reviewed by the ACNFP demonstrated there were no significant differences between the processed products produced from the GM tomatoes and their non-GM counterparts. This was further evidence that no secondary effects had occurred as a result of the genetic modification. The ACNFP also considered the introduction of the nptII gene into the GM tomato. To verify that the FLAVR SAVR™ tomato was safe, this report went through a period of public consultation, and reasoned objections received within a period of 60 days were reviewed before the applicant was informed that the product could be placed on the market.

The assessment of the safety and nutritional value of the FLAVR SAVR™ tomato by the ACNFP, involved an initial evaluation of the effect of production processes applied to the novel food, including addressing all intended products and the processing for each. Standard checks on the genetic factors such as stability, specificity of expression and characterization of the vector, were then carried out. Furthermore, issues involving transfer of genetic material from the GMO were considered in order to show that processed tomatoes will not contain any functional DNA, due to thermal processing, and that transfer to gut flora would be irrelevant because the donor organism, E. coli, is already present in natural human gut flora. The antibiotic selectable marker was shown to not pose a risk to efficacy of orally taken aminoglycoside antibiotics. An assessment of the anticipated intake/extent of use of the GM tomato was carried out to determine the average consumption of processed tomato products within EU. This demonstrated that there were no envisaged new uses or markets for the processed tomato products. Information from previous human exposure to the GM tomato was considered. History of safe use information was also included to show that processed products derived from GM tomato hybrids grown in California had been on sale in UK and no adverse health effects had been reported.

A comparative approach was used to address nutritional information. Nutritional analysis of the GM line of fresh and processed products was compared to non-GM controls and commercially available products to show that no change to nutritional profile was brought about through the transformation. Toxicological tests to compare known toxins of tomatoes between the GM line and commercial cultivars gave evidence for an equivalent toxicity in both. The inserted construct was shown to be inherently non-toxic. Furthermore, it was shown to be degraded rapidly in in-vitro digestion studies, proving a low allergenicity threat.

This risk assessment procedure differs from that used by EFSA because it considers first the processing and products that the GM crop would be involved with. Additionally, this procedure considers the risks involved with consumption and exposure levels earlier in the risk assessment process. Only after these issues have been addressed is a comparative process used to compare compositional data between the GM products and a non-GM control, taking into account the risks imposed by the product processing and intended usage levels.

See ACNFP (1998) for more information.

4.3.5. Risk assessment in the USA

The Food and Drug Administration published a Memorandum in May 1994 as a response to a request for an advisory opinion by Calgene Inc. in 1991 The Office of Premarket Approval responded to this request. The FDA also used data submitted by Calgene to determine the conclusions of this report as a basis for safety assessment. The nutritional profile submitted for the GM tomato and conventional counterparts was concluded to show that the differences in the concentration of vitamin A and C could be accounted for by the differences in environmental conditions of the locations grown. Furthermore these differences were within the normal, expected range, as reflected in literature data.

This assessment studied the differences in known tomato toxicants, glycoalkaloids, and showed that the levels in ripened fruits were equivalent to those in commercial tomato varieties. The report also

assessed the safety of the use of the selectable marker. Animal feeding studies were used to verify the safety of the whole food. Gastric erosions identified in some animals were further investigated in a second and third feeding study which showed that the gastric erosions were incidental and not test article related. The report concluded that, based on the information provided by Calgene Inc., the FLAVR SAVR™ tomato is as safe as other commonly consumed tomatoes.

The first USA risk assessment of the FLAVR SAVR™ tomato used a fairly similar process to EFSA for safety assessment. Differences included using multiple sites with varying environmental conditions to take into account environmental influences on compositional variables within the GM crop; (current EFSA guidelines do state the minimum requirement for data from at least three growing sites or one site over three seasons). Additionally, any differences in the level of a nutrient or anti-nutrient/toxin were considered safe if they were within the range expected as normal in relevant scientific literature, as opposed to only considering a crop to be safe when it is equivalent to a single conventional cultivar or isogenic comparator.

See FDA (1994) for more information.

4.3.6. Conclusions for risk assessment of tomato with delayed ripening/softening

The FLAVR SAVRTM tomato was the first commercially cultivated GM plant in the world, and for many bodies, the first experience of risk assessing a product derived from agricultural biotechnology. The trait was achieved by suppression of the polygalacturonase enzyme, which delayed the ripening process in the plant. Whilst this incurred a physiological/morphological change in the plant, there was no introduction of foreign compounds, or material not usually present in the human diet. For all risk assessment bodies, the applicant was able to provide enough information to satisfy that the modified tomato was as safe as its comparators. For the UK ACNFP, the end processed product (a tomato paste) was primarily assessed, rather than the GM tomato itself as is now done for other novel foods. The ACNFP were satisfied that the tomato paste was as safe as its non-GM counterparts.

5. Risk assessment criteria for GM plants with ‘novel’ traits

This section details risk assessment criteria for ‘novel’ GM plants based on the results gathered from the scientific literature. Criteria and methods for assessment are defined for each stage of risk assessment (hazard identification, hazard characterisation, exposure assessment and risk characterisation). Based on the literature gathered, this section proposes where further information may be needed for ‘novel’ GM traits based on the results of the literature review. Taking into account further information, Section 7 ‘foreseeable scenarios for risk assessment,’ details the project team’s view of how current risk assessment guidance could be made more appropriate for safety assessment of ‘novel’ GM traits. The literature review found that the elements of risk assessment where authors suggested different approaches for ‘novel’ GM traits were primarily at the hazard identification stage. The challenge rests in accurately identifying hazards and predicting levels of exposure for ‘novel’ GM traits (especially where plants have quality/output traits). The project team found that whilst debates in the literature do focus on specific methods for toxicological and allergenicity assessment (hazard characterisation), these are not specifically in relation to ‘novel’ GM traits. As such, this section focuses on methods for hazard identification and exposure assessment, with limited engagement in the debates surrounding the individual methods for conducting toxicological and allergenicity assessment.

The overall approach to risk assessment of 'novel' GM plants is depicted in Figure 1.

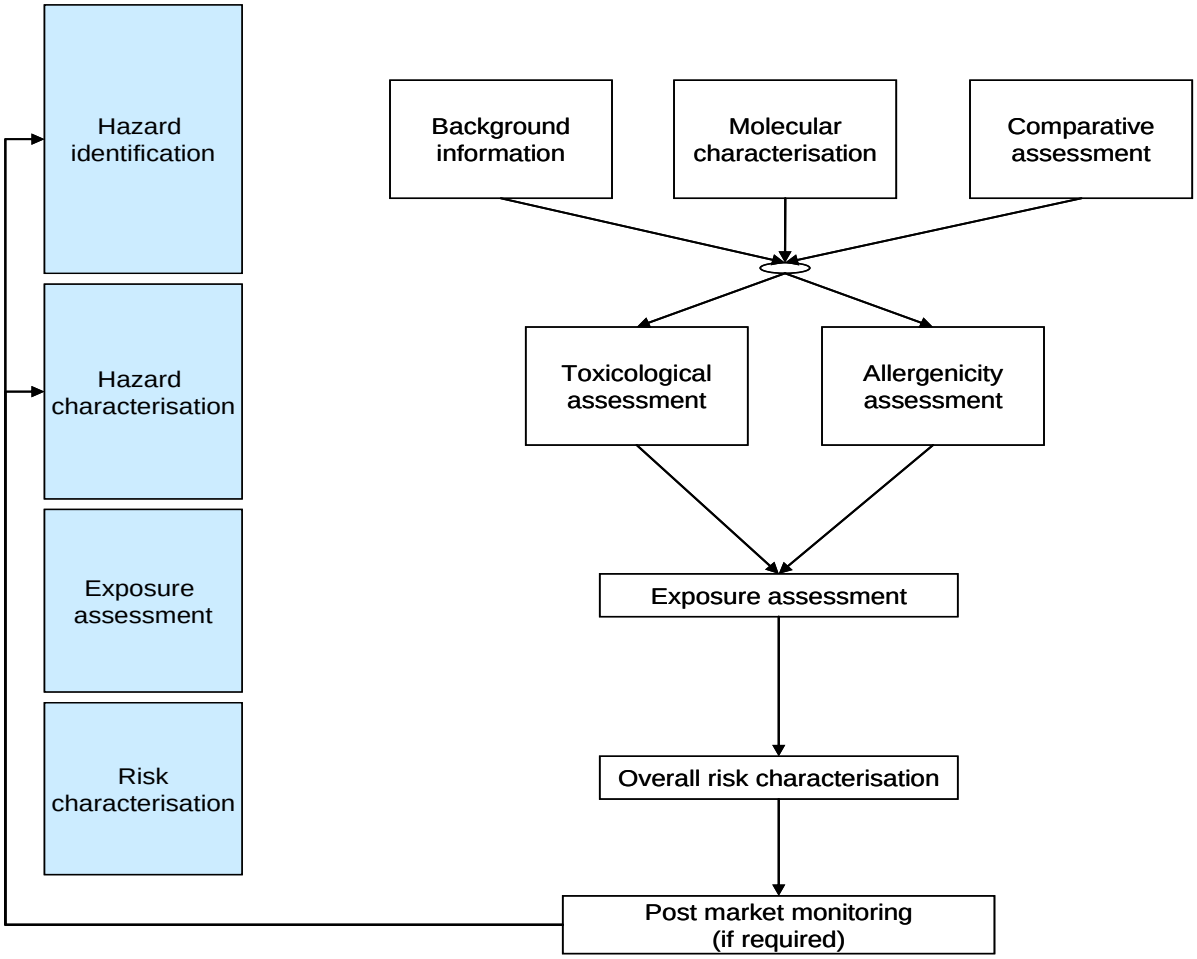

Figure 1: Overall approach to risk assessment

5.1. Hazard identification

Hazard identification is defined as *'the identification of biological, chemical and physical agents capable of causing adverse health effects which may be present in a particular food and feed group'* (Codex Alimentarius, 2009). In the EFSA risk assessment guidelines, hazard identification is the first step in risk assessments to outline potential hazards which should be assessed. This is routinely completed by the applicant giving information on the recipient and parental plans, a molecular characterisation of the plant and the comparative assessment. The comparative assessment aims to identify differences in composition, agronomic performance and phenotypic characterises between the GM plant and its comparator.

5.1.1. Comparative assessment

Much of the literature reviewed in this study suggested that Substantial Equivalence (SE) is an adequate means to risk assess a GM plant with 'novel' traits. As part of the project, the project team found few, if any scientific literature, or international risk assessment bodies that suggested or used

approaches that did not use some form of SE, or comparisons as the basis for safety assessment. Given this, it can be concluded that the term 'comprehensive risk assessment' does not currently refer to any specific approach, and that risk assessment without some form of SE is an unexplored area. A key message arising from the literature is that the definition of SE is not always the same between different stakeholders, and may be interpreted differently. In many cases stakeholders will refer to 'substantial equivalence', the 'comparative approach' or similar, but will not define what is meant by this phrase.

It is clear that comparing GM plants to appropriate comparators is a valuable tool for identifying hazards for further risk characterisation. Given what has been found in the literature review, for GM plants with 'novel' traits it is expected that at some level comparisons will have to be made to accurately identify hazards associated with the GM plant. The project team found no examples of radically different approaches to risk assessment that did not rely on some form of comparison. However, for some authors the current guidelines for selection of appropriate comparators by EFSA may not be wholly appropriate for GM crops with 'novel' traits.

Within the literature, various authors propose that GM plants with 'novel' traits should be compared to the range of natural variation within the host plant species rather than 'comparators' per se. Comparing the plant to the range of natural variation, as contained in reference databases for specific plant species is likely to take into account wider species variation, which may be caused by environmental factors. However, debate exists between authors over which approach is most suitable (different 'omics' approaches). With the change in composition in the host plant that may result from a 'novel' trait, it is thought that comparing to a range of database reference values, rather than a few comparators may be a method better suited to identifying variables which may pose a hazard. However, these approaches are not currently well developed, and at the present time complete databases do not exist for all plant species. Issues may also arise relating to statistics and finding accepted approaches to compare to reference data. For example, the range of natural variation in an individual plant species across the world may be large, considering the range of environments in which it is grown. As such, care must be taken in interpreting values against a database.

Where distinct parts of the plant have been modified (particularly for nutritionally enhanced plants) authors also suggest it may also be appropriate to compare this to an appropriate feed or food with a history of safe use as routinely applied in the assessment of novel foods. The approach to comparative assessment of 'novel' GM plants is given in Figure 2.

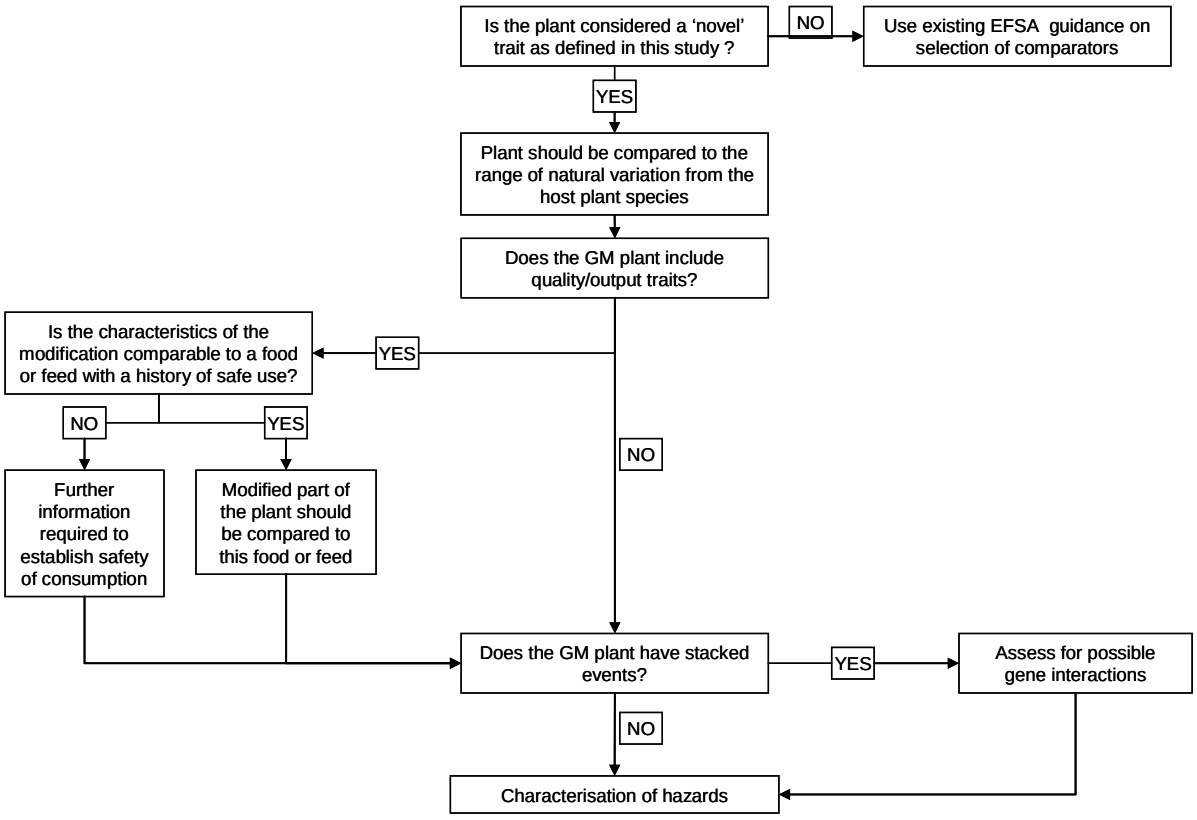

Figure 2: Approach to comparative assessment for 'novel' GM plants

5.1.2. Exposure assessment

Exposure assessment is 'the quantitative estimation of the likely exposure of humans and animals to the food and feed derived from GM plants' EFSA (2011). For GM plants with 'novel' traits that significantly change the composition of the plant, this step is particularly important to ensure that any changes will not cause adverse effects when consumed at different levels as food or feed.

Methods currently employed include probabilistic methods, the use of consumption data and import and production quantities (EFSA, 2011). For 'novel' GM traits with nutritionally enhanced qualities it will be necessary to understand whether any newly expressed proteins, other new constituents and endogenous constituents with altered levels are broken down by food processing. Food processing may also reduce, increase or not affect the toxic or allergenic effects of certain proteins. As such, it will be important to have a good understanding of the intended use of GM plants with 'novel' traits.

5.1.3. Stacked traits

It is viewed by the project team that in the future, GM plants with 'novel' traits are likely to be stacked with traits that have been approved already such as those with herbicide tolerance or insect resistant traits. Currently, guidance states that for stacked traits '*the primary concern for risk assessment is to establish that the combination of events is stable'* (EFSA, 2011). There is some debate as to whether a GM plant, where all traits stacked have been previously approved for food and feed use need undergo a safety assessment as a new approval. The literature reviewed suggests that this should be the case,

but that risk assessment should be based upon the completed risk assessments and data for single events already approved. Further criteria for risk assessment of a 'novel' GM plant with stacked traits would be based upon any potential interactions between the traits combined, as stipulated in the current EFSA guidance (EFSA, 2011).

5.2. Hazard characterisation

Hazard characterisation is the qualitative, and/or quantitative evaluation of the nature of adverse health effects (biological, chemical and physical) which may be present in food or feed (EFSA, 2011). Hazard characterisation represents the testing phase of the hazards identified in the previous hazard identification stage, to give an overall level of risk from the hazard. Established methodologies for toxicological and allergenicity assessment are put forward by organisations such as Codex and EFSA. The aspects of the GM plant tested are determined by the results at the hazard identification stage. For 'novel' GM traits, identifying hazards and unintended effects will be the priority when thinking about any change to current risk assessment criteria. Broadly speaking the debates regarding protocols for allergenicity testing, and toxicity testing are relevant to any GM plant, and not specific to the risk assessment of 'novel' traits. Toxicological assessment and allergenicity assessment are discussed further below taking findings from the literature review.

5.2.1. Allergenicity assessment

The literature review found allergenicity to be a prime area of debate within risk assessment approaches for food and feed safety. Various authors suggest alternatives to the current approach of allergenicity assessment, including decision trees and validation of allergenic potential of GM plant products. They also support the recommended weight-of-evidence approach. For 'novel' GM traits, allergenicity assessment must be appropriately predicated by hazard identification.

5.2.2. Toxicological assessment

The literature review found significant debate in the methodology for GM feeding trials. Internationally agreed methods for toxicity tests have been developed by the OECD and are currently used as the basis for protocols for toxicity testing. For GM plants with 'novel' agricultural/input traits, current toxicity testing methodologies for food and feed safety are adequate, as these will categorise hazards. For GM plants with 'novel' quality/output traits, particularly those which alter the nutritional content of the plant, toxicological studies may need to be amended to take into account the likely exposure of the new food/feed in human/animal populations.

5.3. Risk characterisation

Risk characterisation is based on the findings from hazard identification, hazard characterisation and exposure assessment (EFSA, 2011). At risk characterisation stage, any uncertainties in the risk assessment of the GM plant should be declared, and an estimated risk given. Based on existing EFSA guidelines the risk assessment should demonstrate that:

- The GM plant is demonstrated to be as safe as the non-GM host plant species;
- Any modified parts of the plant are comparable to a similar food or feed with a history of safe use (particularly relevant for 'novel' GM traits);
- The food or feed will not be nutritionally disadvantageous for consumers based on expected usage;

- The food or feed will not be nutritionally disadvantageous by displacing something else in the diet; and

- The feed derived from GM plants will not harm or mislead the consumer by impairing distinctive features of the animal products compared to conventionally produced feed (as in EFSA, 2011).

- The applicant should propose suitable strategies for labelling end products to ensure intended use by consumers.

5.4. Post market monitoring

Post market monitoring (PMM) can be used on a case by case basis to complement the pre-market risk assessment under certain circumstances. For example, an initial estimate of exposure to the food may be variable and subject to uncertainty before the product is marketed. PMM is to be used where appropriate but does not substitute thorough pre-market toxicity testing but complements and reinforces it. PMM aims to evaluate specific unintended effects that may not be fully understood at risk assessment stage (EFSA, 2011). PMM may be particularly relevant to nutritionally enhanced plants where, for example, consumption patterns of these plants may differ to expected patterns, and this may only be realised after a period of use. For example dietary exposure may change due to popularity in the market, or perceived health benefits of the trait. Existing EFSA guidance on food and feed safety PMM is not concrete. In the cases when PMM is required, it will be important to have a standardised set of guidelines. Authors in the literature review suggest that PMM may be used to validate exposure levels in different populations, as predicted in the exposure assessment.

For example, the specific cases where PMM may be required on GM food/feed could include:

Health claims/modified nutritional value of a GM product which may mean consumption increases from the initial expectation from pre-market monitoring. PMM could be used to assess this to ensure the product is still safe; and

To increase the probability of detecting rare unintended effects in certain individuals with specific genetic/physiological characteristics and disease states.

6. Risk assessment approaches for novel foods

In this section of the report the project team reviewed the approach to assessing novel foods, made without the use of agricultural biotechnology in order to provide comparisons with the approach for risk assessing GM plants.

6.1. UK Novel Food Assessment

The risk assessment of novel food and feed in the UK, is carried out by the Advisory Committee on Novel Foods and Processes (ACNFP) under Regulation (EC) 258/97. This panel makes decisions on the safety of any novel food destined for human consumption to be placed on the market in the European Union.

All ACNFP risk assessments take their basis in the guidance provided by the European Commission in Commission Recommendation 97/618/EC. In this guidance document the type of information that are likely to be required to establish safety of a novel food are given. This includes 13 categories:

Specification of the novel food;

Effect of the production process applied to the novel food;

History of the organism used as a source of the novel food;

Effect of the genetic modification on the properties of the host organism;

Genetic stability of the GMO used as a novel food source;

Specificity of expression of novel genetic material;

Transfer of genetic material from GMO;

Ability of the GMO to survive in and colonise the human gut;

Anticipated intake/extent of use of the novel food;

Information from previous human exposure to the novel food or its source;

Nutritional information on the novel food;

Microbiological information on the novel food; and

Toxicological information on the novel food

Within the guidance it is recognised that no formalistic approach can cover adequately all novel foods, and the steps above are provided for guidance only. Prior to Regulation (EC) 1829/2003 on genetically modified food and feed, risk assessments of GM plants were carried out by individual member states under the novel foods regulations, and steps E to H details specific information required for assessment of GMOs. All risk assessments by the ACNFP are carried out on the product in its processed form. Data requirements under each heading are different for each novel food application, and the process is not prescriptive in this manner. An applicant seeking authorisation of their product to the market is supported by the Food Standards Agency (FSA), regarding the information that they must provide to the ACNFP to complete a safety assessment. The FSA would highlight any product

contents which could be of particular concern, and also point the applicant to a previous risk assessment dossier for a product which is relevant to their application. As such the FSA advises the applicant on data to include in the assessment on a case-by-case basis, and leads to submission of a dossier to the ACNFP before their initial consultation on the safety of the product. The data provided for the novel food will review previous exposure, and the safe level of consumption of comparable food products. In each case, a dialogue about product use recommendations and feasible restrictions on its use will occur throughout the risk assessment. Companies are encouraged to recommend an upper limit for safe use, and consider how to ensure that use of the product results in most consumers staying below this limit. The ACNFP can consider possible restrictions of use, or conditions of authorisation (including post-market monitoring) to ensure safe levels are not exceeded.

Following a decision by the ACNFP, a written opinion is submitted to the European Commission and other member states for comment before a final decision on authorisation of the novel food is made (see Figure 3 for the full process).

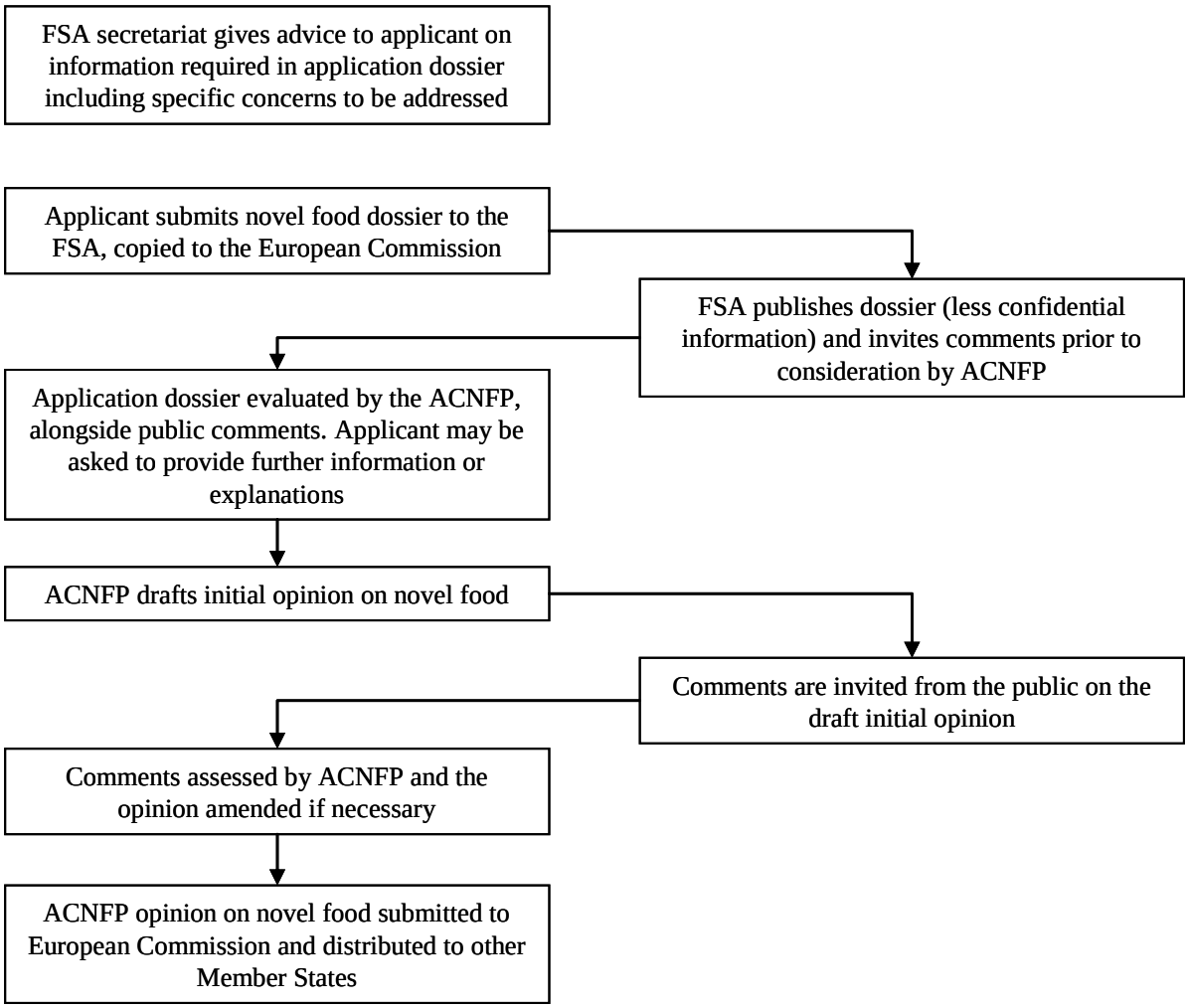

Figure 3: The UK novel foods process

The risk assessment procedure by the ACNFP, in all cases, differs from that used by EFSA. This is because it considers first the processing the GM crop would undergo and the products which would be

manufactured or marketed to consumers. Additionally, this procedure considers the risks involved with consumption and exposure levels earlier in the risk assessment process. Only after these issues have been addressed is a comparative process used to compare compositional data between the GM products and a non-GM control. The risks imposed by processing the product and intended usage levels are taken into account.

6.2. Novel foods and feeds in Canada

6.2.1. Novel foods

Health Canada is responsible for establishing the standards and policies which determine the safety and nutritional quality of all foods sold in Canada. Control via a mandatory pre-market notification procedure is described under Division 28 of the Food and Drugs Regulations – Novel foods regulation[9]. The safety assessment procedure is set out in the Guidelines for the Safety Assessment of Novel Foods (see Health Canada, 2006). These guidelines also cover GM plants, and this is covered in Section 0 of this report. Novel food assessment is triggered by the presence of the trait itself, rather than the method used to achieve it (i.e. the plant, rather than the process is subject to regulatory oversight). The safety criteria for the assessment of novel foods were created using the scientific principles outlined by the OECD. These guidelines have been developed to be flexible, allowing widely variable novel foods and food products to be assessed on a case-by-case basis. Novel whole foods/food ingredients may need to be assessed in Canada due to new products being imported, a new species being introduced as a food, new processing techniques, or GM plants from which foods are derived. This assessment involves:

- Describing the name, intended use, intended manufacture, preparation, preservation, packaging and storage;
- Reviewing information regarding its history of use as a food in a country other than Canada - information about history of human exposure will be important where there are traditional handling or cooking requirements;
- Reviewing information about estimated levels of consumption/usage patters (a dietary exposure assessment). An exposure assessment to discover the estimated changes in the dietary intake distribution of micro-constituents, and their differential impact in subgroups of the populations (e.g. children, infants, elderly, ethnic groups, susceptible populations) and well as the impact on the population as a whole; and
- A safety assessment data package is also required to detail: characterisation of the derived strain/line, nutritional, toxicological, allergenicity, chemical and microbiological considerations and, if GM, any specific GM considerations.

Post-market monitoring is only used under specific circumstances when it is deemed an appropriate risk management measure. Novel foods are generally not given market approval if there is any evidence which indicates that they are not safe for consumption. Therefore post-safety assessment (the need for post-market monitoring) is considered on a case-by-case basis. For each of the data submissions, all that is required of the applicant is data collected from experiments designed with sound scientific concepts and principles. Assessment of nutritional quality involves a consideration of vulnerable groups (young children, pregnant and lactating women, those with specific metabolic characteristics, adolescents and others who consume large amounts of food, and the elderly who consume small amounts of food) as mentioned above.

[9] Food and Drug Regulations C.R.C., c.870. Food and Drugs Act, Regulation Respecting Food and Drugs, 04.01.13

A review of the information about a substances history of use as food is a fundamental part of the risk assessment process. Under the novel foods guidelines a substance may be considered to have a history of safe use as food if it has been an ongoing part of the diet for a number of generations, in a genetically diverse human population, used in ways and at levels similar to those expected in Canada. If it can be demonstrated that a product has a history of use as food under this definition, the confidence in the evidence presented is increased. For novel foods where a history of use cannot be demonstrated, specific information would be required to determine:

- The dietary exposure of the food, including how much of the food is likely to be consumed, its potential impact on the dietary intake of nutrients, and if there are any anti-nutrients, toxins, contaminants or novel substances and their potential exposure;
- Information on nutritional composition and quality to determine how the food could be used in the diet;
- Toxicological and allergenicity testing on a case by case basis; and
- Identification and levels of chemical contaminants, and a comparison of these levels with similar food products.

6.2.2. Novel Feeds

The Feed Section of the Canadian Food Inspection Agency (CFIA) provides a national livestock feed program, which is regulated by the Feeds Act and Regulations, to ensure that livestock feeds (including novel feeds) are safe, efficacious and properly labelled. The safety assessment procedure is set out in the guidelines for the assessment of novel feeds (plant sources) (see CFIA, 2012). Both 'conventional' feed ingredients, and novel feeds are regulated in the same manner by CFIA if they differ significantly from conventional ingredients. Feeds or feed ingredients are considered to be novel if they:

Are not approved as livestock feed in Canada; and/or

Contain a novel trait.

As with novel foods, and GM plants in Canada, it is the presence of a difference in the product or plant that triggers the need to pre market assessment, not the process used to obtain it. In order to carry out a risk assessment for novel feed products, a range of data requirements have to be fulfilled. Specifically these are:

- Characterisation of the plant source;
- Nutritional data;
- Dietary exposure;
- Toxicology data;
- Allergenicity data;
- Laboratory Animal/Livestock feeding trials;
- Evaluation of environmental safety; and

- Method of detection and identification requirements.
- The process is conducted on several internationally recognised principles:
- Assessments are conducted on a case-by-case basis- data requirements determined on the individual feed being assessed;
- Familiarity- the degree of information required is based on the familiarity with the product (i.e. history of use);
- Comparative approach- novel feed products are compared to those with a history of safe use;
- Valid scientific rationale- a valid scientific rationale supported by data or references may negate requirements for specific studies;
- Weight of evidence- the sum of the overall data submitted determines whether a novel feed should receive authorisation;
- Feed definitions and labelling- feeds are labelled appropriately to ensure safe use;
- Substantial equivalence- is a concept used as defined by the OECD; and
- Unintended effects- the assessment considers the likelihood that unintended effects may be present.

6.3. Differences Between GM and Novel Food Risk Assessment approaches

The approach used to assess novel foods and feed by the UK and Canada are broadly similar to the approach used to assess GM plants by the EFSA GMO panel, however a few key differences exist between the two processes. Overall novel food and feed assessments consider assessment of risks that are inherent within the derived products of the novel trait, and risks are determined by the use and predicted use of these products. This constitutes an assessment process that is 'near market', rather than the GM risk assessment process by EFSA which considers GM plant material at point of harvest to be produced into a number of derived food and feeds.

The concept of 'history of use' (or familiarity as described by CFIA) of a food or feed is a significant part of the risk assessment approach for both the UK and Canada. The basic principle infers that where an applicant can demonstrate that a novel food or feed has a history of use in a large population, for a number of years and at levels similar to what would be expected from the new product, more confidence can be given to any evidence presented. This effectively means that if a novel food or feed has been consumed before, or is 'familiar' to assessors, less data is required. For GM plants as risk assessed by EFSA, an approach is used whereby GM plants are compared to traditionally cultivated crops with a 'history of safe use' to identity differences and equivalences.

Within novel foods and feed assessments, risk assessment is carried out on a case by case basis, to account for the wide variety of novel foods and feeds that may require authorisation. The dossiers produced by applicants to demonstrate safety of novel food and feed differ in the levels of data presented, depending on the risks posed by individual GM plants. On the other hand the EFSA process for GM plants is more prescriptive in nature, following a standard process to be followed for all applications with specific data requirements.

Novel foods and feed are risk assessed focusing on the product in its processed form. This takes into account the intended manufacture, preparation, preservation, packaging and handling of the product.

GM plants are risk assessed usually at the point of harvest. For GM plants which undergo a standard processing procedure (equivalent processing for the GM and traditional crop) the implications of processing are often not considered, and the GM crop is compared to the traditional cultivated plant in its unprocessed form. Implications of processing are primarily important for novel food risk assessment. However, EFSA guidelines do also state that elements of the risk assessment of GM plants and derived food and feed includes looking at the, 'influence of processing and storage on the characteristics of the derived product'.

For novel foods and feed, where valid scientific rationale for safety can be provided, backed by existing data or references, this may negate the need for specific tests of safety to be carried out. For GM plants risk assessed under the current EFSA guidance, this approach is not necessarily practiced to assure safety.

Information about dietary exposure will direct risk assessment priorities when analysing novel foods, whereas this is a lesser component of the current risk assessment of GM plants. Dietary intake and potential nutritional impact is, however, still a part of current EFSA guidelines for assessing GM plants, however this does not necessarily predicate hazards to be assessed at the hazard identification stage.

Post-market monitoring is often used on a case-by-case basis post-risk assessment of some novel foods and feed. The novel food risk assessment process will often involve advising the applicant on a correct data package to include the relevant safety information, and discussion in a panel-scenario about the safety issues of relevance to the specific product being risk assessed. The amount of post market monitoring required can be dependent on the extent to which the applicant can prove that the novel food or feed has a history of safe use. If novel food or feed is produced with a trait not previously seen in the human or animal diet, then uncertainty about its post market use is greater. Post market monitoring would aim to track use of the product by consumers in case this led to further risks. The novel food risk assessment process considers risk assessments previously carried out which are of relevance to the product being assessed, to direct safety data package requirements.

Overall, the novel food and feed risk assessment approach is a flexible approach to assuring safety, requiring data from applicants on a case by case basis. Each dossier submitted to the relevant authority will usually contain varying levels of data, depending on the level of existing evidence on the product in question (history of use, and existing scientific evidence). Where a novel food or feed has undergone a form of processing not experienced before, data requirements will include the effect of processing on the product. For the risk assessment of GM plants, the emphasis of risk assessment is on the effect of the modification, obtained through the use of biotechnology on the plant. As such, hazards identified often are predicted on whether the modification has produced any unintended effects in the host plant. Proving a history of safe use for a GM plant is not always appropriate, as by its very definition it will be unlike other plants currently in the food chain. However, the concept is useful for proving the safety of any expressed proteins inserted into a plant (particularly for 'novel' quality output traits). There is no widely accepted definition of "history of safe use", and EFSA does not define the term, or use the concept.

7. Foreseeable scenarios for risk assessment

7.1. Background

This section considers all evidence gathered from the literature review, other sections of the report, and discussions held between all members of the project team to put forward a foreseeable scenario for risk assessment. The foreseeable scenario is relevant to all plants deemed to have 'novel' GM traits, and for other cases where the current food and feed risk assessment guidance provided by the EFSA GMO Panel may not be suitable. Please note that this section is based on the judgements and discussion of members of the project team, in response to the findings from the literature review and other sections. It aims to provide a first step to thinking about how risk assessment guidance could be amended to ensure its suitability for 'novel' GM plants.

The emphasis of the project brief as provided by EFSA, was to review current strategies for risk assessment of GM plants expressing 'novel' traits, and for 'comprehensive' risk assessment approaches for GM plants *per se* (i.e. not based on the comparative approach). The emphasis of this was on strategies for risk assessment where the selection of appropriate comparators (as defined in EFSA, 2011) may be difficult. On reviewing the literature the project team found that at some level, comparisons were always a cornerstone of food and feed risk assessment of GM crops. No evidence was found where stakeholders were evaluating the characteristics of GM plants and the derived products without comparing to an appropriate comparator or a database. As such, it is recognised in the foreseeable scenario for risk assessments that approaches to risk assessment will be based on using a comparative approach as a starting point, with differences to the current EFSA guidance to make the process effective at assuring the safety of plants with 'novel' GM traits. The approach advocated here is designed to be workable, i.e. a method for risk assessing 'novel' GM traits which means a decision can be made in a timeframe and within a cost that does not exceed the current approach used by EFSA.

It is recognised that for plants with 'novel' GM traits, the parts of the existing risk assessment that may need revision are principally hazard identification (i.e. how do you accurately identify hazards for analysis) and exposure assessment (particularly for 'novel' quality output traits which provide benefits to the consumer). As such, this section focuses principally on these two areas. The literature review of this project found substantial debate surrounding individual protocols for hazard characterisation such as toxicological and allergenicity assessments. In this report the project team has put less emphasis on hazard characterisation methods as it is assumed these will be the same for all GM plants, and that engaging in debates on specific methods of hazard characterisation is not a principle objective of this project.

7.2. Overall approach to risk assessment

For plants with 'novel' GM traits the overall approach to risk assessment currently employed by EFSA (identification of hazards, hazard characterisation, exposure assessment and risk characterisation) is unlikely to change significantly. The two areas focussed on in this section are hazard identification and exposure assessment, whilst the methods for hazard characterisation and risk characterisation are envisaged to remain unchanged.

7.3. Hazard identification

7.3.1. Background information

Hazard identification is the first step in risk assessment to outline potential hazards which should be assessed. The first step of hazard identification in the current EFSA guidance is to provide comprehensive information on the recipient and donor organism. For 'novel' traits, the donor organism which the gene has come from may not be a plant. In this case, information would be required on this donor organism such as name, family etc., its geographical distribution and data on past and present use (see below). For quality/output traits, as part of this approach, information is required to demonstrate the history of the expressed protein as a food or feed. In this case the food or feed can fall into two specific categories:

Food or feed (expressed proteins) with a history of safe use: the applicant can demonstrate that the safety of the expressed protein in food or feed is confirmed from experience of use and continued use in the normal diet of a large part of the population of a country or in farmed/domestic animals for a number of generations, consumed at levels foreseen to be similar to its use in the GM plant. [10]

Food or feed (expressed proteins) without a history of safe use: the applicant cannot demonstrate history of safe use in food or feed, and thus presents data on any use of the expressed trait as food or feed (i.e. traditional use in a population sub group) on a case by case basis.

Where applicants can demonstrate a history of safe use of the novel protein in food or feed, the confidence in the safety of the GM plant expressing this trait would be increased. Confidence levels in safety of the expressed trait for food and feed without a history of safe use would decrease depending on the strength of evidence presented. Confidence levels may be determined by specific criteria, ranging from expressed traits with a full documented history of safe use, down to expressed traits with little representative data to prove history of use as a food or feed. If an expressed protein results in changes to levels of a metabolite in plants (such as higher levels of vitamin A in 'Golden Rice) the previous history of the metabolite in food or feed will also need to be documented along with the expressed protein. Where a history of safe use cannot be proven, risk assessment may then require further information on the nutritional and toxicological properties of the expressed trait (such as a specific fatty acid or protein expressed in the plant) to assure its safety, on a case by case basis. Where possible, this information would be taken from information about the foods/feeds in which the ingredient already exists, and studies have already been carried out. If information is lacking in this area, the applicant may be required to prove that the expressed trait is comparable to other similar foods or feeds with an established history of safe use.

7.3.2. Comparative assessment

It is envisaged that a comparative assessment would be carried out on the GM plant, using a comparator as outlined in the current EFSA guidance on the selection of comparators for the risk assessment of GM plants (EFSA 2011a). In the current EFSA guidance (EFSA, 2011), two tests are carried out. The test of difference is used to confirm whether the GM plant, apart from the intended modification, has any differences with the comparator(s) that may be considered a hazard. The test of equivalence is used to verify whether the agronomic, phenotypic and compositional characteristics of the GM plant fall within the normal range of natural variation, estimated from a set of non-GM reference varieties with a history of safe use. This is currently carried out using field trials of the GM plant and its comparator, with each field trial replicated at a minimum of 8 sites which are representative of the range of likely receiving environments where the plant will be grown.

[10] Definition based on various definitions used by existing risk assessment bodies principally assessing novel foods, such as 'Health Canada' [http://www.hc-sc.gc.ca/fn-an/legislation/guide-ld/nf-an/guidelines-lignesdirectrices-eng.php#a4.1.1.1]

For GM plants with 'novel' traits the receiving environments in which the crop is grown are likely to vary markedly, especially for complex agricultural/input traits where crops may be designed to grow under extreme environmental conditions that are markedly different than those in which the conventional comparator is normally grown. An example of this would be drought tolerant maize (MON87460) as discussed in the risk assessment case studies which is likely to be grown in arid areas, and areas that are at risk of suffering from drought. As in the existing EFSA guidance, drought tolerant crops could be compared directly with comparators on the same site (even if in some conditions a lower yield would be expected from conventional varieties). It is appreciated that there may be more extreme examples in which the GM plant may be grown in receiving environments in which comparator is unable to survive. At this stage, given that the GM plants are likely to require authorisation worldwide, it is envisaged that this is an unlikely scenario. However guidance on appropriate field trial design may need to be developed to take this into account. A hypothetical example could be a GM plant with tolerance to highly saline environments, where conventional comparators cannot naturally be grown. In this case, there could be different approaches taken: For example, a GM plant grown in a high saline environment may need to be compared to the range of natural variation of control plants (comparators) grown under 'normal' conditions, but with the GM plant also grown in a more extreme (i.e. saline) environment, and its performance compared between the two environments. A second approach may be devised by employing a field experiment to test the response of the GM crop and its comparator to increasing degrees of salinity, in the background of a 'normal' (i.e. non-saline) environment, where other sources of environmental variation could be managed, as far as possible. An experimental approach could then be employed whereby increasing rates of salt could be used to generate treatments of increasing salinity, and each salinity treatment applied to both cultivars in a fully factorial design. A further example is a GM plant tolerant to heat stress, grown in conditions where a conventional comparator could not. In this case the applicant may wish to use an environment such as a glasshouse to simulate heated conditions. Again, a fully factorial design could be used to generate treatments of heat stress on the GM plant and its conventional comparator. These considerations imply that the existing EFSA guidance on experimental design of field trials may not always be appropriate, and more sophisticated experiments may need to be designed on a case by case basis, to adequately assure safety, by comparing to the range of natural variation. Other considerations in the risk assessment process for instance the safety and fate of the leaves which may be enriched in salt, would also need to be considered, if it is normally fed to livestock.

For GM plants with 'novel' traits that induce major compositional changes to the host plant, it is clear that the GM plants will most likely not fit into the range of natural variation of the non-GM reference varieties tested.

Various other approaches of comparing to databases or other means of comparing to ranges of natural variation are described in the literature review of this project. However, difficulties exist when it comes to defining what the 'range of natural variation' actually encompasses. Potentially, the range of natural variation for an individual crop can be extensive, if it is cultivated in a number of different environments. Quality-controlled datasets do exist, and an example is the ILSI Crop Composition Database (see https://www.cropcomposition.org/query/index.html), however, this is currently only available for maize, cotton and soybean. As such, a chief conclusion is that using this approach to assure food and feed safety is not likely to be very helpful for GM plants with 'novel' traits, which, by the nature of their modifications, are likely to fall outside the range of natural variation. It is however, appreciated that comparing to the range of natural variation may be a useful tool to give a confidence level in the extent to which the agronomic, phenotypic and compositional characteristics of the plant have been changed, and aid for identifying levels of unintended variance that should be the subject of further testing. This further testing would ultimately have to be carried out on a case by case basis, depending on the trait expressed. This approach is similar to that used in the assessment of novel foods by various EU member states. The overall approach to hazard identification is given in Figure 4.

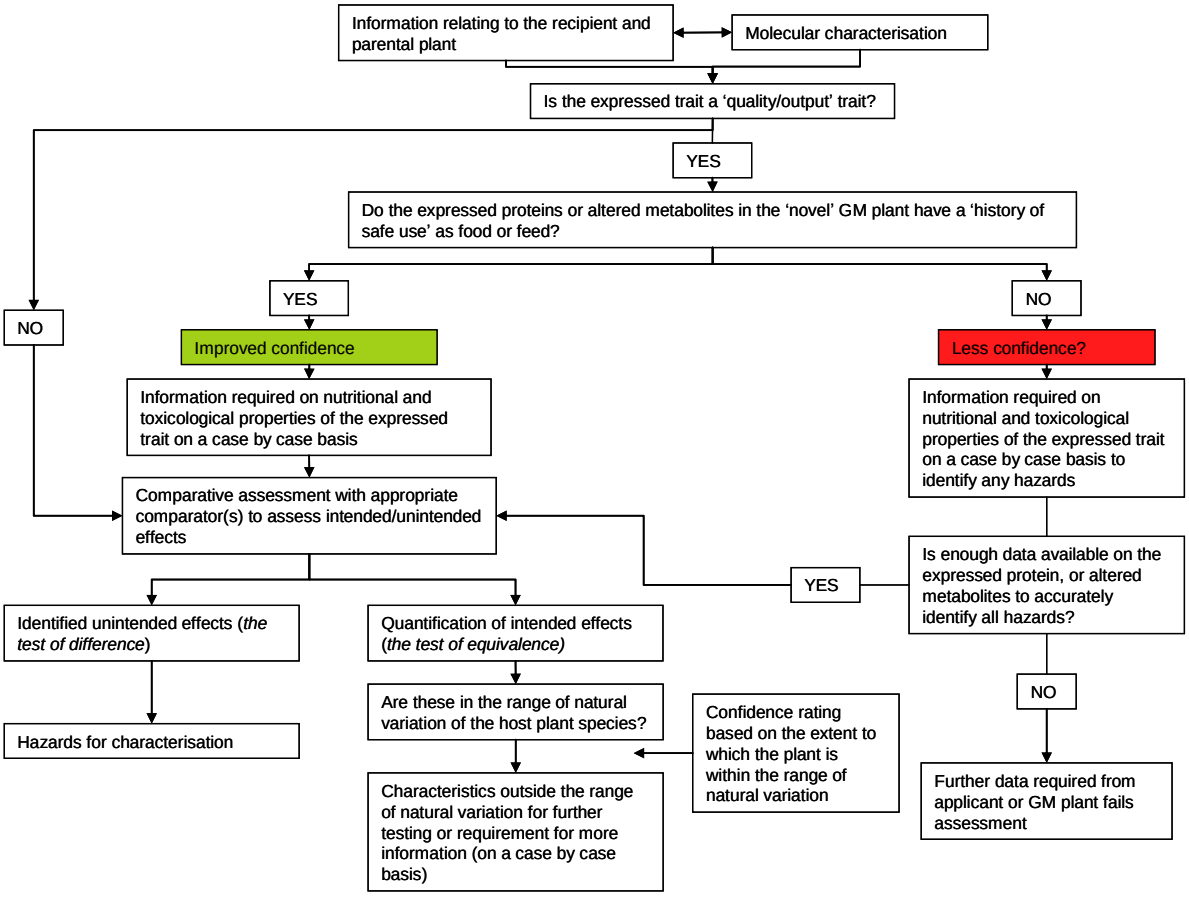

Figure 4: Overall approach to hazard identification for GM plants with 'novel' traits

7.4. Exposure assessment

Exposure assessment is the estimation of the exposure of humans and animals to the food and feed derived from GM plants. This step is particularly important for GM plants expressing 'novel' quality/output traits that are intended to provide a benefit to the consumer. For GM plants expressing agronomic/input traits, it is likely that consumption of the derived food and feed will not change significantly. However, consumption may change if certain traits are desirable and hence more crop is produced, which may mean it replaces food and feed derived from other crops already on the market. The principle objective of the exposure assessment is to 'estimate the anticipated average and maximum intake levels of the food and feed based on representative consumption data for products derived from the respective conventional plants' (see EFSA, 2011). For 'novel' GM quality/output traits, this may be a more complex task than for previous GM crops assessed by EFSA due to the increased likelihood of changes to consumption patterns. Exposure assessment should be question-based, on a case by case basis depending on the plant being assessed. For exposure assessment, applicants should aim to answer the following broad questions:

1. How much of the food or feed is likely to be consumed and at what frequency (including average and high intake scenarios);
2. What role is it likely to play in the diet, and is this different from the food/feed it is intended to replace;
3. What are the potential impacts of the food/feed on the dietary intake of nutrients based on the findings of Q1;
4. Will it be clear to consumers that the product(s) are different from those already available on the market, and will consumers understand the implications of the change to make informed choices about consumption;
5. Based on any hazards identified previously, what is the likely intake of any bioactive substances, anti-nutrients or toxins present in the food/feed;
6. Are there any particular at-risk groups, and what are the potential effects; and
7. Would average consumption of the product likely to be different between EU member states?

It is important to note that within the literature there is little guidance on how exposure assessment should be carried out, and the methodology used. As such, it is proposed these questions would be answered using representative consumption data for comparable food/feedstuffs as is current method used. Applicants should detail the likely derived food and feeds on which they base assumptions on the data given in the exposure assessment. On a case by case base, the GMO Panel may wish to base its approval on a maximum level to be contained in derived food or feed. Information should be given on whether the availability of the GM trait may lead to other derived food and feeds not immediately foreseen for market.

7.5. Post market monitoring

Within the literature there was little information found on post market monitoring found in the context of risk assessment strategies for 'novel' traits,, and in particular the conditions in which this should be undertaken. For 'novel' quality/output traits, a degree of post market monitoring may be necessary in order to track the use of the GM plant in derived food and feed to understand whether exposure levels have changed over time. Various authors in the literature review have recommended the use of PMM for use on a case-by-case basis, under certain conditions where a better estimate of dietary exposure and/or nutritional consequence of a food is required. It is recommended here that PMM may be required on a case by case basis, however further work is needed in this field to give better guidance on the cases when this may be required.

7.6. Management of the risk assessment process

Given the emotive nature of the subject of GM plants across Europe, it is important to ensure that a transparent and straightforward risk assessment process is followed. Within the UK, the Advisory Committee on Novel Foods and Processes (ACNFP), supported by the Food Standards Agency (FSA), gives opinions on novel food applications under the Novel foods regulations (Regulation EC No 258/97), and uses a system as detailed in Figure 5. Under this system, the typical time for an opinion to be produced and submitted to the EC is approximately 18 months from initial registration of intent to the FSA/ACNFP.

As can be seen from Figure 5, at each major stage of the process views are sought from the public on any matters of concern on the application. These responses are then filtered by the FSA secretariat, and ones of particular note raised at ACNFP meetings. If, given the comment or information presented, the ACNFP feel that more information is required from the applicant to satisfy the concern, this is then requested. Once the ACNFP are satisfied that the applicant has provided all necessary information to make a decision, a draft opinion is then produced for public comment. If concerns are then raised again, this is reviewed by the ACNFP and either more information is requested from the applicant or the comments are viewed as not applicable or relevant. Once the ACNFP is satisfied that all information has been presented and views taken on board, a final opinion will be sent to the EC, who will then make a decision on authorisation.

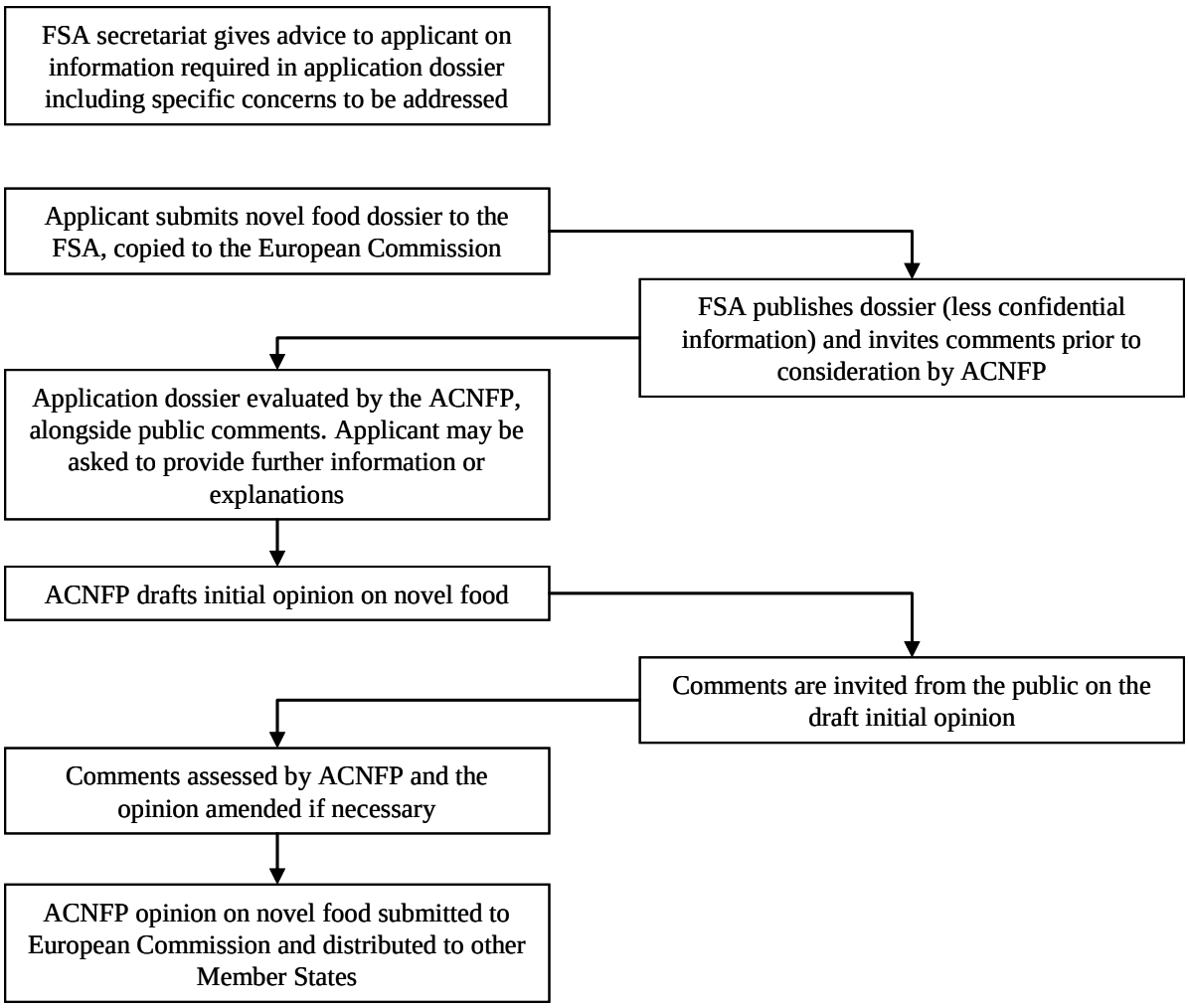

Figure 5: The UK novel foods risk assessment process

GM plants are a contentious issue within the EU, with polarised views between different groups. As such, making the risk assessment process as transparent as possible would be worthwhile to minimise the extent to which claims of potential risk are made on any GM plant after an assessment has taken place and decision on authorisation made. Publishing outputs of the assessment process and seeking consultation may also help to ease the view of uncertainty that exists within wider stakeholders in the EU over GM technology. In particular the project team recommend that:

- Dossiers (less confidential information) are published and comments invited before consideration by the GMO Panel; and
- Comments are sought from the public on the initial opinion of the GMO Panel.

Clearly, the comments received on the application would be wide-ranging, and not all could be presented to the GMO panel due to sheer constraints of time. Screening of comments by the GMO unit at EFSA would ideally be carried out to separate viable concerns on the safety of the GM plant, from general comments of support or opposition without any sound basis for estimating impact.

Timing of risk assessments is also an issue, as any process for risk assessment must guarantee reasonable time to come to a decision. If GM technology takes too long to be appropriately risk assessed, it may be that its applicability for market may have reduced by the time a final opinion is

given. It is recommended by the project team that introducing a time-bound risk assessment process would be worthwhile to ensure the process of risk assessment were workable, whilst still ensuring robust safety of the product. Time constraints would ideally be given on:

- The time for the public to give comments on the GM technology at the two consultation phases detailed above;
- The time taken for the GMO Panel to give feedback on application dossiers (either a draft opinion, or request for further information); and
- A maximum time that the overall risk assessment approach could take before a final opinion was given by the GMO Panel for consideration.

What has been proposed in this section is a risk assessment process for GM plants, which is more similar (in part) to the framework for assessment of novel foods. It should be noted that Regulation (EC) 258/97 on novel foods is currently being revised to bring about improvements and in particular to form a centralised body for the assessment of novel foods in the EU. If the novel foods authorisation process is centralised within EFSA, it will be important to ensure that this has a clearly defined relationship to the assessment of GM plants. In particular, going forward, new 'novel' GM traits such as the soybean example given below are likely to be similar to the types of food and feed authorised under the novel foods regulations.

8. Foreseeable scenarios – example risk assessment process

This section gives an example of how the approach to risk assessment detailed by the project team in Section 7 could be applied to a GM plant with 'novel' traits. Here we use a hypothetical example of a GM plant, a soybean producing a long chain docosahexaenoic acid (DHA) and eicosapentaenic acid (EPA).

8.1. Background to the case study

Long chain polyunsaturated fatty acids (Omega-3 oils) have gained much attention in the wider consumer media for the potential health benefits for consumers. Polyunsaturated fatty acids such as docesahexaenoic acid (DHA) and eicosapentaenic acid (EPA) are currently only consumed through marine fish oils, and farmed fish fed on meal from marine fish. These acids can be synthesised in humans from α linolenic acid (ALA) which is found in plant based products, but efficiency is low (about 5% in men and slightly higher in women) (Halford, 2012). EPA and DHA oils are accumulated throughout the marine food chain, but are made by marine algae. As stocks of marine fish are in decline, and farmed fish is not considered a sustainable source of these fatty acids, it is clear why biotechnology companies may look to feature a plant based product that offers an alternative source of these acids. Evidence suggests that biotechnology companies may be developing soybeans with enhanced omega-3 oils for the market[11], however at the current time a variety with highly expressed levels of DHA and EPA has not been submitted for authorisation around the world. DHA and EPA oils produced from microalgae and sold as a nutritional ingredient for foods have currently received authorisation in the EU under Regulation (EC) No 258/97 as a novel food.

8.2. Hazard identification

To genetically engineer a conventional soybean (or other oilseed plant) to produce oils containing long chain polyunsaturated fatty acids such as EPA and DHA, a number of different strategies have been considered to extend the metabolic pathway that is already present in plants, by introducing genes from suitable algal sources (Venegas-Caleron *et al* 2010). Once engineered the host plant would have significant changes made to its oil composition (accumulation of EPA and DHA oils, and intermediates) which would make it unlike any conventional soybean currently grown. The steps taken to identify hazards would follow the logical order as depicted in Figure 4. The first two steps of hazard identification, information relating the recipient and parental plant/donor organism, and molecular characterisation would remain the same as is currently described in the EFSA guidance (EFSA, 2011). Typically this would involve providing information relating to the recipient and parental plant/donor organism to evaluate all issues of potential concern, and provide sufficient information on the genetic modification.

The trait expressed would be classed as a 'quality/output' trait, as it chiefly benefits the consumer rather than farmer/grower. As such, in order of the flow diagram, the applicant would be required to provide the history of food use of the trait expressed. DHA and EPA rich oils are typically synthesised by marine algae, such as Schizochytrium sp. from the kingdom Chromista, accumulated through the marine food chain and have been consumed for millennia by people in the form of fish oils. DHA- and EPA-rich oils obtained directly from algae have also been authorised under the novel foods regulation (Regulation (EC) No 258/97) for direct consumption as food or feed as a nutritional supplement in the last 10 years. However, prior to this no direct human consumption of the algae or its oil has been known. Given this information it is likely that an applicant would be able to demonstrate that the traits expressed (DHA- and EPA-rich oils) have a history of safe use given their widespread consumption in fish, and as supplements such as 'cod liver oil'. Confidence in their history of use as food or feed is

[11] See http://www.monsanto.com/products/Pages/how-omega-3-works.aspx

likely to be high given the fact that humans are known to have eaten fish, high in DHA and EPA for generations.

For DHA- and EPA-rich oils, information on nutritional and toxicological properties would most likely be given by citing existing information and studies that have been included to assess their safety for approval as novel foods. Within these assessments, nutritional equivalence is shown between DHA- and EPA-rich oils in comparison with existing foods (algal oil derived from Cryptocodinium cohnii). The applicant may also wish to consider the similarities of the DHA- and EPA-rich oil in comparison with foods such as cod liver oil nutritional supplements, and/or oily fish such as mackerel as DHA and EPA accumulated through the marine food chain. Toxicological information has previously confirmed the safety of the oil through using the intact, dried microalgae or the DHA and EPA-rich oil using sub-chronic feeding studies, developmental toxicity evaluation in rabbits and rats, single generation rat reproduction toxicity, mutagencity studies, laying hen study and a broiler chicken study[12].

After providing information on the expressed trait, a comparative assessment, comparing the GM plant containing high levels of DHA and EPA against an appropriate comparator under the existing EFSA guidance (EFSA, 2011) would then be carried out. In this case the GM plant would be compared with conventional soybean plants. It is envisaged that the modified plant would be grown under the same conditions as conventional soybeans, as there are no modifications that mean the grower would treat it any differently. As such, the existing EFSA guidance on field trial design would be employed to identify any unintended effects of the modification.

As per existing EFSA guidance, the test of difference and test of equivalence would be carried out to identify unintended effects, and to quantify intended effects. The test of equivalence aims to verify whether the agronomic, phenotypic and compositional characteristics of the GM plant fall within the normal range of natural variation. For a GM plant with DHA- and EPA-rich oils, the phenotypic characteristics of the plant would differ from the comparator(s), in terms of the fatty acid profile of the plant and any enzymes engineered into the plant to covert plant fatty acids to DHA and EPA, which would fall outside the range of natural variation. A confidence rating would be given to the GM plant, depending on the extent to which it may fall outside the range of natural variation. For values that fall outside the range of natural variation further testing would then be required on a case by case basis in the hazard characterisation stage. Further testing would then aim to demonstrate that this altered profile is safe, using tests or presentation of additional information on a case by case basis. For the GM soybean this may be a demonstration that the levels of DHA and EPA in the plant are within the range that would be expected from the donor organism (micro algae). The test of difference would aim to pick up unintended effects, such as a newly expressed protein as a result of the modification. All unintended effects would then undergo further testing at the hazard characterisation stage.

8.3. Exposure assessment

As a nutritionally-enhanced quality/output trait, the exposure assessment stage of risk assessment is particularly important. The overall aim would be to estimate the anticipated average and maximum intake of the soybean with high levels of DHA and EPA. DHA and EPA is thought to benefit both humans and animals, and as such it is likely that its uses may be in derived food and feed products. It would most likely be intended to replace conventional soybeans in the diet, and as such modelled intake would likely be based on established consumption data for conventional soybeans. However, simply assuming that consumption will be the same as conventional soybeans may be simplistic, and particular consideration should be given to:

[12] For more information see the individual application dossiers submitted to the UK ACNFP: http://acnfp.food.gov.uk/

- Whether intake is likely to be higher due to the enhanced nutritional properties of the derived food and feed (i.e. will consumers eat more due to the health benefits of high DHA/EPA);
- Whether the derived food/feed may replace other foods/feeds in the diet (i.e. would consumers reduce intake of fish due to the availability of DHA/EPA in soybeans which may be a cheaper alternative);
- Would there likely be a difference in consumption between EU member states (i.e. countries that eat less fish in the diet may be more likely to want to consume DHA/EPA enriched products);
- Will consumers understand the changes made to the derived food and feed, and be able to make informed choices about the use of the product (i.e. will consumers understand that there are recommended daily intakes of DHA and EPA oils, and adjust their intake of products derived from the GM plant accordingly); and
- Are there any at-risk groups sensitive to higher consumption of fish oils?

The GMO panel may wish to base approval for the plant on the basis of a recommended daily intake of the DHA/EPA oils. As such, if after an approval an applicant wanted to develop a food product with higher levels of the plant included, it would likely need further qualification that at a scenario of higher intake, there would be no detrimental effects. The exposure assessment should consider any unintended effects found at hazard identification stage, and the likely exposure of these to consumers considering the data gathered. At this stage, post market monitoring should be considered on a case by case basis to determine whether patterns of exposure/intake change with the development of derived food and feed products. However, for DHA and EPA fish oils, there are no perceived at-risk groups. If this could be qualified with data then post market monitoring would be likely not to be required.

9. Conclusions

9.1. Headline conclusions

This project aimed to review strategies for risk assessment of food and feed safety of GM plants, with reference to GM plants with 'novel' traits, and 'comprehensive' risk assessment strategies. Overall the review of scientific literature and approaches used by international risk assessment bodies demonstrated that using comparative approaches, to some extent were always the basis of risk assessments. Currently a 'comprehensive food and feed safety and nutritional assessment of GM plants, and derived food and feed' without the use of a comparator (as stipulated in EFSA, 2011a) is not an approach which is used by international risk assessment bodies, neither is it proposed by the scientific literature as a way of risk assessing GM plants. Hence, it can be concluded that methods for risk assessing GM plants without using a comparator are not currently well understood, and are not applied. As such, little information is available on how these techniques may work in practice. In particular the terms 'novel' GM trait, and 'comprehensive' risk assessment do appear in the literature, and evidence suggests these terms are not explicitly defined and/or used outside of EFSA.

9.2. Findings from the literature review

At some level identifying unintended and intended effects must use a comparative approach. This may be achieved in different ways by; comparing the GM plant to a conventional comparator, to a processed food/feed, or using other tools such as 'omics' approaches. These must be used to identify hazards which will be assessed in the risk assessment process. The review has found that the approach of most international risk assessment bodies is based on OECD guidelines for risk assessment using substantial equivalence. However, individual interpretations may differ slightly; different international risk assessment bodies use slightly different methods to risk assess GM plants. Strategies range from fairly prescriptive and more rigid processes (such as those used by EFSA), to those where the risk assessment process is conducted on a more flexible basis according to the GM trait under assessment (such as the process used by Australia and New Zealand). 'Novel' GM plants which have already been approved were risk assessed using the same framework as 'first generation' agricultural/input traits. No specific modifications were made to risk assessment criteria.

Profiling techniques such as 'omics' approaches (including metabolomics and proteomics) have received much attention in the scientific literature. These approaches are less targeted than comparing to an appropriate comparator, and serve as a method to identify unintended effects. However, their accuracy for detecting unintended effects has been the subject of debate in the academic literature. These methods have the potential to be used in the future, however at the present time these methods are not well understood, with a lack of available databases.

The project team found much debate in the scientific literature regarding exact protocols for allergenicity and toxicity assessments (hazard characterisation). For allergenicity assessment the major studies proposed by EFSA and Codex are supported by a number of authors. Authors discuss the ways of improving the strength and accuracy of allergenicity assessments including: clinical aspects, structural aspects, in silico approaches, IgE binding studies, cell-based methods, profiling techniques and animal models. For toxicity testing the debates revolve around the specific methodology of carrying out individual tests (such as rat feeding studies). In particular there is marked debate between the use of *in vivo* versus *in vitro* tests.

Methods for carrying out exposure assessments received comparatively little attention in the scientific literature; however this is regarded as a particularly important aspect of the risk assessment approach by the project team. For 'novel' quality/output traits of particular importance is to consider the likely

consumption patterns of the derived food and feed. Nutritional assessment similarly received little attention in the literature and should consider nutritional relevance of any new constituents, changed levels of endogenous constituents and potential alterations in the total diet for the consumers/animals.

Post market monitoring is also an area where no consensus exists as to when it is required. Some authors suggest that PMM can be used to validate predictions of exposure levels within different populations. The exact situations in which PMM should be employed as part of safety assessment are not clear, and for food and feed risk assessment remains an unexplored area.

9.3. Foreseeable scenarios for risk assessment

Overall, the approach currently used by EFSA (comparing to appropriate comparators and identifying differences and equivalences) is regarded as a robust one. In the foreseeable scenario for risk assessments, the project team propose that the overall approach to risk assessment should not be fundamentally changed to take account of GM plants with 'novel' traits. The key area of risk assessment that may require revision to make it suitable for the range of 'novel' traits that may require authorisation in the future is hazard identification. This is largely because of the difficulty of comparing 'novel' traits, which may have induced major changes to the host plant, with a conventional comparator. The current 'case by case' approach to risk assessment is important for 'novel' traits, given the wide variety of modifications which may require authorisation. A flexible approach will need to be employed for hazard identification in order to tailor risk assessment methods to accurately identify hazards. The comparative approach, as currently used by EFSA is deemed to be the most suitable method to accurately identify unintended effects, however it is seen that the design of field trials to achieve this may need revision for risk assessment of 'novel' traits. In particular, for plants where it may not be possible to grow them side by side, field trial design will need to be tailored in order to compare them. This may involve growing the GM plant in a different location than its conventional comparator, or using more 'sophisticated' methods such as experiments which simulate the effects of the environment in which the plant would be grown. In the foreseeable scenarios for risk assessment the example of a salt tolerant plant is used in which the GM plant could be compared to its comparator in a 'normal' (i.e. non-saline) environment and subject to increasing degrees of salinity artificially.

The concept of 'history of safe use' is also seen as a valuable tool, particularly for 'novel' quality/output traits which may express a trait that is nutritionally beneficial to the consumer. The principle of this concept is that if an applicant can prove the trait expressed has a 'history of safe use' as food or feed then this may lead to greater confidence in the data presented (however does not negate the applicant from completing a full risk assessment). Whilst this concept is used by many international risk assessment bodies, it is seldom given a specific definition. In particular the exact time period that would constitute a history of safe use is not given by risk assessment bodies. In this project we have used the below definition, based to some extent on the definition used by Health Canada for the risk assessment of novel foods:

Food or feed (expressed trait) with a history of safe use: the applicant can demonstrate that the safety of the expressed trait in food or feed is confirmed from experience of use and continued use in the normal diet of a large part of the population of a country or in farmed/domestic animals for a number of generations, consumed at levels foreseen to be similar to its use in the GM plant.[13]

[13] Definition based on various definitions used by existing risk assessment bodies principally assessing novel foods, such as 'Health Canada' [http://www.hc-sc.gc.ca/fn-an/legislation/guide-ld/nf-an/guidelines-lignesdirectrices-eng.php#a4.1.1.1]

If the applicant cannot prove that the trait has a 'history of safe use', then on a case by case basis data may be presented on any use of the expressed trait as food or feed. This may be, for example a traditional use of the food in a population subgroup or similar.

Exposure assessment is a key stage of the risk assessment process for these types of 'novel' traits: particularly quality/output traits and appropriate data must be gathered to prove that the derived food and feed of the GM plant will not be consumed in ways that may be unsafe (such as over consumption of particular proteins or metabolites). Specific methods for exposure assessment were not covered heavily in the literature; however in the foreseeable scenarios the project team outline a number of questions which may require answers from applicants. For 'novel' GM quality/output traits, exposure assessment may be a more complex task than for previous GM crops assessed by EFSA due to the increased likelihood of changes to consumption patterns. In particular exposure assessment should question whether the new 'novel' trait may induce changes in consumption patterns, displace any other food/feed in the diet, and question whether consumers will know enough about the derived food and feed to make informed choices over consumption (i.e. will they understand that the plant has been modified and what this means).

Public consultation is viewed as an important element of the risk assessment process to increase openness and transparency, given the contentious nature of GM technology in the EU. Publishing outputs of the assessment process and seeking consultation may also help to ease the view of uncertainty that exists within wider stakeholders in the EU over GM technology. In particular the project team recommend that:

- Dossiers (less confidential information) are published and comments invited before consideration by the GMO Panel; and
- Comments are sought from the public on the initial opinion of the GMO Panel.

Regulation (EC) 258/97 on novel foods is currently being revised to bring about improvements and in particular to form a centralised body for the assessment of novel foods in the EU. If the novel foods authorisation process is centralised within EFSA, it will be important to ensure that this has a clearly defined relationship to the assessment of GM plants (particularly given the relative similarity between some quality/output traits and other novel foods such as the soybean example given in the foreseeable scenarios).

9.4. Recommendations for further work

This project reviewed the scientific literature, and the strategies of international risk assessment bodies for risk assessment of GM plants with 'novel' traits, and for comprehensive risk assessment strategies. This was a broad question, and as such has raised further questions, and highlighted areas in which the project team believe future work may be required;

Review of wider risk assessment strategies

Using the principles of a systematic review methodology meant that records reviewed were specific to the question asked (i.e. existing strategies to risk assess 'novel' traits and for comprehensive risk assessment). It is possible that wider risk assessment strategies (risk assessments used outside the food and feed safety sector) may also be useful to inform the approach for risk assessing 'novel' GM traits and of GM plants and it is recommended that further work be carried out in this area. This could be a valuable way to identify other potential methods of hazard identification which do not rely on the comparative approach ('comprehensive' risk assessment). For example, the use of modelling to guide risk assessment strategies could be a useful tool and has been used elsewhere such as in environmental risk assessment.

Post market monitoring

PMM is an area that has received little discussion in the literature, and there is no rigorous guidance on when this should be a requirement resulting from risk assessment. PMM may be particularly relevant for quality/output traits in order to validate the results of exposure assessment. However further work is needed to develop guidance and highlight specific scenarios where PMM should be carried out, and could be informed by the approaches of international risk assessment bodies, and from other risk assessment approaches (for example novel foods and processes). In addition, work on PMM should detail the methodologies for carrying it out accurately, and the frequency and extent to which it may be required.

Field trial design

A finding of this report is that design of field trials for the comparative approach will need to be taken on a case by case basis, with a degree of flexibility employed to allow for accurate assessment of 'novel' traits. It is recommended that further guidance on this subject be developed, including the specific circumstances in which field trial design may need to be amended, with suggested approaches. A review would aim to look at approaches taken to field trial design in other specialist areas (such as efficacy studies of pesticides, nitrogen response trials in crops etc), how they may be applied to the food and feed risk assessment of GM plants and under what circumstances.

History of safe use

In the foreseeable scenarios for risk assessment the project team suggest that history of safe use is a valuable stage of the safety assessment process, particularly for 'novel' quality/output traits which may express a trait that is nutritionally beneficial to the consumer. However, only one of the international risk assessment bodies reviewed define what constitutes a history of safe use. Health Canada defines this term, but the time periods used ('a number of generations') is still relatively vague. It is recommended that more work should be carried out in this area with the aim of providing a more exact definition of what constitutes a history of safe use, what data should be used to support such a claim, and if the applicant cannot prove a history of safe use what other data should be presented (i.e. traditional use of the food or feed in a subset of a population).

Risk management and GM risk assessment process

The foreseeable scenarios for risk assessment detail recommendations on the extent to which public consultation should be included as part of the risk assessment of GM plants. The recommendations are based on what is carried out during the novel foods risk assessment process by the UK ACNFP. The Novel Foods Regulations (258/97) are currently being revised to bring about improvements and in particular to form a centralised body for the assessment of novel foods in the EU. Given the similarity between some of the expressed traits occurring in 'novel' quality/output GM plants, and the types of foods seeking authorisation under the Novel Foods Regulations, it will be important to ensure that the two processes have a clearly defined relationship. It is recommended that further work be carried out to identify the similarities and differences between the risk assessments of novel foods and GM plants, with a particular emphasis on how the overall risk assessment approach is managed. This could be used as a basis for giving recommendations on how risk assessments for novel foods and GM plants would interact.

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APPENDICES

A. CRITERIA FOR THE DEFINITION OF GM PLANTS WITH 'NOVEL' TRAITS

Purpose of document

The purpose of this deliverable was to put forward criteria to define the range of traits under analysis ('novel' traits) for Work Package 1. The criteria developed in this document were used as a point of reference throughout the project.

Background

The European Commission defines novel food, and food ingredients as those which have not been used for human consumption, to a significant degree in the EU before 15th May 1997[14]. When discussing GM plants, the term 'novel' has no specific definition when referring to modifications made to the host plant. For the purposes of this project, Table 5 gives criteria for GM traits that should be considered 'novel'. Please note that we classified 'novel' GM traits according to the nature of the trait itself and not the methodology used to achieve the genetic modification.

Table 5: Definition of 'novel' GM traits

'Novel' traits	**Non-Novel traits**
These include any GM plant which has undergone: Alterations to concentration of storage compounds or nutritional content (e.g. starch in tubers/ sucrose in fruit/ fatty acid in seed); Introduction of 'foreign' storage compound(s); Physiological/ morphological change to plant, e.g. changes to protein or metabolite abundance to alter plant processes; and Alterations in metabolite concentrations to enable plant to tolerate stresses such as frost, cold, salt etc.	Non-novel traits are taken to be when the composition of harvested organs or products is unaffected by the transformation. Therefore plant physiology, morphology and processes can be directly compared to a conventional counterpart. Examples of traits previously risk assessed using substantial equivalence include: Genes targeted to confer tolerance to herbicides; and Genes introduced to produce insecticidal proteins.

Examples of 'novel' GM traits

The below list provides examples of GM plants which can be considered 'novel' according to the criteria in Table 5. All the examples below are GM traits that are envisaged to require market authorisation worldwide at some point in the future, and are at varying levels of commercial development.

Please note these examples do not constitute a definitive list of novel GM traits.

[14] European Commission webpages on novel food and food ingredients http://ec.europa.eu/food/food/biotechnology/novelfood/index_en.htm

NOVEL TRAITS:

Compositional Trait Modification

Amino acid content

Maize, *Zea mays*. CordopA gene from *Corynebacterium glutamicum*; elevated free lysine in the grain

Seed fatty acid content

Arabidopsis thaliana. Delta6-desaturase from *Primula vialii*; delta6-unsaturated C18 omega 3 fatty acids

Oilseed rape, *Brassica rapa*. Thioesterase from *Ulbellularia californica*, neomycin phosphotransferase from *Escherichia coli*; high oleic acid, low linolenic acid

Vitamin/ mineral content

Tomato, *Solanum lycopersicum*. Lycopene beta-cyclase from *Narcissus pseudonarcissus*, phytoene desaturase (crtI) from *Erwinia uredovora*; provitamin A content

Rice, *Oryza sativa*. Phytase-encoding gene from *Aspergillus fumigatus*; iron biofortification

Altered carbohydrate metabolism

Reduction in NAD-malic enzyme

Soybean, *Glycine max*. Galactanase/UDP glucose glycosyltransferase

Enhanced starch accumulation

Rice, *Oryza sativa*. Fructose-1,6-bisphosphate aldolase (FDA) from *Escherichia coli*

Prevention of degreening

Oilseed rape, *Brassica rapa*. Antisense RNA of type I chlorophyll a/b binding protein LHCII

Developmental Trait Modification

Ripening control via ethylene

Melon, *Cucurbitaceae*. S-adenosylmethionine hydrolase from *Escherichia coli* bacteriophage T3 (sam-k)

Tomato, *Solanum lycopersicum*. Aminocyclopropane cyclase (ACC), neomycin phosphotransferase II

Tomato, *Solanum lycopersicum*. S-adenosylmethionine hydrolase from *Escherichia coli* bacteriophage T3 (sam-k)

Ripening control via degratative enzymes

Tomato, *Solanum lycopersicum*. Polygalacturonase (PG)

Extension of vegetative growth

Arabidopsis thaliana. CCA1 promoter overexpression; disruption of circadian rhythm

Resistance to senesence

Arabidopsis thaliana. Farnesyl transferase and inhibitors of naturally occuring form

Abiotic Stress Tolerance Traits

Enhanced drought tolerance

Maize, *Zea mays*. Shock Protein (CopB) from *Bacillus subtulis*, NptII from Tn5 of *Escherichia coli*

Tomato,*Solanum lycopersicum*. Osmotin gene from *Nicotiana tabacum*

Enhanced salinity tolerance

Tomato,*Solanum lycopersicum*. Osmotin gene from *Nicotiana tabacum*

Eggplant, *Solanum melongena*. MtlD from *Escherichia coli*; mannitol accumulation

General abiotic stress tolerance

Tobacco, *Nicotiana tabacum*. Mannitol-1-phosphate dehydrogenase (Mtl1) from *Escherichia coli*; mannitol accumulation

Rice, *Oryza sativa*. Late Embryogenesis gene (LEA) from barley, *Hordeum vulgare*

Rice, *Oryza sativa*. ADC gene from *Datura stramonium*; modified polyamine biosynthesis

Biotic Stress Tolerance Traits

It is not yet known whether these traits affect composition of the harvested parts/ organs and thus whether they are classed as novel traits under our criteria.

Enhanced resistance to fungal disease

Tobacco, *Nicotiana tabacum*. Mannitol-1 phosphate dehydrogenase (Mtl1) from *Escherichia coli*; mannitol accumulation

Enhanced resistance to viral disease

Potato, *Solanum tuberosum*.Cry3A from Bacillus thuringiensis and ORF1, ORF2 from potato leafroll virus; resistance to potato leaf roll virus

Enhanced resistance to bacterial disease

Arabidopsis thaliana. WRKY45 ; activation of salicylic acid (SA) signalling pathway

B. LITERATURE REVIEW RESULTS - RISK ASSESSMENT STRATEGIES PROPOSED IN THE SCIENTIFIC LITERATURE

The following sections detail risk assessment methods that have been discussed in the scientific literature for assessing either GM plants with 'novel' traits as defined in the project teams criteria (Appendix A), or for where a comprehensive risk assessment approach (i.e. not based on the comparative approach) is applied.

Comparative approaches to safety assessment

A number of papers discuss the relevance of SE to the risk assessment of GM plants with 'novel' traits. Kok *et al*. (2008) discuss how the development of 'novel' GM traits will have impacts on the safety assessment of the plant products. The paper proposes the use of toxicological and nutritional characterisation of novel plant varieties, where hazards have been identified and when there is no conventional counterpart available for comparative assessment. Additionally, the paper discusses the importance of safety assessments of food products derived from new plant varieties closely following the developments in plant breeding. The aim here is to ensure that safety assessments are suited to the novel food products that may be on the market in the future. Additionally, Parrott *et al*. (2010) suggest that compositional differences between 'novel' trait GM crops, such as soybean and oilseed rape with altered oil fatty acid profiles, may not be an indicator of a hazard. This paper asserts that unless the compositional change is shown to cause adverse effects, like reduction in a key vitamin or nutrient, the change may not alter the safety of the food or feed.

Chassy *et al*. (2007) indicate that the comparative safety assessment process is a valid approach for evaluating the safety and nutritional impact of nutritionally improved food and feed crops ('novel' GM), but that examinations must be carried out on a case-by-case basis. They go on to say that further studies are warranted if significant unintended and unexplainable differences between the modified crop and an appropriate comparator are found. Heinemann *et al*. (2011) also assert that any information which can help in a risk assessment, even if it falls outside current routine, should be included on a case-by-case basis at the discretion of the regulator. Talas-Oğraş (2011) argues that the risk assessments of GM plants need to be carried out on a case-by-case basis with multidisciplinary approaches, covering fields such as molecular biology, toxicology, dietetics, and genetics. The case-by-case approach was put forward to address regulatory challenges associated with food and feed safety assessments. The aim of this was to ensure that safety assessments are proportionate to the risk; use expert scientific advice; allow transparency and public participation; and establish international safety standards (Jaffe *et al*. 2004). Kier and Petrick (in press) assert that 'novel' GM traits should be assessed on a case-by-case basis, and should focus on the modified metabolic pathways. Additionally, the paper discusses how modifications in regulatory proteins that control endogenous plant gene expression are identical to those that have been commonly selected through conventional breeding practices. Therefore they suggest that there is no reason that novel GM crops should have any special safety assessment procedures when compared to those modifications already selected for conventional breeding. The paper suggests that a comparative assessment is still applicable to novel GM traits, but that it should include an assessment of plant morphology, agronomic characteristics, plant composition and the safety assessment of the inserted protein.

Kuiper *et al*. (2002) comment that the application of substantial equivalence may have practical difficulties due to: the availability of near-isogenic parental lines for comparison; limited methods for detecting unintended effects and limited information on natural variations in levels of crop constituents. The paper suggests characterising the expression and toxicity of new proteins; alterations in metabolism; potential for gene transfer from GM food/feed to human/animal gut flora; potential allergenicity of the new traits and the role of the new food in the diet. This approach is the same as the

existing method, but with further development. Despite this, Kuiper *et al*. (2010) state that the comparative assessment approach is applicable to nutritionally or quality enhanced GM crops. They also add that biochemical and physiological modifications induced through these transformations should be well understood in order to ensure that synergistic or antagonistic effects on the nutritional status of the food or feed are identified.

Results from Llorente *et al*. (2011) have supported the use of the current evaluation criterion for novel crops even if they are not substantially equivalent. In this study, the comparative approach was used to evaluate the safety of quality improved potato lines with silenced polyphenol oxidase (Ppo) transcripts and reduced tuber browning, for which the transgene itself encodes an enzyme of unknown biological function that alters compositional characteristics of the host plant. For this reason, the paper suggests there is potential for developing unpredictable unintended metabolic changes to the host plant, but that these can be safely assessed using the comparative approach. In this case, unintended effects were identified in the primary metabolism, implying that the lines were not compositionally equivalent. The unintended effects were assumed to be caused in part by the high compositional heterogeneity, highlighted despite the compositional comparison being assessed under controlled environmental conditions. Additionally, Schmidt *et al*. (2011) suggest that the production of indicator compounds in GM (generally) could be compared to a conventional counterpart.

A number of 'novel' GM traits have been risk assessed via comparing to a conventional counterpart. For example high lysine maize for poultry and swine feed, sweet potato enriched with provitamin A, Golden Rice 2, and ASP-1 enhanced sweet potato have all been assessed by the comparative approach with an appropriate comparator with a history of safe use by Glenn, (2007). Compositional analysis of the crops with known toxins and anti-nutrients formed part of a tiered assessment, which allowed the case-by-case assessment of the crop depending on the perceived risk of the protein. This assessment also considered crops that were grown in locations representative of normal production. Additionally, comparative analysis was used to verify the safety of GM Rainbow papaya with resistance to ringspot virus. In this assessment the crop was shown to be 'substantially similar' to a conventional counterpart (Tripathi *et al*., 2011). Transgenic Volveriella volvacea (straw mushroom) with tolerance to cold stress (through the additional of an anti-freeze protein) was risk assessed using the comparative approach (Wang *et al*., 2009). Aumaitre *et al*. (2002) also question the use of SE to risk assess crops with altered starch composition as a result of the introduction of antisense technology genes. They recommend that a nutritional equivalence approach might be more appropriate. Additionally, Chen *et al*. (2003) safety assessed cucumber mosaic virus resistant sweet pepper and tomato using a comprehensive method that addresses safety information such as genotoxicity (via micronucleus tests, aberration tests, and Ames tests), nutritional value (animal feeding studies), toxicity and allergenicity. However, Chassy (2010) questions the demand for equivalence of composition, saying it ignores the role of the food in human and animal nutrition.

Furthermore there are a number of studies that discuss the harmonisation of risk assessment approaches. For example Brent *et al*. (2003) propose to develop internationally agreed protocols and arrangements for the regulation of GM foods to allow sharing of work load for aspects of regulatory activity and safety assessment. Additionally, Schmidt *et al*. (2011) suggest the use of eight modules for risk assessment as a cost effective method of risk assessing novel and non-novel GM:

Molecular characterisation;

Agronomic traits;

Compound analysis;

Target and non-target organisms;

Toxicology;

Soil and micro-organisms;

Allergenicity; and

Post-market monitoring (PMM).

Heinemann *et al.* (2011) propose that the development of reproducibility standards would eliminate disagreements over the appropriateness of emerging or old techniques, and concerns over whether results come from non-industry laboratories. They conclude that this would bring more confidence to the safety assessment of GM plants.

Furthermore, Kok *et al.* (2008) suggest that GM crops are disproportionately more heavily assessed than conventionally bred crops. They conclude that there should be a general screening frame for all newly developed plant varieties to select ones which cannot be considered as safe as plant varieties already on the market. Chassy (2010) concludes that regulation of GM crops is more rigorous than is justifiable as they do not present new risks compared to traditional breeding, and are more precisely defined and understood than non-transgenic equivalents. Chassy (2010) questions the focus of risk assessments on demonstrating the composition of a GM variety is virtually identical to its conventional counterpart (which the authors say is often called a 'comparator' although as mentioned, EFSA guidelines distinguish the two) when this is not required for newly developed conventional counterparts themselves.

Profiling techniques

Xue *et al.* (2012) support non-targeted techniques to improve the detection of unintended effects through comparing to the non-GM counterpart for a range of compounds. Non-targeted methods could be more appropriate as they extend comparative analysis and reduce uncertainty whilst screening for effects in an unbiased way. Chassy *et al.* (2007) recommends that compositional analysis of crops with known toxicants and anti-nutrient compounds should include analysis of those specific analytes. Also, if warranted, an evaluation of the targeted metabolic pathway should be carried out to find metabolites which should undergo compositional analysis due to safety and/or nutritional considerations. The paper comments that appropriate phenotypic properties of the nutritionally improved crop need to be assessed when grown in representative production locations as part of the overall comparative safety assessment process.

Davies (2010) states that there is not a strong case for the routine use of -omics technologies in risk assessment and that a case-by-case approach is required. Where endogenous plant metabolism is modified ('novel' GM), or where novel foods arise in other ways, specific deployment of profiling can complement traditional targeted analyses, but it should not be seen as a replacement for them. The authors state that a benchmark is needed for risk assessors and risk managers, which takes into account natural variation from crop management, interactions between genotype and the environment and from non-GM breeding systems. Davies (2010) argues that current analytical approaches are too targeted as they analyse a small subset of compositional variables. The author states that unbiased, larger scale analysis of gene and protein expression should be carried out as well, using transcriptomics and proteomics respectively. The paper also suggests that the limitations of the -omics approaches include: a lack of compositional datasets for GM and conventional varieties; poor correlations between gene and protein expression (influencing the interpretation of transcriptomic data); the fact that low abundance transcripts/proteins may not be detected and that hybridisation-based arrays do not provide information on alternative splicing, which may affect the functionality of the protein expressed, and allow limitations to the number of compounds that can be screened (Davies, 2010).

Glenn (2007) also suggests a comparative approach using -omics technologies and says additional studies should be carried out where suitable on a case-by-case basis. Studies may focus on levels of components in the biosynthetic/degradative pathways of the increased nutrient in the novel crop. The example of nutritionally enhanced lysine maize for poultry and swine is discussed. This case study is said to show that despite each product needing to be considered on a case-by-case basis, the comparative approach of both safety and nutritional assessment can be applied successfully to 'novel' trait GM crops. This nutritionally enhanced maize was thus deemed as safe as and substantially equivalent to conventional maize, apart from the intended increase in lysine and resulting increase in two lysine catabolites.

Heinemann *et al.* (2011) discuss the comparative approach and emphasise how, as more types of GM plants and novel traits are developed, a broader use of molecular profiling in risk assessments is required. They discuss how molecular profiling may supplement the comparative approach to risk assessment, by increasing confidence in risk assessments. It warns that the profiles used should: be designed to address relevant risks, be applied at the correct assessment stage, and be clearly set out for regulators. They say the development of reproducibility standards would eliminate disagreements over the appropriateness of emerging or old techniques, and concerns over whether results come from non-industry laboratories. They add that while profiling is generally based on robust and reproducible techniques, there are probably some target classes that are more important than others. This is especially important when there is significant concern that the novel transgenic proteins, or their post-translationally modified isoforms, might be found in GMOs as biologically active immuno-modulators. The authors can see no justification for not using proteomics to describe all isoforms of such proteins in the GM plant. The authors add that non-targeted profiling would require access to test materials which may be hard to obtain from developers.

Kuiper *et al.* (2002) highlight a need for new profiling methods to screen for changes in the modified plant at: genome level, during gene expression, during protein translocation, and at cellular metabolism level. The authors support the implementation of DNA microarray technology to analyse the expression of a large number of genes in a sensitive and quantitative manner. The proteomic tool Sodium Dodecyl Sulphate- Polyacrylamide Gel Electrophoresis (SDS-PAGE), followed by protein digestion and analysis by Mass Spectrometry (MS) allow for the identification of new proteins or the alteration of existing ones. Chemical fingerprinting may indicate whether intended/unintended effects have taken place as a result of the modification. The authors say further thought is needed with respect to uniform selection of critical compounds, and conditions under which GM crops should be grown. They comment that the targeted (single compound analysis) approach has its limitations with respect to identification of unknown anti-nutritional factors and natural toxins and conclude that the development of profiling methods at different levels (genomics, proteomics, and metabolomics) should be encouraged.

Talas-Oğraş (2011) discusses how the risk assessment methodology is focussed on substantial equivalence and an assessment of potential unintended effects. The paper suggests that due to global trade and use of GM plant-derived food/feed, profiling techniques need to be defined for the characterisation of GMOs. Talas-Oğraş (2011) says that the current improvements of new instruments mean sensitive profiling technologies (including genomics, proteomics, transcriptomics and metabolomics) have been used for GM plants in order to evaluate specific information about the metabolic pathways in order to detect unexpected effects in nutritionally improved, 'novel' GM crops. Profiling of the plant's metabolites, gene expression, and proteins could be useful for detecting unexpected changes in GM plants. They also mention that there is a need for validated profiling methodologies before they are considered for safety assessments of GM crops.

Metabolomics

Metabolomics allows a general overview of the primary metabolism and its modifications. For this reason, Llorente *et al.* (2011) suggest metabolomics as the most useful approach, compared to transcriptomics and proteomics. Metabolomics was used as a targeted profiling approach to provide evidence that the observed metabolic differences are as a result of a reduction in the activity of the down-regulated enzyme for a novel trait, and therefore to quantify and characterise the unintended effects.

Catchpole *et al.* (2005) discuss how hierarchical metabolomics has been used to demonstrate substantial compositional similarity between novel-GM and conventional potato crops. Rapid metabolome fingerprinting and data mining methods were used to allow metabolites responsible for differences between potato genotypes to be identified. Only anticipated metabolites were found in GM lines and glykaloid levels were normal. Gas Chromatography – Time of Flight analysis (GC-TOF) data analysis was used. There was a large variation in metabolome profile between conventional cultivars, hence GM potatoes fit into the natural variation, apart from introduced genes. In the comparative assessment framework, metabolic side-products might undergo more detailed investigations into toxicity, abundance and chemical structure.

Kusano *et al.* (2011) discuss the comparative technique and the use of metabolomics for charting the unintended effects of genetic modification. They discuss multi-platform metabolomics as an approach that is both sensitive and robust and constitutes a good starting point for characterising GM plants.

However, Chassy (2010) discusses how metabolomic profiling does not produce data which is useful in a safety assessment, and suggests that there is no current reason to think -omics can add value to safety assessments for novel and non-novel GM. If future technology can accurately determine the content of many plant components more efficiently than conventional analysis, metabolomic screening could be an attractive tool for compositional analysis alongside conventional analysis. -Omic technologies measure analytes, including unidentified ones, but Chassy, (2010) reports that the analysis often sacrifices at least one of the characteristics of validated analytical methods currently used, including the fact that there is not an internationally validated and standardised measurement technique for most compositional variables, or robust datasets that establish safety intervals (although there are standards for microarrays).

Proteomics

Proteomics can detect changes in protein expression level in the *proteome* (all proteins expressed by a genome). Proteomics can be used as part of a comparative risk assessment with the aim of improving the detection of unintended effects (D'Alessandro and Zolla, 2012). It is suggested that proteomics can be used to complement the existing safety assessment. Proteomics has been used to assess the food safety of transgenic maize, and has been used with metabolomics approaches (identify metabolites with Nuclear Magnetic Resonance (NMR). The paper suggests that substantial equivalence should be assessed at multiple levels and that proteomics might provide insight into aspects that are not necessarily linked to other biological parameters such as metabolism.

Xue *et al.* (2012) support the idea of an integrated risk assessment of transgenic *Bacillus thuringiensis* (Bt) and Phosphoenolpyruvate Carboxylase (PEPC) rice (*Oryza sativa*). They discuss a comparative, proteomics approach. Comparative proteomics involves two-dimensional electrophoresis, Matrix-Assisted Laser Desorption/Ionisation – Time of Flight (MALDI-TOF-MS). In the development of GM crops, plants respond to modifications in their genomes. Any genomic change should be reflected at protein expression level (in the proteome), and therefore proteomics can detect these changes. Hence analyses of the proteome of GM plants compared to their non-GM lines should enable the

identification of changes occurring due to the modification. Although this is useful for studies on biosafety the paper warns that this does not lead to final evidence of harm.

Comparing to a Database

The current comparative system involves assessing a number of compositional variables. The transgenic crop is compared against a background level for each of these variables in a conventional counterpart. Compositional analysis has been described as the 'cornerstone for the nutritional assessment of food material' (Varzakas *et al.*, 2007). Nutritional assessment aims to ensure the food/feed is not nutritionally disadvantageous, and is separate to the food and feed safety assessment. New GM crops which have undergone modification of the plants metabolic pathways in order to improve agronomic vigour, or to increase the nutritional/ health value of the crop, may not have a suitable conventional counterpart available for a comparative assessment. In this case, it has been suggested that it could be assessed against a database describing quantitatively, the natural variation for each designated compositional variable of the conventional crop species (Varzakas *et al.*, 2007; Chassy *et al.*, 2007). This would constitute a targeted approach which would involve the simultaneous screening of specific compounds. This would form part of a comparative approach, assessing against the background natural variation to determine meaningful differences (Chassy *et al.*, 2007). This method is supported by Catchpole *et al.* (2005), who discuss the issue of unintended effects, commonly occurring at a level that sits within the natural variability of that compound in the conventional crop varieties. Furthermore, Chassy *et al.* (2007) suggest that a database could act as a safety standard to assess many components of the crop composition without prior identification in the future. Most have supported the idea that this database would include metabolic information, but Dijk *et al.* (2010) suggest the development of a database comprising expression levels of the transcriptome.

Davies (2010) states that comprehensive mass spectral databases for plant compounds are growing and that this will assist the identification of metabolites which differ between the GM crop and its comparators. The paper says the development of comprehensive databases on gene, protein and metabolite profiling of 'conventionally' grown crops and 'conventionally' bred crops grown under a range of environmentally variable conditions, will provide a highly desirable benchmark for the safety assessment of alternative production systems or breeding practices.

Chassy (2010) comments, that the composition of transgenic crops may fall within the range that is seen in conventional crops. The author says this is not too surprising as acceptable agronomics are a requirement for selecting from transgenic events (as for selections using traditional breeding) and plants with meaningfully altered compositions are likely to appear abnormal. It has been shown that the natural variation in the contents of a number of widely tested compositional markers in both parental and modified plants is significant, especially due to environmental influences such as drought stress (Novak and Haslberger, 2002).

Peer-reviewed databases for both 'novel' and 'non-novel' traits, such as the crop-composition database compiled by the International Life Sciences Institute (ILSI), could include a list of key components (including nutrients, anti-nutrients, toxins, allergens, and secondary plant metabolites) that are identified to have possible health impacts; all with appropriate ranges of natural variation (Sesikeran *et al.*, 2008; Novak and Haslberger, 2000). This approach has been suggested to provide additional benefits to risk assessments other than the continued possibility of carrying out a comparative evaluation framework, including the provision of a more rigorous detection method for unintended effects. The development of this aspect of the safety assessment lies in the potential of these methods to detect unintentional effects with higher sensitivity than non-targeted approaches (Sesikeran *et al.*, 2008). In common with toxicity testing, it has been proposed that this database could include reference intervals to describe safe intake levels for a healthy, heterogenous population (Kaeppler, 2000). One limitation to this method may be that data might not be directly comparable due to differences in analytical methods or sample preparation (Novak and Haslberger, 2000).

The use of compositional data from a range of conventional cultivars was discussed in Schmidt *et al.* (2011). Substantial equivalence was determined using baseline threshold values calculated from the four non-GM potato cultivars, firstly through a Gas Chromatography, Mass Spectrometry (GC-MS) method for the detection of chosen metabolites. If the threshold values were exceeded by the GM potato line, a further detailed risk assessment would be carried out to show whether or not the values exceeding the threshold were biologically relevant. The compound analysis and molecular characterisation create the fundamental data to localise the necessary research for a case-specific risk assessment on: environment, human and animal health and also consider post-market monitoring. The content analysis of key nutrients was performed with conventional methods known for food and feed examinations. Baselines and thresholds would take into account natural variability when comparing the potential risk of transgenic plants with those already present in cultivation or consumption. They say this baseline-threshold approach should help to quantify the risk as well as rank GM plants within the range of wild and cultivated plants. The paper therefore suggests an alternative comparator: to use baselines and thresholds which are assigned due to natural variability of crop populations.

Barber *et al.* (2008) conclude that there is a large variation in metabolome profile between conventional cultivars. Therefore 'novel' trait GM potatoes can easily fit into the natural metabolite range of classical cultivars, for all apart from introduced genes. In the comparative assessment framework, such metabolite side products might eventually be subjected to more detailed investigations if deemed necessary with respect to toxicity, abundance and chemical structure. Dijk *et al.* (2010) also suggests that this would be the case if assessing transcriptome variation to act as a benchmark for safety assessment, since variation in transcriptome make-up as a result of environmental factors is great.

Comparing to food product

Current risk assessment objectives aim to compare the transgenic crop with a conventional counterpart. It may be that a number of comparators could be used to address issues of biosafety. Constable *et al.* (2007), suggest comparing novel GM crops with similar products which have a 'history of safe use'. For example if a crop is modified to produce an oil or protein, it might be reasonable to compare the modified crop trait levels to an existing food oil, or protein. This existing product would therefore act as a replacement for the conventional counterpart for this particular crop characteristic. This would fulfil requirements for risk assessing the introduced trait itself, but not any unintended effects of the trait. These comparators would act as a starting point for safety assessment in a similar way to a conventional counterpart. Constable *et al.* (2007) also encourages a comprehensive/ holistic approach to safety evaluation based on mechanistic insights, nutritional safety and toxicology where necessary.

Varzakas *et al.* (2007) also suggested that GM plant material should not only be compared with material from the parent plant but with material from the parent plant genetically modified to express the empty construct (a construct without the transgene).

GM crops with non-novel agronomic traits such as herbicide tolerance and insect resistance are discussed by Delaney (2007). As with food ingredients, bioengineered foods are characterized analytically as one aspect of the safety assessment. In contrast to food ingredients which are discrete organic entities, bioengineered foods are complex mixtures of nutritional and non-nutritional components. They do have nutritional value and because they are typically whole foods (as opposed to fractions of foods), are also likely to have effects on satiety. Accordingly, there are considerable differences in the analytical characterization of the test substances between these types of studies. Although analytical characterization of food ingredients can be used to define the substance in terms of absolute purity, the analytical characterization of bio-engineered foods is conducted by comparison of the concentrations of a number of known nutrient, non-nutrient, secondary metabolites, and anti-nutrient components of the bioengineered food with its closest non-GM isogenic comparator, and

additional non-GM reference substances obtained from the same field trials. This paper reviews the safety assessment of bioengineered foods, comparing them to testing strategies used for typical food ingredients. They conclude that strategies used to assess safety of bioengineered foods are at least as robust as those used for typical food ingredients (Delaney, 2007).

Allergenicity assessment

Delaney (2007) concludes that further progress should be made in developing definitive methods for the identification and characterization of proteins that are potential allergens.

Talas-Oğraş (2011) discuss how identification of allergenic potential of a GM plant product should be validated, ensuring it is robust, and reproducible. In cases where the GM plant differs from a conventional counterpart by one or more novel protein, the protein should be assessed for potential adverse effects. Bioinformatic databases (such as allergenonline.org) containing protein allergens are used to detect global sequence similarity and short contiguous amino acid sequence identity. In vitro cell culture screening systems might be used as an additional system for risk assessing food-derived compounds. The application of this for preliminary screening of transgenic food might decrease the number of animal experiments

The weight of evidence approach to allergenicity assessment has been widely accepted as the method of reducing risk of allergenicity in GM foods, based on Codex guidelines (2009) (Snell *et al.*, 2012; Goodman *et al.*, 2005 and Singh *et al.*, 2009). This is true of both 'novel' and 'non-novel' foods. For example correlations between sequence homology and stability have been identified to be useful for overall safety assessment, but neither approach provides direct evidence of allergic potential (Atherton, 2002). The development of allergies is considered to be unpredictable due to the natural variability in human subjects. The current risk assessment process takes this into account by considering allergenicity prediction methods, in order to minimize any uncertainty about proteins in question (Davies, 2005). Allergenicity assessments must consider two main issues; the transfer of a known allergen from a crop into a non-allergenic target crop and the creation of a neo-allergen where *de novo* sensitisation might occur in the population. The second allergenicity risk has been commented to be of no greater risk in GM crops than in traditionally bred crops (Lack, 2002). A number of studies have commented that allergenic potential in 'novel' GM traits is equivalent to that in non-GM novel foods (Lack, 2002).

These assessments are therefore carried out via a range of standard protocols, to carry out an initial characterization and safety assessment of the protein itself, and then a characterization and safety evaluation of the whole transgenic plant. They include:

- *Protein Source investigation - evaluation of the history of human exposure to the source of the gene and considers whether there is evidence of allergy associated with the protein or the source.* Snell *et al.* (2012) and Goodman *et al.* (2008) propose not only considering the source of the gene but also to take into consideration the potential for post-translational modifications to alter the structure and antigenicity of a protein when expressed in a new host. Snell *et al.* (2012) suggest full characterization of the protein of interest expressed in the native and transgenic state might also be warranted for assessment of allergenicity;

- *Amino acid sequence similarity, bioinformatic analysis.* Snell *et al.* (2012) recommend protein allergen databases such as AllergenOnline (Version 11, Feb 2011) as the primary method of testing amino-acid sequence similarity, to predict allergenicity through conserved allergenic sequences, but adds that sequence comparison analysis with known allergens may have limited predictive ability. Goodman and Tetteh (2011) suggest this approach is non-validated: it may reveal false positive hits due to matching of short peptide sequences;

- *Potential allergenic cross-reactivity.* This was assessed using *in silico* analysis of the construct sequence. *in vivo* safety evaluation showed no adverse effects on physiological parameters (Llorente *et al.*, 2011 and Hothorn and Oberdoerfer, 2006);

- *Stability studies*. Some issues involved with homology testing and proteolysis testing have been highlighted in literature. Meredith (2005) suggests that tests for resistance to proteolysis using simulated gastric juices, may not identify all the allergenic compounds within a GM crop since many known allergens are susceptible to proteolysis;

- *Cross-reactivity with IgE from food-allergic individuals*. Meredith, (2005) states that the potential allergenicity of transgenic plants can be determined to a reasonable degree using specific screening with sera of subjects allergic to the source of the gene, as supported by Lack, (2002). On the other hand, Goodman *et al.* (2005) identifies that the use of broadly-targeted IgE binding assays in animal model systems is not supported by current evidence as a sufficiently predictive method of investigating allergenicity of compounds. Goodman and Tetteh, (2011) also suggest that this approach is non-validated, and that it poses a potentially high risk of false positive results and low probability of true positive results. Aalberse, (2008) proposes that broadly targeted IgE studies may not identify cross-reacting compounds. US EPA/ORD initiated a targeted research effort to develop appropriate specific and targeted IgE serum tests to assess potential allergenicity, as described by Goodman *et al.* (2008) and Ladics and Selgrade (2009);

- *Animal Studies.* Snell *et al.* (2012) recommend animal models to screen proteins for allergenicity but Varzakas *et al.* (2007) propose that new *in vitro* methods and animal models need to be developed, evaluated and validated in order to assess directly the inherent sensitising potential of new-formed proteins. Goodman and Tetteh (2011) suggest that this approach is non-validated: it has questionable applicability to human sensitivity and hence might not be able to be extrapolated to humans; and

- *Human Skin Prick Test*. This test protocol was used in the allergenicity assessment of leaf curl virus resistant GE tomato (Singh *et al.*, 2009). This test was used to demonstrate a lack of allergenicity with respect to a positive control of histamine diphosphate. Skin reactions equal to or greater than the positive control in wheal size were graded as an allergenic reaction to the sample.

Results from Singh *et al.* (2009) suggest that GM tomato with replicase gene is safe with respect to toxicity and allergenicity, as assessed using sequence analysis, protein extraction, mice testing studies (IgE response), human skin prick tests and sera collection. The joint FAO/WHO consultation set out in 2001 that a protein would be considered an allergen when positive results for either a sequence homology, or specific serum screen were validated. In the case of a negative result for either of these tests, a combination of pepsin-resistance tests and animal modeling would be used to define a high, intermediate and low probability of allergenicity (Lack, 2002). Many of these standard assessment methods for compound allergenicity have been questioned as to their value in a risk assessment strategy, including animal models, and targeted serum screens, which have been identified in Goodman and Tetteh (2011), due to the fact that predictive values have not been validated. For this reason Goodman and Tetteh (2011) and Chassy (2010) consider that the inclusion of these non-validated tests may result in the rejection of safe and beneficial products, excessive costs and the disruption of trade without any reduction in risk. Furthermore it has been suggested that an allergenic assessment should include an assessment of history of safe use, for example previous safe consumption of grain amaranth was considered when assessing maize modified with an Amaranth 11s globulin by Sinagawa-Garcija *et al.* (2004). This is supported by Young *et al.* (2012), who suggest that bioinformatic analysis often will not detect allergenic proteins, and that these tests should be reconsidered if many food products with a history of safe use are failing allergenicity assessments.

Potential improvements to allergenicity assessment

Newly expressed proteins: Barber *et al.* (2008) suggests a tool for substantiating serum banks for allergenicity assessment. The patient selection criteria should take into account the geographical location of patients, the intensity and nature of the environmental allergens in the area and the potential cross-reactivity among allergenic molecules. Sera for serum banks, obtained from patients with demonstrated food allergies, should be subjected to a further characterisation by screening with a panel of relevant allergenic molecules. A representative panel of these sera should be used in the allergenicity assessment. Barber *et al.* (2008) also adds that *in vitro* methodologies should have the adequate specificity and sensitivity, and the integrity of the molecules tested should be guaranteed.

Schmidt *et al.* (2011) conclude that improvements to bioinformatic search algorithms and scoring criteria for potential cross-reactivity are needed. Including broadly targeted allergic serum IgE binding studies, animal sensitization and provocation studies and extensive pre- market clinical evaluation to look for potential minor quantitative differences in allergens.

Selgrade *et al.* (2009) suggest more sophisticated structure-activity tools may prevent the unnecessary elimination of potentially useful products. More detailed protocols for serum testing should be applied so that the data are reliable. Liquid Chromatography (LC)/mass spectrometry may be a viable alternative to more labour intensive approaches to quantify proteins. Data generated using the Bowman mouse model suggest that pepsin digestibility is a good indicator of potential allergenicity. The authors also suggest that the food matrix may play an important role in the sensitisation process. All the above rely heavily on IgE as the indicator for potential allergenicity. It is possible for IgE responses to occur in the absence of an adverse reaction in both humans and mice. To date, assessments have largely focused on the sensitisation process, but work presented here suggests that the ability to sensitise and the ability to evade oral tolerance are not one and the same. Pepsin stability is clearly important for sensitisation, but resistance to both pepsin and trypsin appear to be required for oral tolerance.

Decision trees may be used as a potential tool for allergenicity risk assessment, as proposed by Meredith (2005). This is supported by Sinagawa-Garcija *et al.* (2004), who supports the use of a decision tree which takes into consideration multiple features of the novel protein. This paper uses a decision tree approach to assess the safety of maize modified with an Amaranth 11S globulin gene. This tree addresses the souce of the gene, the homology of newly introduced proteins to current allergens, IgE binding studies, digestion of the protein in vitro, and the immuno-reactivity of the novel protein in appropriate animal models.

Allergenicity is now assessed during gene selection, taking into account: their source, previous consumer exposure, history of safe use of the source material, the gene, the product of the gene and any ethical issues that might arise. This aims to prevent the generation of a 'novel' transgenic crop that had a high potential of eliciting allergenic effects (Taylor, 2001 and Taylor, 2003).

Whole GM plant: Assessing the protein as expressed within the plant, as well as the transgene itself, can be important. Proteins of a non-plant origin found expressed within plants might show different patterns of glycosylation. This will affect the properties of the protein, and therefore should be considered when assessing protein allergenicity (Kusano *et al.* 2011). Also the allergenicity of the Cucumber Mosaic Virus (CMV) coat protein expressed in transgenic tomato was assessed via investigation of the expression of the transgene source of the protein by Lin *et al* (2010) This paper concluded that the transgenic protein itself should be studied as expressed within the plant rather than as a transgenic protein expressed in prokaryotic systems. This also applies when proteins are expressed in different species.

A number of studies highlight the issues surrounding stringent allergenicity assessment, proposing the idea that inclusion of non-validated tests in the risk assessment process can lead to the rejection of safe and beneficial products, excessive costs and, in the extreme, a negative impact on trade with no significant reduction of risk. Furthermore, these non-validated tests may bring about the introduction of a product that does pose a substantial risk to an allergic consumer (Goodman *et al.*, 2008). Goodman *et al.* (2008) suggest that the stringency used to assess the allergenicity of GM crops should be in line with that of commercially bred varieties, which show the natural variability in allergenicity currently consumed. Goodman & Tettah (2011) suggest that the current allergenicity assessment could be strengthened by an explanation of probable hazards and an estimation of at-risk groups, alongside how exposure and risk assessment should be evaluated.

For any novel food risk assessment process investigating allergenic potential, a number of protein characteristics are considered. These include the identity of the individual protein components, and their relationship to known food allergens. Also assessment may extend to serum screening for cross-reactive, skin-prick tests in previously sensitised individuals and double-blind placebo-controlled food challenges. Furthermore the quantity of protein that might be consumed is considered. Constable *et al.* (2007) suggest that homology screening is a less appropriate test of allergenicity because there is not a specific transgene to sequence. For proteins that are related to a food allergen, a specific serum screen to identify potential IgE-binding capacity is appropriate. Qualitative analysis is used to estimate whether a product component is likely to present an allergenic hazard. Positive results would indicate the need for a skin-prick test, or a double blind placebo-controlled food challenge.

Mouse Models

Mice can be used as models for compound allergenicity in humans. There are a number of alternative methods of testing compound allergenicity using these models, which vary mostly in the method they use to administer the compound into the systems of the mice. Mouse models are considered favourable because of their suitability for testing; a small size, short breeding cycle, well characterised immunology, and widely available immunological and molecular reagents used to study allergenicity in this species. Mice can be models for allergenicity in humans via oral sensitisation tests, intraperitoneal sensitisation tests, and transdermal sensitisation models (Aldemir *et al.*, 2009). More recently the use of intratracheal challenge models has been suggested as a suitable method of administering the test compound to mice (Goodman *et al.*, 2008).

The applicability of mouse models to assess allergenicity of compounds from GM plants is widely debated (Goodman *et al.*, 2008 and Bowman and Selgrade, 2008). Oral sensitisation models have been suggested to be the most appropriate route of testing for allergenicity in mice, especially considering the role of oral tolerance in regulating IgE responses. Additionally, foods containing digestion-resistant proteins provoke allergic responses in this model, therefore supporting the use of pepsin resistance testing in a weight of evidence approach to allergenicity testing. The subcutaneous route has been shown to be inadequate to distinguish allergens from non-allergens (Bowman and Selgrade, 2008).

Improvements to the allergenicity assessment process have been suggested for mouse oral sensitisation models, including the expansion of the test panel of known allergenic and non-allergenic foods to be verified by independent laboratories prior to its wide scale use as an allergenicity assessment method in a risk assessment procedure. Bowman and Selgrade (2008) suggest that it is vital that this model identifies all compounds which elicit IgE responses after oral exposure with the adjuvant, as these compounds are associates with a greater allergenic risk (such as peanut, Brazil nut), but not identify false positives, or foods which induce no IgE response due to it's suppression through oral tolerance induction (for products such as egg white).

Prescott and Hogan (2006) has suggested via animal models that the consumption of a GM plant containing an allergenic protein does not always result in predisposition to an allergic hypersensitivity response. They concluded that consuming a GM crop can sometimes bring about the production of a T-cell response that will protect against subsequent development of allergenicity specific to that particular protein.

Toxicity Assessment, Nutritional Assessment and Animal Feeding Trials

Toxicity Assessment

The first step for safety and nutritional assessment of modified plants is the compositional analysis of potential food/feed and including the newly expressed proteins and other new constituents, and the comparison with conventional counterparts. Toxicity assessments for food and feed safety most commonly involve laboratory animal feeding studies alongside *in vitro* studies for safety assessment, whereas target animals are most often used for nutritional assessment to ensure the procuct is not nutritionally disadvantageous to consumers/animals (Flachowsky and Wenk, 2010). Further toxicity assays include those proposed as to be a substitute for animal testing (appropriate *in silico* or *in vitro* test methods, including *in vitro* genetic toxicology tests and screening for point mutation, chromosomal mutation and DNA damage/ repair) (Yen *et al.*, 2011). These studies attempt to investigate the short and long term effects of consuming the whole GM product. Chassy *et al.* (2007) suggests that studies with laboratory animals can backup observations from other parts of the safety assessment, although they may not be sensitive enough to show unintended minor changes. Feeding studies with target livestock species are suggested to be important in demonstrating expected nutritional benefits of nutritionally enhanced feed crops.

Chassy *et al*. (2007) agree with the use of a 90 day rodent toxicology study where appropriate. They also discuss simultaneous screening of many components without prior identification in the future, whereas previously specific compounds have been looked at (a targeted approach). Talas-Oğraş (2011) says an *in vitro* ruminal epithelial cells system developed to study the effects of the insect specific Bt toxin could provide an appropriate tool for safety evaluations before *in vivo* studies. Also gastrointestinal cell culture models of the digestive tract are exemplified as important future tools for screening GM crops. Schmidt *et al.* (2011) suggest that toxicology could be assessed via a novel highly sensitive *in vitro* system simulating the transport of substances from the gut into the blood, that detects risk of incorporation in humans or animals at an early time point, although the model remains to be developed.

Due to the requirement for reliable information, longer-term, case-by-case studies are advised to evaluate GM plants for human/ animal consumption (Talas-Oğraş, 2011).

Many studies (Domingo *et al.*, 2011; Atherton, 2002 and Zhou *et al.*, 2012) indicate concerns about the effectiveness of whole food laboratory feeding trials, especially over a 90-day period. Snell *et al.* (2012) concludes that there is, based on evidence from risk assessments of non-novel trait GM, no reason to suggest that a 90-day trial is insufficient. Flachowsky and Wenk (2010) argue that studies with target animals should also be used more intensively for safety assessment in future as more representative candidates especially for GM feed safety. To improve GM food risk toxicity risk assessment, it has been suggested that a safety assessment carried out using a cultured human cell system may be more appropriate (Momma *et al.*, 2000).

Whole food trials have been criticised by Atherton (2002) and Flachowsky *et al.* (2007) due to the inherent variation in the composition, nutritional value and the bulkiness of the whole food product, which would mean that any unintended toxic compound would be fed at such low multiples that any toxicity effects would not be identified. Furthermore, feeding at any higher levels would compromise nutritional value of the food and balance of the diet (Sesikeran and Vasanthi, 2008). Hollingworth *et*

al. (2003) states that this might also be exacerbated by the fact that animals would consume an article at much lower levels to those that would be eaten in the human diet. In common with this, Hollingworth *et al.* (2003) asserts that testing of multiple combination of previously identified unintended chemicals remains a difficult scheme for toxicologists.

Longer term whole food animal trials have been considered in the literature. Snell *et al.* (2012) concludes that studies lasting two years do not seem necessary other than when doubt remains after a 90-day study. To allow for an evaluation of the effect of consumption of a transgenic (in particular for high lysine rice) on reproduction and development in the consumer, Zhou *et al.* (2012) suggest the use of a three-generation reproductive toxicology study to complement the 90-day feeding trial. Momma *et al.* (2002) also consider the use of long-term trials, stating that short-term studies would not be long enough to investigate malformations, reproductive disorders, mutatagenicity or carcinogenicity caused by consumption of any GM product.

Llorente *et al.* (2011) used dietary interventions in a murine model to assess the safety of unintended effects of GM consumption. Organ and blood physiological parameters were analysed: no toxicity effects, alterations on metabolism, body weight changes, or adverse effects on gut micro-biota were observed between the GM fed and non-GM controls.

Flachowsky *et al.* (2007) indicate that routine feeding studies with target animal species add little to nutritional assessment of feed from GM plants of the first generation, but they will play a more important role in nutritional and safety assessment of feeds from GMP with output traits such as altered fatty acid profiles in rapeseed or inulin potatoes. Strategies for nutritional and safety assessment which are developed for those of the first generation cannot directly applied for GMP with substantial changes of the constituents such as 'novel' GM traits. This paper suggests that case by case studies with animals will gain more importance in 'novel' GM plant risk assessment, to combine safety studies and nutritional assessment in target animal species. These animal studies, suggested to include reproductive assessment in a four-generation experiment with laying hens, would also allow the examination of unintended effects in this case, where an avian model is appropriate. Additionally, Yen *et al.* (2011) showed that although some substances may present no mutagenic effect, animal experiments or epidemiological investigations are valuable as they have proven them to be carcinogenic.

Herman *et al.* (2010) have proposed that equivalence limits, especially for toxicity testing, may be a poor model for comparing transgenic crops with reference crop varieties. This paper suggests the use of reference intervals, constructed for a healthy heterogeneous population using appropriate sample sizes and many varieties and environments, to which the levels of specific compounds can be compared. It is indicated that these intervals could be calculated using publically available compositional data such as is found in the International Life Sciences Institute Crop Composition (ILSI) Database.

A range of quantifiable variables have been suggested to be relevant to animal feeding toxicity trials, including growth, body weight, food consumption and urea, and cholesterol levels, and histological variables such as lung histology and release of splenic cell culture supernatant (Singh *et al.*, 2009). Further quantifiable variables suggested for long term feeding trials include reproductive data, hematology, serum chemistry, relative organ weights and pathologic section (Zhou *et al.* 2012). Feeding studies in rats have been used to assess transgenic rice (*Oryza sat*iva) modified with Soybean Glycinin (Momma *et al.*, 2000), which involved studying body weight, general signs, blood samples for blood count and chemical components in blood serum, and necropsy. Chao and Krewski (2008) suggests traditional toxicity testing methods, such as an acute study, a 14-28 day feeding study and an animal growth study, in its graded risk assessment strategy, with additional organ targeted toxicity of hormonal proteins and immunotoxicity of protein expressed at high levels for GM crops for which there is a perceived high toxicity risk.

Chassy (2010) discusses results from feeding transgenic crops to laboratory animals. The paper states that although none of these studies have revealed a negative effect of the transgenic breeding process, none of these studies have been repeatable either. The paper says studies that claim adverse effects on consumption of GM crops are often: poorly designed; inadequately documented/conducted; have experimenter bias, and draw conclusions without meaningful biological differences. *Mutagenic tests* (as specified by the national standards of People's Republic of China via Ames test), involving the evaluation of mutagenic effects on micro-nucleated cells and sperm, can also be carried out (Flachowsky *et al.*, 2007).

Flachowsky *et al.* (2012) recommends new experimental designs for 'novel' GM plants (for nutritional and safety assessments). These should take into account number of locations, growing seasons, geographical spread, replicas and statistical analysis. Apart from the transgenic and its near isogenic counterpart (control), the studies should preferably have four or more conventional (commercial) reference varieties to help explain any unexpected differences or confirm any expected differences observed between the test and control plants (ILSI, 2007). In such a case it is possible to compare the composition and nutritive value of GM plants with commercial lines.

Nutritional Assessment

GM food: Chassy *et al.* (2007) proposes that pre-market studies in humans might be suitable depending on the case, to assess the nutritional effectiveness of the nutritionally improved GM crops in cases where alteration by conventional breeding would prompt similar studies.

The nutritional assessment of GM foods should consider: nutrient composition; the biological efficacy of nutrient components in the foods and the assessment of dietary intake and nutritional impact (Varzakas *et al.*, 2007). Snell *et al.* (2012) suggest that the investigation of nutritional equivalence of GM crops to their non-GM counterparts should involve biochemical analysis, histological examination of specific organs, haematology and detection of transgenic DNA.

GM Feed: Flachowsky *et al.* (2005) suggests a case-by-case approach to (long term) feeding trials in order to compare GM-feed with variously supplemented isogenic counterparts. For example assessment of the nutritive value of feed from GM plants with substantial changes to their chemical composition ('novel' trait GM plants, characterised by intended beneficial nutritive properties). Standardised comprehensive test procedures and studies are necessary, such as digestibility and/or bioavailability of nutrients, influence on animal health, welfare and fertility, quality of foods of animal origin and occurrence of unintended or unexpected effects. This paper also proposes that specific animal feeding studies should be conducted with the target species to confirm the expected nutritional properties of the modified crops and their components or co-products depending on the type of modification. This is supported by Domingo *et al.* (2011), who raise doubts as to the relevance of substantial equivalence approach for assessing GM crop suitability for consumption in the diet, since equivalence in nutritional capacity does not substantiate two crops having the same health risk.

Flachowsky *et al.* (2012) suggest that when considering substantial similarity between GM and non-GM-feed, animal feeding studies do not add substantially to the nutritional and safety assessment of GM feed/food. It proposes that specific studies with target animals may contribute more substantially to nutritional assessment of feed and could help safety assessment.

DNA Transfer

GM Feed: El-Sanhoty *et al.* (2006) have investigated the transfer of DNA into animals feeding on GM crops. This paper showed that DNA is partially resistant to mechanical, chemical and enzymatic activities within the rat gastro-intestinal tract (GIT). This was investigated through DNA extraction and purification from organs, digesta of different parts of GIT and faeces of rats. This DNA did not

lead to transgenic gene expression in somatic cells or detectable integration into the germ line of animals when provided orally.

Recombinant plant DNA has not been detected in eggs, or milk of animals fed GM maize or soybean, although DNA fragments of multicopy chloroplast genes could be detected. Animal feeding studies have shown a small amount of fragmented DNA may not be digested. Mammals can take up dietary DNA but stable integration/expression of the DNA has not been shown. The paper discusses how no study has yet conclusively demonstrated the transfer of recombinant DNA from recombinant DNA plants to naturally occurring bacterial/ host cells in the GIT of mammals. Studies investigating traceability of DNA in food and feed use polymerase chain reaction (PCR) based methods.

GM Food: Rizzi *et al.* (2012) also review the persistence and fate of ingested DNA in the GIT, and investigate the possibility of Horizontal Gene Transfer (HGT) to bacteria/ DNA uptake in the host, and potential implications for risk assessment of GM food. The paper mentions studies using *in vitro* simulations of human digestion which have been carried out to evaluate the degradation level of recombinant DNA (from GM soya and GM maize). Recombinant DNA was rapidly degraded when present in a purified form, but no degradation of DNA within GM soya and maize was seen. Similar results came from an in an *ex vivo* rat system simulating the human gut. The paper discusses GM generally and does not necessarily discuss a specific example of GM, rather the concept of gene transfer from GM crops to other organisms more generally. The assessment of the risk of GM plants currently requires the analysis of possible effects of incorporation into human or animal organisms. A method for *in vitro*-digestion has shown that reabsorption processes have a key role in the assessment. The paper discusses how toxicology could be assessed via a novel, highly sensitive in vitro system simulating the transport of substances form the gut to the blood. It detects the risk of incorporation in humans/ animals at an early stage.

Rizzi *et al.* (2012) discuss how a prediction of the fate of dietary DNA is currently unachievable and that the effects must be evaluated on a case-by-case basis. Dietary DNA can affect the exposed mammalian host in many ways, but they state that plant tissues contain less DNA (around 0.6–3 g DNA/ kg dry matter) than animals. The exposure of the GIT to dietary DNA is related to the extent of food processing, food composition, and to the level of intake: some highly refined plant-based food ingredients (starch/vegetable oil) are frequently assumed to not contain DNA and that this assumption is not necessarily always correct. In Europe, measures to restrict the general use of (but not ban completely) Antibiotic Resistance Marker Genes (ARMGs) have been taken. Directive 2001/18/EC (EC, 2001) dictates that GMO's containing ARMGs that could compromise medical treatment will be considered when during the risk assessment of GM plants. EFSA's scientific panel (EFSA, 2004) have classified ARMGs in three groups with recommendations on managing associated risks. This was updated and the 2009 version recognises the need to assess GM case-by-case.

Post market monitoring, exposure assessment and vulnerable groups assessment

Post-Market Monitoring

Hlywka *et al.* (2003) say the PMM aspect of a comparative risk assessment should be used in a standardised way, and implies PMM could, where relevant, be used to look into consumption levels further. The paper discusses how the use of PMM may be appropriate under certain conditions (not routinely without prior evaluation) where a better estimate of dietary exposure and/or nutritional consequence of a food is required. For example, a case where this may be appropriate is when a potential safety issue (allergenicity for example) cannot be addressed fully with pre-market studies.

The authors also consider that PMM may sometimes be necessary to substantiate evidence on dietary intakes of a nutritionally improved food (novel GM trait) with beneficial effects on human health. It is noted that monitoring programmes must be hypothesis-driven, and rely on the availability of accurate

consumption data. They say exposure assessment methods include deterministic and probabilistic estimates of intakes calculated from food supply data, individual dietary and household surveys, or total diet studies. In the development of a monitoring approach, the authors recommend that resource allocation should depend on the desired level of conservatism and the endpoint of interest. They discuss how the cost of monitoring varies greatly and hence determining cause may be limited. Hlywka *et al.* (2003) and Chassy *et al.* (2007) say there is a need to consider levels of consumption which are confirmed by PMM in some cases. As Chassy *et al.* (2007) notes: PMM should not be relied upon to assess safety, as this could both undermine the pre-marketing risk assessment process and decrease public confidence in GM products.

Exposure Assessment

Hammond and Jez (2001) highlight the requirement for exposure assessment for proteins introduced into crop species, whether they are for 'novel' or 'non-novel' traits. The Cry1Ab protein can be readily detected in plant tissues, but not in processed food products, suggesting that it is broken down on food processing. The paper proposes heat treatment during normal food processing can either; reduce, increase or do not affect toxic or allergenic effects of certain proteins introduced to form 'non-novel' traits. Additionally, thermal processing, changes in pH, reducing agents and mechanical shearing will all have an effect on the dietary exposure to functionally active proteins in processed food products, rendering them below levels of any safety concerns. Araya-Quesada *et al.* (2010) support the inclusion of a food processing assessment as part of the risk assessment process.

Lack, (2002), suggests that once the presence of increased levels of a protein has been detected, the hazard and risk assessment should to be made in terms of the quantity of protein that might be consumed in the diet, alongside issues such as protein allergenicity/ toxicity. They note the importance of carrying out assessments in the context of the proposed use of the product and considering dietary exposure. Chassy *et al.* (2007) discuss how pre-market assessment to look at the impact of introducing an improved nutrition ('novel') plant on the nutrient intake of consumers may be appropriate. For example, there could be changes in agricultural practices or in dietary intakes. They discuss the use of additional studies to assess health from higher nutrients

Chassy (2010) discusses how non-novel GM crops such as insect-resistant sweet corn, and 'novel' GM plants such as virus-resistant squash and papaya are intended to be consumed whole by humans. However, the author says the proportion of the diet represented by these crops in industrialized countries is so small, it is hard to see how a change in their composition could adversely affect health/nutrition. The paper discusses the major GM crops in the world (soy, cotton, maize, and canola) saying they are consumed as highly purified ingredients like starch or bleached distilled oils which provide food energy but are not major nutrient sources for consumers.

As well as mentioning animal studies and dietary intake (Xue *et al.* 2012) mentioned that the approach used to establish an acceptable daily intake level for additives and pesticides is based on the definition of a 'no observed adverse effect' level and accounting for uncertainty factors and human variability. This has a long history of use for foreign compounds. A population-distribution approach has been proposed to define the magnitude of any risk at intake values above the guidance value. Other problems the Hellenic Food Safety Authority has identified include: the estimation of average rate of consumption of GM; estimation of future consequences to human health from an increase in the amount of GM food consumed after approval of the GM product; the shortage of epidemiological data for the increased use of different varieties on the effects to human/ animal health as conventional products are replaced by GM; safety limits; research studies to confirm positive effects from the GM food and more research on insert detection and characterisation and confirmation of DNA sequence analysis.

Huggett and Conzelmann, (1997) also support the assessment of the likely degree of exposure to vulnerable groups and the likely processing that would be applied to the GM crop when considering its safety. In the case of a greater predicted dietary exposure, it is thought that the safety program should be made more extensive, so that the assessment is proportionate to the risk.

Stacked traits

De schrijver et al. (2007) examined the safety of stacked traits including two stacked traits which could be non-novel. They reference the industry platform, EuropaBio which has published a document on the evaluation of GM stacked events (EuropaBio, 2005) formulating some guidelines for risk assessment. One-way GM stacked events, where two transgenic traits are combined, and three-way GM stacked events, have been notified for authorisation in the EU. GM stacked events combining more transgenic traits can be expected in the future.

Whether extra data are needed for risk assessment of GM stacked events, has been argued by Advisory Committee on Releases to the Environment (ACRE, 2004) in the UK. If compounds with a synergistic toxic potential for animals and/or humans are combined in the GM stacked event, additional toxicity testing is considered relevant.

De Schrijver *et al.* (2007) summarise that risk assessments for single traits should be taken into account. Also genotypic data required to verify molecular structure of the DNA inserts remains unchanged, and data on expression levels to verify the expression level of the traits remained unchanged should be collected. Additionally, comparative data, including agronomic performance, compositional analysis and wholesomeness studies using broiler chickens or rabbits, was also a requirement for the risk assessment process for stacked traits as part of the EFSA guidelines. For most a 90-day rat feeding trial was instructed, but for a number of cases (i.e. MS8XRF3, NK603XMON810 and 1507XNK603) this not required it as it was determined that sufficient information had been provided to demonstrate the safety of the whole food/feed.

Whether risk assessment for GM stacked events, where each single trait has been separately risk assessed, has to take place, has been argued by ACRE (ACRE, 2004) and De Schrijver *et al.* (2007), as also concluded by a study by RIKILT Institute of Food Safety, Netherlands (Kok *et al.*, 2008). Since non-GM hybrids need no risk assessment, people may query why GM-stacked events do. Risk assessment of GM events could be less exhaustive than single events therefore, when each single event has been positively assessed for its biosafety, forming a platform for further risk assessment Additional information to be collected should both prove the validity of the previous studies as well as providing data proving the safety of the GM stacked event. For example the minimum requirements to be able to extrapolate data from parental GM risk assessments are: evidence of the presence and copy number of the parental inserts, evidence that expression levels are similar in parental and stacked lines, and proof that the insert is conserved during the breeding process (De Schrijver *et al.* 2007)

It is also recommended that agronomic, morphological and compositional studies are carried out on the GM stacked event. This serves to address the potential adverse effects that might result from interbreeding of GM cultivars. These studies will justify that the phenotype and composition of the GM stacked event are equivalent to its comparators. Any changes should be further risk assessed in a similar framework as in a single trait GM risk assessment (De Schrijver *et al.*, 2007), This paper also adds that if compounds have a synergistic activity in humans and/or animals, further toxicity risk assessment tests would be required.

Ridley *et al.* (2011) supports the compositional equivalence of stacked events in GM crops combined through conventional breeding. They suggest therefore that the compositional evaluation of a single trait GM crop is sufficient to determine whether that trait in combination with other GM traits is compositionally equivalent to conventional counterparts.

Risk proportionality: graded approach/ tiered approach

An ILSI publication recommends a tiered, weight-of-evidence approach to safety assessing transgenic proteins. Chassy *et al.*, 2007 state that the assessment of the potential consequences of adopting nutritionally improved crops should balance potential risks and the potential benefits of alleviating nutritional deficiencies in many people: a risk–benefit analysis.

A risk-based classification scheme is suggested to be a more efficient use of testing resources: to focus more on those GM foods of higher concern (Chao *et al.*, 2008). This is supported by Deng *et al.* (2008), who suggest a focus on the degree of risk and impact of different GMOs, so as to develop hazard grades allocated to toxicity, allergenicity, anti-nutrition effects, and unintended effects, so that risk assessment is proportionate to the hazard. The paper demonstrates the feasibility of this hazard grade allocation through the assessment of GM capsicum, modified with the cecropin DB gene that gives resistance to bacterial wilt (*Ralstonia solanacarum*).

Chao and Krewski (2008) conclude that a graded approach offers potential for more efficient use of testing resources by focusing less on lower concern GM foods, both 'novel' and 'non-novel'. Transgenic crops would be risk assessed based on a tiered system depending on type of substance, prior dietary history, estimated exposure level, prior knowledge of toxicity, and the nature of concern related to unintended changes. These tiers have been assigned as below:

TIER 1 (baseline) : 1. Protein gene products (Bioinformatics analysis, Pepsin digestibility, Functional tests) , 2. Protein and non-protein gene products (Physical–chemical analysis, Limited toxicity testing (acute, 14–28 day) Assessment of dietary intakes (tier 1), 3. Unintended Donor organism and host plant (prior knowledge of safety), 4. Genetic material transferred (Molecular characterization), 5. Gene product (prior knowledge and where applicable, experimental evidence of the function and effect or activity in the host plant), 6. Metabolites (biochemical analysis of known toxicants, key nutrients and anti-nutrients endogenous to the host plant), 7. Allergenic effects (protein/gene products), 8. C-intended and unintended (similar tests to those for toxic and anti-nutritional effects).

TIER 2: (depending on type of substance, prior dietary history, estimated exposure level, prior knowledge of activity) 1. Gene Products (assessment of dietary intakes (tier 2), physiochemical-characterisation, metabolism and toxicology), 2. Metabolites (tiered approach to metabolite fingerprinting/profiling- biochemical analysis, animal growth study), 3. Protein gene products (Serum screening for IgE binding; functional assays; immunochemical quantification of endogenous allergenic proteins).

TIER 3: Metabolic fate in plant metabolism of LMW compounds, Digestion/absorption/bioavailability of HMW compounds, organ targeted toxicity of hormonal proteins, immunotoxicity of protein expressed at high levels, synergistic effects among gene products where indicated. Chao and Krewski (2008)

A review carried out by Durham *et al.* (2011) has highlighted issues with the support of increased stringency in the risk assessment process. A flexible 'de minimus' approach has been proposed to address the processing time to take a new GM crop through the risk assessment process. This model would address risk proportionality by emphasising a trait-based risk assessment which would minimise regulatory bottlenecks for crops which are determined to be of lower risk, and add precaution to GM crops for which it is inferred there is higher risk. This review also supports the use of evaluating the endpoint trait, not the generative methodology, when considering the safety of a GM crop.

Cranor (2004) discusses the U.S. regulatory experience with chemical substances to provide some background for locating the strengths and weaknesses of different legal structure - pre-market screening structure. It is a "tiered" legal structure with quite minimal review for plants that, on the basis of submitted evidence, appear to pose no risks to the environment or other crops and more elaborate review of transgenic plants that pose greater risks. This structure thus burdens regulation differentially, depending upon the properties of the plants involved. Although there is a place for a comparatively quick review of transgenic plants when there is good evidence that they will not pose problems, the existing procedures appear to have features that undermine the goals of the existing regulatory structure. Moreover, there appears to be a need for better scientific and regulatory guidance to demarcate which transgenic plants should be routed through the notification procedure and which should be screened under the permit process. A too rapid a review by too few scientists with no external checks on the quality and rigor of the review undermines the pre-market and protective aims of the current regulatory structure. The existing regulatory structure at the USDA for transgenic plants, although generically a pre-market structure and holding the promise of better detecting risks that can arise than would post-market laws, appears to be in need of improvement in order to assess and identify the range of risks that could easily arise from transgenic plants.

Cockburn *et al.* (2002) suggest that further international integration of the food and feed safety assessment procedure must be applied in order to ensure international agreement of food safety.

Statistical analysis

Herman *et al.* (2010) comment on the comparative approach and explain equivalence limits, or the values at which a difference between two compositional variables is small enough to be considered insignificant, or pose no safety risk, or are a poor model for comparing transgenic crops with an array of reference crop varieties. They suggest an alternate model (statistical approach), analogous to that used in clinical medicine, where reference intervals are constructed for a healthy heterogeneous population, which are developed using previous results of safe levels experiments, or safe levels of intake values, to act as a frame of reference for compositional variables. Specifically, they advocate the use of distribution-free tolerance intervals calculated across a large amount of publicly available compositional data such as is found in the ILSI Crop Composition Database. The use of tolerance intervals using appropriate sample sizes, and covering many varieties and environments, represents a valid statistical approach for assessing the composition of transgenic crops in relation to their conventional counterparts.

Ward *et al.* (2011) discuss the comparative approach and the role of statistics. They say the statistical methodology that EFSA expects an applicant to adopt when making a GM crop regulatory submission, includes proposed methodology with inclusion of reference varieties in the experimental design to provide a measure of natural variation amongst commercially grown crops. They say that while taking proper account of natural variation amongst commercial varieties in the safety assessment of GM plants makes good sense, (Ward *et al.* 2011) the methodology described by the authors is shown to be flawed and cannot be considered fit for purpose currently. They say there is failure to take proper account of interactions in the statistical model. Dijk *et al.* (2010) also suggest that the current statistical tests which could be used to detect differences between compositional or transcriptomic variables might lead to a high false discovery rate due to multiplicity errors within the univariate statistical test system. This drawback might mean that novel ways of testing differences should be designed,

Hothorn *et al.* (2006) say the common approach for demonstrating safety of novel foods (by SE) is problematic if done by revealing a non-significant p value for all or most nutritional components. They say the concluding something is safe based on a non-significant t test can be biased, for example: if the variance is high and measurements are missing. Even with the use of the additional criterion for "biological relevance," being within a tolerance interval (estimated from analysis of commercial

lines/database normal ranges) still does not overcome the issues. They say by fixing the less important false positive error rate to the common a = 0.05 quite different component-specific false negative rates result in this proof of hazard approach, which are of higher priority in demonstrating safety. They suggest defining an international sample size requirement for field trials. However, different designs (block, competently randomized or split plot design) and experimental heterogeneity between locations, will influence the false negative rate. The paper notes the appeal of the proof of hazard: non-significant p values, effect consistency between years, regions, additional controls and the normal ranges does not ensure a component-specific control of some level of the important false negative error rate. They note that without defining safety thresholds, no proof of safety with a primary control of the more important false negative rate is possible. Thresholds are suggested to be defined as absolute or scale-variant by testing differences between GM and conventional varieties, or as percentage change by the ratio to control (the authors say the best approach appears to be to define component-specific safety ranges proportional to the component-specific variance of the non-GM control grown in the same field trials). They suggest that the calculation of safety ranges should be based on a multi-site analysis and say the scenario is in line with the requirements of regulatory bodies.

Kuiper *et al.* (2002) suggest that statistical analysis and the interpretation of differences in the composition of GM foods compared to traditional counterparts should be further developed.

C. LITERATURE REVIEW RESULTS- RISK ASSESSMENT METHODS TAKEN BY INTERNATIONAL RISK ASSESSMENT BODIES

The following sections detail risk assessment methods that are currently used by international risk assessment bodies for GM plants with 'novel' traits.

European Union

Risk assessments for food and feed safety of GM plants for the EU are carried out by the European Food Safety Authority (EFSA) on behalf of all 27 member states. The main principles of the process are in line with the guidelines put forward by the Codex. This process is discussed further at intervals throughout this report; however food and feed risk assessment follows 4 principal steps (see EFSA, 2011 for more details):

a) Hazard Identification

Hazard identification is the identification of hazards that should be the subject of further tests, and classification throughout the risk assessment process. Principally it is any biological, chemical, and physical agents that may cause adverse health effects and are present in the GM plant. Hazard identification focuses on identifying differences between the GM plant, and a comparator, by comparing compositional, agronomic and phenotypic characteristics into account. The identification of any differences determine the additional studies that may be required throughout the assessment.

b) Hazard characterisation

Hazard characterisation is the qualitative and/or quantitative evaluation of the nature of adverse health effects identified at hazard identification stage. Hazard characterisation attempts to quantify potential toxicological and/or nutritional effects of the identified differences.

c) Exposure assessment

Exposure assessment aims to quantify the likely exposure of humans and animals to food and feed derived from GM plants. This will document the nature and size of the populations exposed to food and feed derived from GM plants, and the magnitude, frequency and duration of such exposure.

d) Risk characterisation

Based on the previous 3 steps of risk assessment, risk characterisation is the qualitative and/or quantitative estimation of the probability of occurrence and severity of known or potential adverse health effects. At this stage, more information may be required to complete the overall risk characterisation, otherwise this step should illustrate whether the identification and characterisation of hazards is deemed to be complete.

USA

Risk assessments of GM food and feed safety in the USA are carried out by the Food and Drug Administration (FDA). GM plants are regulated within the same framework as plants derived from traditional plant breeding. The FDA follows a standard procedure to address safety principles which have some characteristics in common with those of conventionally bred crops. For recombinant DNA modified crops, the assessment scheme follows the basic outline of addressing characteristics of the new plant variety, based on a) characteristics of the host plant, b) donor species, c) the nature of the genetic change, d) the identity and function of the newly introduced substances, and e) unexpected effects that accompany the genetic change.

The risk assessment also focuses on the following considerations:

The potential presence of toxicants known to be characteristics of the host and donor species;

The concentration and bioavailability of important nutrients for which the crop is ordinarily consumed;

The safety and nutritional value of newly introduced proteins; and

The identity, composition and nutritional value of modified carbohydrates, fats and oils

The scientific concepts applied to any GM crop currently follow the concept of substantial equivalence as applied by OECD. The potential effects of food processing are considered at each stage in the safety assessment process.

Faust, (2002) discusses the comparative approach used in the USA to risk assess plants differing in composition ('novel' traits) and/or intermediate compounds and other potential nutritional value, for example canola with high laurate or incorporating the phytase enzyme, and soybeans with high oleic acid. Experimental data needed to carry out this process are clearly defined by a decision-tree, which specifies the information and protocol requirements for risk assessment of "novel" foods in the USA.

The risk assessment procedure in USA

The risk assessment procedure in the USA follows a distinct path of risk assessment protocols which can be summarised as below (for more information see FDA, 1992). This attempts to define both the characteristics of the host plant, and the donor plant, as well as assessing the safety of the introduced trait and any unintended effects that result from it. A comparative assessment is used to determine any unintended effects of both 'novel' and 'non-novel' trait GM crops. Furthermore, a case-by-case approach is used to determine whether feeding trials are appropriate to ensure the full safety verification of an individual GM crop.

a) ***Characteristics of the host plant***

This considers: taxonomy, other species that have previously contributed to the genetic information of the host, the history of safe use, the extent of previous experience, the part of the plant used as food, the presence of potentially harmful constituents (toxicants and anti-nutrients), typical methods of processing and impact of processing on reduction/ enhancement of effects from potentially harmful constituents, and the identity and level of nutrients for which the food is consumed.

b) ***Characteristics of donor plant***

This considers: taxonomy, other species that have previously contributed to the genetic information of the donor, the history of safe use, the part of the donor used as food and the presence of potentially harmful constituents (toxicants and anti-nutrients).

c) ***Nature of the genetic change***

This considers: association of transferred genetic material with harmful constituents (toxicants, anti-nutrients and allergens), the history and deviance from molecular constructs, known activities of regulatory sequences and the presence of extraneous open reading frames.

d) ***Identity and function of the newly introduced substance***

The safety assessment of the substances introduced should address the specific risk associated with that substance. Therefore the assessment will vary depending on the substance altered:

Protein – should be based on: the presence and level in food product, origin, known or suspected allergenicity, evidence of consumption in other foods at similar levels and under similar conditions, the effects of processing, biological function, known or potential toxicity, chemical differences and similarities to edible proteins, and the presence of host specific post-translational modifications;

Carbohydrate – should be based on the nature of carbohydrate or modification; or

Fats and Oils – should be based on the composition of the fat/oil and the presence of any unusual components at levels that would cause safety concern.

Toxicology feeding studies (or other toxicological tests) are encouraged only when the characteristics of the plant, or the nature of the modification, raise safety concerns that cannot be resolved by analytical methods. The FDA recognises that animal feeding studies with whole foods have limited sensitivity and hence encourage companies to consult informally with the agency about test protocols. The FDA also considers the stability of inheritance of the transgene.

f***) Stacked traits generally receive no additional evaluation***, when single traits have been recognised by the FDA as safe.

Japan

The structure of the risk assessment procedure in Japan has been investigated using the risk assessment documents describing the procedure used to risk assess five separate 'novel' trait GM crops. These GM crops include High Oleic Acid Soybean (DD-Ø26ØØ5-3), Mavera™ Maize (high lysine, REN–ØØØ38-3), Mavera™ YieldGard Maize (high lysine and insect resistant, REN–ØØØ38-3 x MON–ØØ81Ø6), modified fatty acid soybean (Event 305423, DP-3Ø5423-1), modified thermostable alpha-amylase, insect resistant and herbicide-tolerant maize (SYN-E3272-5 X SYN-BTØ11-1 X SYN-IR6Ø4-5 X MON- ØØØ21-9)[15]. These risk assessment procedures are defined to assess the safety of crops for provision as both food and feed, as well as to address the processing, storage, transportation, and disposal acts that are applicable to them.

The risk assessment procedure in Japan

Risk assessment procedures in Japan follow a formulaic set of protocols which assess, as they do in USA, information about the host crop, the donor crop and differences between the GM and a conventional counterpart, via the comparative approach. Despite similarities to the USA risk assessment process, Japan does not consider the type of trait added when addressing risk assessment protocols for a specific GM crop. Therefore no specialist approach is taken in terms of risk assessment when considering the type of trait added. The protocol for risk assessment is as follows:

a) **Information concerning preparation of the GMO**

Information on the GM trait, and its potential use as food or feed.

b) **Information concerning donor nucleic acid**

The composition and origins of component elements and the function of component elements are fully assessed, including characterisation of the sequence, characterisation of the proteins produced and characterisation of any metabolic system changes that will occur as a result of transgene insertion. For example this included profiling of soybean storage proteins for assessment of metabolic system changes for a modified fatty acid event.

c) **Information concerning vectors**

This considers the name, origin and properties of the vector (characterisation of sequence, function of sequence and presence or absence of infectivity of vector), to include a characterisation of the method of GMO preparation (such as transferring the nucleic acid, rearing of the crop).

d) **State of existence and stability of nucleic acid**

The genetic location where the replication product exists, the number of copies of replication product and stability of inheritance, the position relationship in the case of multiple copies existing in chromosomes, and the stability of expression under natural conditions.

e) **Methods of detection and identification of GMO**

Information on how the specific GM line may be detected in the food chain, it's sensitivity and reproducibility.

[15] Full text risk assessments of these crops as assessed by Japan are held on the Biosafety Clearing House databases, http://bch.cbd.int/database/organisms/ (by searching for the individual GMO).

f) **Difference between GMO and host organism**

This considers physiological or ecological characteristics such as: total novel compound content, unintended effects in metabolite concentrations and nutritional safety of the whole food (feeding study in broiler chickens to test identified 'problem' compounds). It also considers the differences between a conventional counterpart in morphological and growth characteristics.

e) **Stacked traits**

To address issues involved with trait stacking they consider: pathway-specific activity of proteins, differential enzyme activity and independent function within the recipient organism. ELISA can ensure lack of protein interaction, and efficacy of each trait is analysed in field trials. For example the stacked Mavera™ YieldGuard maize was assessed by inferring that LY038 and Cry1Ab function independently from each other, i.e. do not interact in a metabolic pathway or the metabolic system of the recipient organism. Therefore it was assumed that these proteins to not affect each others activity, and confirmed by testing free concentration of lysine, and the efficacy of insect resistance.

Canada

The regulatory body responsible for food and feed risk assessment of GM plants is Health Canada, who implement the 'Novel Foods Regulation', where a novel food can include those derived from biotechnology, amongst other 'novel foods' such as those that do not have a history of safe use of food (see Health Canada, 2006; B.28.001 for the complete list). Approval of GM products for feed is carried out by the Canadian Food Inspection Agency. The risk assessment is a product based system, and assessment is triggered by new characteristics of a product, rather than the process used to obtain this product (i.e. genetic modification). In this guise, just because a plant has been genetically modified, does not necessarily make it eligible for a full risk assessment.

Food safety assessment

Food safety assessment by Health Canada involves the evaluation of foods relative to conventional counterparts, taking both intended and unintended effects into account. As with the EFSA risk assessment processes, any hazards identified at this stage would be assessed to determine its relevance to human health. The evaluation involves an assessment of:

Genetic modification considerations

Description and characterisation of the introduced trait if achieved using genetic modification

History of use

History of both donor and host organism is used to provide information about the likely toxin production under normal manufacturing conditions,

Dietary exposure

A full exposure assessment is carried out only when the change that has taken place is large enough. In all cases therefore the magnitude of the change should be assessed against the expected nutritional value of the unchanged food. Where there is an intentional nutritional or health-related modification, the impact of how it will be promoted would also be considered,

Nutritional consideration

This investigates unintended nutritional effects, alongside substitution of food ingredients of significant nutritive value with less nutritious ingredients, excessive intake of nutrients or bioactive substances, and new of increased levels of anti-nutrients in the diet. Nutrient bioavailability is considered. An evaluation of intended nutritional modifications is also required,

Toxicology considerations

To demonstrate that there are no known or unknown substances which pose a safety risk,

Allergenicity considerations

The assessment of the potential for foods containing novel proteins to cross-react with known food allergens or to lead to the development of *de novo* hypersensitivity,

Chemical considerations

The identification of chemical contaminants is reported on.

Where no conventional counterpart is available, the novel food is evaluated using data taken from historical experiences of experimental studies.

The role the food will play in the diet can be used to focus the risk assessment on the nutritional implications of the food.

The nutritional considerations take in to account a broad range of issues with assessing the nutritional value of GM crops including; substitution of foods and food ingredients of significant nutritive value with less nutritious components, excessive intakes of nutrients or other bioactive substances because of greatly increased levels in the novel food, or new/increased levels of anti-nutrients that can have implications for the nutritional value of the food. Decisions about the nutritional quality of the food will be made based on nutrient intake recommendations, the role of the food in the diet of the population, and their role of diet and nutrition in reducing the risk of developing diet related disease and health promotion. Furthermore the food is subject to higher levels of analysis if unexpected nutritional changes have occurred as a result of the transformation. If a novel food has no history of safe use, then the composition of the food is studied in order to determine its potential role in the diet.

Feed safety assessment

A standardised process is used to formally identify any risks that the GM crop may pose to livestock nutrition.

The procedure used in Canada to risk assess ‘novel’ trait GM crops addresses the novel trait, the unintended changes that result from the transformation and the nutritional value of the GM crop. It does not specifically assess safety issues regarding the host or donor organism, as seen in most other country risk assessment procedures. Approaches taken to ensure nutritional value and food and feed safety are carried out using the comparative approach. Each GM crop is compared to a conventional counterpart. All risk assessment procedures include a feeding trial to ensure the nutritional value or ‘bioefficacy’ of the GM crop, and few elements of the protocol for this are applied on a case-by-case basis. The procedure addresses the introduced protein in its *in planta* processed state.

a) Identification of novel traits

To describe the novel trait, and verify the stable integration of the gene into the plant's genome,

b) Potential impact on livestock nutrition

This considers nutritional equivalence of the GMO to traditional soybean (amino acid profile, concentration of fibre, minerals etc.) and is assessed within tolerance levels,

c) Safety of introduced substances

Broiler studies are used to assess degradation in the animal's liver and impacts on bird performance or health, with comparison to a conventional counterpart,

d) Presence of anti-nutritional factors and unintended changes in secondary metabolites

As compared to a conventional counterpart,

e) Bioefficacy

17 day broiler feeding trials with poultry are used to assess: performance, carcass quality, meat composition and chick and adult mortality, by comparing with a conventional counterpart. Trials with dairy cattle and swine are also used to assess performance and milk fatty acid level differences between GMO and a conventional counterpart.

f) Potential impact on livestock and workers/bystanders

This considers toxicity and allergenicity using: acute oral toxicity studies, simulated gastric fluid digestion studies, bioinformatics studies - of biologically relevant structural or immunological similarities - and poultry feeding trials with a vastly overestimated dose,

g) Nutritional Equivalence

This considers differences in nutritional value between GMO and traditional soybean using a 42 day broiler feeding study to assess performance, mortality, weight gain, feed efficiency and organ and carcass yield differences between those fed with GMO and those with conventional counterpart,

h) Processing of proteins

This considers glycosylation of the transgenic protein.

Philippines

The risk assessment procedure applied to GM plants in the Philippines has been investigated using the documents describing the risk assessment procedure for Mavera™ Maize (high lysine, REN-ØØØ38-3), and Mavera™ YieldGuard Maize (high lysine and insect resistance)[16]. This risk assessment procedure assesses whether the trait added is already part of the diet, and therefore has a history of safe use, to inform the risk assessment procedure for that trait. The comparative approach is used to verify a safe, nutritional composition. This nutritional assessment incorporates an assessment of the anti-nutritional characteristics of the crop that may be appropriate due to the nature of the host or the trait. The elements of the risk assessment as described below are carried out on a case-by-case basis.

The risk assessment procedure in the Philippines

a) **Identification of novel traits**

Considers a description of GM traits, and stable integration into a plant's genome.

b) **History of safe use**

This considers whether a protein/ substance is naturally present in feed and food of other crop varieties.

c) **Nutritional composition**

Comparative analysis of transgenic and conventional counterpart of compositional information.

d) **Anti-nutrition characteristics**

This considers the levels of anti-nutrients (e.g. phytic acid and raffinose in high lysine Mavera™ Maize).

e) **Stacked traits**

This considers efficacy performance only with comparison to the single traits and no further safety assessments are carried out.

[16] Full text risk assessments of these crops as assessed by Japan are held on the Biosafety Clearing House databases, http://bch.cbd.int/database/organisms/ (by searching for the individual GMO).

China

There is limited information on risk assessment of GM plants in China, however a few authors discuss the process in broad terms.

Deng *et al.* (2008) discuss the Ministry of Agriculture (MOA) and Ministry of Health regulations in China. Here, unintended effects are ranked as an important index in the safety assessment of GM Plants. There are suggestions that there needs to be an outlined path by which unintended effects are studied to prevent GM safety being questioned.

Zarrilli (2005) talks of criticism of GM crops including food-safety aspects, which led to changes in the Chinese legal framework on agro-biotechnology. In 2001, the Government enacted a framework: Regulation on the Safety Control of Agricultural GMOs, to protect human/animal health. Implementing regulations were issued on three areas including Biosafety Evaluation which established procedures for handling applications for GM cultivation and set up an advisory body (the Biosafety Committee) and a decision-making body (the Biosafety Administration Office) under the MOA to handle applications. Applicants must provide information on risk assessment and GMOs are classified into four classes depending on their potential for safety concerns.

Australia and New Zealand

Risk assessment strategies are governed by FSANZ (Food Standards Australia New Zealand) in these countries. The risk assessment approach is based on OECD guidelines, and all information (apart from a small amount of commercial in confidence data) is made publicly available. It is a similar approach to the ACNFP. There are two rounds of public consultation during the assessment of applications before an opinion is finalised. FSANZ do not commission their own scientific studies; it is the responsibility companies who develop GM foods to demonstrate its safety and provide raw data. Where no established history of safe human consumption can be documented, a cautious approach is used.

The risk assessment procedure in Australia and New Zealand

Brent *et al.* (2011) describe the safety assessment undertaken by FSANZ to evaluate new GM crops. They evaluate according to five main principles. These include:

Safety assessments use scientific, risk based methods, taking data from a variety of sources (applicant, literature, general technical information, independent scientists, regulatory information and international bodies);

Safety assessments are conducted on a case-by-case basis, taking a cautious approach to assess components of the individual GM varieties used as food or in food preparation. Other characteristics of the food, such as the levels of nutrients and naturally occurring allergens, toxins and anti-nutrients are also considered in details;

New genetic material, new proteins and other characteristics of the GM food are considered. The new genetic material is considered separately and completely, and other new characteristics of the food are assessed in detail;

Intended and unintended effects of the genetic modifications are analysed. When appropriate, comparisons are made to conventionally produced foods, with a history of safe use. The comparisons are usually made to verify differences in levels of allergens, toxins, nutrients and anti-nutrients. Significant differences observed between a GM crop and a conventional counterpart are used to assign compounds for assessment for adverse health effects; and

Where appropriate comparisons are made to conventionally produced foods with a history of safe use. This is used to identify any differences in the levels of naturally occurring allergens, toxins, nutrients and anti-nutrients, with any significant differences assessed for their adverse health effects.

Brent *et al.* (2011) also discuss further issues considered by the assessment body, including:

a) **Nature and stability of genetic modification**

A description and molecular characterisation of the genetic modification identifying the relevant parameters requiring assessment in the new food.

b) **General safety issues**

History, extent of use, nature and level of expression of ay novel protein in the GM plant, and potential for DNA-transfer.

c) **Toxicology and allergenicity**

Consideration is given to the similarity of the novel protein to any known toxins, and the results of any toxicity tests and likely human exposure to the novel protein. For allergenicity, the assessment considers whether transfer of allergens during genetic modification may make foods previously considered non-allergenic, to become allergenic. In addition the assessment considers whether expression of a novel protein in a food may lead to the development of a new allergy in certain individuals. Currently, animal feeding studies are not routinely required by FSANZ.

d) **Nutritional assessment**

This aspect of the risk assessment aims to establish whether the food is nutritionally adequate and will support typical growth and well-being. In most cases this can be achieved by understanding the genetic modification, together with an extensive compositional analysis. Where compositional analysis indicates significant differences in number of important components or nutrients or concern about bio-availability of key nutrients animal feeding studies may be carried out.

In Australia, the OGTR (Office of the Gene Technology Regulator) oversees development and release of GMO under the Gene Technology Act 2000. Licenses for dealing with GMOs will not be issued unless the OGTR thinks any risks can be managed. In New Zealand, the Environmental Protection Authority performs a similar role under the Hazardous Substances and New Organisms (HSNO) Act 1996. When a GMO will be used in food, FSANZ will determine its safety for consumption. GM foods are regulated under Standard 1.5.2 – Food produced using Gene Technology, contained in the Australia New Zealand Food Standards Code (the Code). The standard has mandatory pre-market approval and labelling requirements.

Argentina

Burachik (2010) discusses the Argentinian regulatory bodies the National Advisory Commission on Agricultural Biotechnology (CONABIA), and the Technical Advisory Committee on GM Organisms Use (CTAUOGM). Applications are submitted to the Ministry of Agriculture for analysis and assessment on a case-by-case basis by the Ministry, its regulatory and advisory bodies, and other areas of the government. At the level of both field trials and commercial approvals, the assessments are science-based. The ultimate aim of the process is to ensure the crop is safe with regard to many aspects including food, feed and processing. The applicant must submit information on the GM crop including: phenotypic expression; a description of the agronomic practices; potential changes in the geographical zones (if different from the non-GM counterpart), and the molecular genetic characterisation. Up to 2008, 1511 applications for field trials and related regulated activities had been assessed.

Early regulations for the biosafety assessment of GM crops in Argentina were similar to those of the EU and the US. Argentina has since modified the regulations to take into account new scientific knowledge/developments and incorporate its own understanding of biotechnology and biosafety. Burachik (2010) describes the process as flexible, rational and scientific. Pre-commercial tests (involving extensive sowing) are used as a second phase in the regulatory process in Argentina. After field trials, the pre-commercial tests are carried out to perform local-specific tests and collect material/data for regulatory purposes.

AHTEG Guidance

The Ad Hoc Technical Expert Group on Risk Assessment and Risk Management are an expert group established by Parties to the Cartagena Protocol on Biosafety[17], which provide technical expertise in specific aspects of risk assessment and risk management. Their first meeting, held in April 2009, discusses the development of guidance documents to support countries in protocols for conducting risk assessment of GM crops.

AHTEG have produced guidance on the risk assessment of living modified organisms, to include both environmental release assessment, and an assessment of the implications to human health. They have discussed how to further support countries in conducting risk assessments of GMOs. The finalisation of the document "Guidance on Risk Assessment of Living Modified Organisms" (AHTEG, 2010) included a section on living modified organisms with stacked genes or traits.

The guidance provides proposals for the risk assessment of stacked traits, stating that the level of re-examination of the stacked traits should vary case-by-case but should always take into account the results of the parental GMO risk assessment. They suggest that there should be a level of revaluation of the molecular sequence at the insertion site, and the presence of the full sequence of each transgene could be confirmative to the molecular characteristics of the parental GMOs, but may also be a basis for assessing the potential adverse effects on human health. Furthermore, phenotypic characteristics, including the levels of expression of any introduced gene products or modified traits, compared to the parent GMOs and to relevant non-modified recipient organisms (plants), should be considered. Information can be further collected based on the nature of the combined traits. For example introduced proteins that might be more likely to interact should be further investigated for combinatorial effects such as increased toxicity or allergenicity.

[17] The Cartagena Protocol is an international agreement established under the UN Convention on Biological Diversity to protect biological diversity from the potential risks posed by modern biotechnology, see http://bch.cbd.int/protocol/background/ for more details.

D. GM PLANTS WITH 'NOVEL' TRAITS THAT ARE ENVISAGED TO REQUEST MARKET AUTHORISATION

COMPLEX INPUT TRAITS									
Crop	**Name**	**Trait Type***	**I/A or O/Q †**	**Genes Added**	**Effect**	**Event Name**	**Breeder**	**Approval Status**	**Source**
Apple	*Malus domestica*	BST	I/A	Type 1 alpha-hordothionine precursor	Resistance to fungi	Multiple	Plant Research International	Short/medium term	BCH
Bananna	*Musa sp.*	BST	I/A	Osmotin gene, ß-1,3-glucanase, chitinase	Resistance to fungi	Not classified	Centro de Ingenieria Genetica y Biotecnologia	Short/medium term	BCH
Chicory	*Cichorium intybus*	AST	I/A	Additional copy of SST gene (inulin expression)	Re-synthesis of fructan after a frost period: frost tolerance	None	Plant Research International	Short/medium term. Risk assessed for field trial in Netherlands (2004)	BCH
Maize	*Zea mays*	AST	I/A	Shock Protein (CopB from *Bascillus subtulis) and NptII* from Tn5 of *E. coli*	Drought tolerance	MON 87640	Monsanto	Short/medium term- applied for authorisation	BCH
Papaya	*Carica papaya*	AST	I/A	Papaya ringspot virus coat protein	Papaya ringspot virus resistance	55-1/63-1	Cornell University	Short/medium term. Approved for food in Canada and environmental and food and/or feed in the USA	CERA
Papaya	*Carica papaya*	AST	I/A	Coat protein encoding sequences from PRSV isolate H1K with thymidine inserted to yield frameshift	Papaya ringspot virus resistance	X17-2	University of Florida	Short/medium term. Approved for environmental (2009) and food and/or feed (2008)	CERA
Pea	*Pisum sativum*	AST	I/A	Pea Enation Mosaic Virus or Pea Seed-borne Mosaic Virus coat protein	Pea Enation Mosaic virus or Pea Seed-bourne Mosaic virus resistance	pWell05, pWell08, pWell07	Agritec Plant Research	Long term. Risk assessed in Czech Republic (2011) for field trial	BCH
Plum	*Prunus domestica*	AST	I/A	Plum pox virus (PPV) coat protein	Plum pox virus (PPV) resistance	C5	USDA Agrivultural Research Service	Short/medium term. Approved in the USA for environmental (2007) and food and/or feed (2009)	CERA
Potato	*Solanum tuberosum*	AST	I/A	Rpi-blb1 and Rpi-blb2 genes from *Solanum bulbocastanum*	*Phytophthora infestans* resistance	VCPMA16/19	BASF	Long term. Environmental risk assessment in Czech Republic (2012) and the Netherlands (2006)	BCH
Potato	*Solanum tuberosum*	AST	I/A	Potato Virus Y coat protein	Potato Virus Y resistance	SY230	Tecnoplant (Argentina)	Long term	JRC
Potato	*Solanum tuberosum*	AST	I/A	Stomatal density and distribution 1 (sdd1) was silenced via RNAi	Heat tolerance and increased yield	StSDDhpi	Max Planck Institute for Molecular Plant Physiology	Short/medium: Germany approved import/use with conditions	BCH
Potato	*Solanum tuberosum*	AST	I/A	Stomatal density and distribution 1 gene	Drought tolerance	pBinAR-StSDD1	Max-Plank Institue of Molecular Plant Physiology	Short/medium term. Risk assessed for import/use and environmental in Germany (2010)	BCH
Potato	*Solanum tuberosum*	AST	I/A	Potato leaf roll virus, ORF1 and 2	Potato leaf roll virus resistance	New Leaf: RBMT21-350	Monsanto	Canada, Mexico, Phillipines, Republic of Korea (BCH) (risk assessment)	BCH
Potato	*Solanum tuberosum*	BST	I/A	Osmotin gene, ß-1,3-glucanase, chitinase	Resistance to fungi	Not classified	Centro de Ingenieria Genetica y Biotecnologia	Short/medium term	BCH
Rice	*Oryza sativa*	BST	I/A	Osmotin gene, ß-1,3-glucanase and chitinase	Increased resistance to fungi	Multiple	Centro de Ingenieria Genetica y Biotecnologia	Short/medium term	BCH
Squash	*Curcurbita pepo*	AST	I/A	Cucumber mosaic, zucchini yellows mosaic, watermelon mosaic viral coat proteins	Viral resistance	CZW-3/ ZW20	Asgrow (USA); Seminis Vegetable Inc. (Canada); Monsanto	Short/medium term. Risk assessed in Canada for food (1998) and the USA for environmental (1996) and food and/or feed (1994)	CERA
Sugar Beet	*Beta vulgaris*	AST	I/A	RZM gene	Beet necrotic yellow vein virus resistance	SBVR111	Syngenta	Short/medium term. Approved in Spain for import use (2012). Risk assessed for environmental in Czech Republic (2011) and Spain (2012)	BCH
Sugar Cane	*Saccharum sp.*	BST	I/A	Osmotin gene, ß-1,3-glucanase, chitinase	Resistance to fungi	Not classified	Centro de Ingenieria Genetica y Biotecnologia	Short/medium term	BCH

OUTPUT TRAITS

Crop	Name	Trait Type*	I/A or O/Q †	Genes Added	Effect	Event Name	Breeder	Approval Status	Source
Argentine Rapeseed	*Brassica napus*	CM	O/Q	Inserting 12:0 ACP Thioesterase encoding gene from California bay laurel (*Umberllularia californica*)	High laurate and myristate	23-18-17, 23-198	Monsanto	Canada for environmental, food and feed (1996) and the USA for environmental and food and/or feed (1994)	CERA
Argentine Rapeseed	*Brassica napus*	CM	O/Q	Fatty Acid Desaturase mutant	Modified seed fatty acid content (high oleic acid, low linolenic acid)	45A37, 46A40	Pioneer Hi-Bred	Short/medium term	CERA
Argentine Rapeseed	*Brassica napus*	CM	O/Q	Chemical mutagenesis of FAD2 gene	Modified seed fatty acid content (high oliec acid)	46A12, 46A16	Pioneer Hi-Bred	Short/medium term. Risk assessed in Canada for food (1996)	CERA
Barley	*Hordeum vulgare*	CM	O/Q	Endosperm-specific expression of sucrose transporter HvSUT1	Protein content	HOSUT-lines	Institut für Pflanzengenetik und Kulturpflanzenforschung	Short/medium term	BCH
Linseed	*Linum usitatissimum*	CM	O/Q	Fatty acid desaturase (FAD2) inactivation by RNAi	Increased oleic acid in linseed oil	Not classified	Agritec Plant Research	Long term. Risk assessed in the Czech Republic for field trial (2012)	BCH
Linseed	*Linum usitatissimum*	CM	O/Q	D6-Elongase from *Physcomitrella patens* , D6- and D5-desaturase from *Phaeodactylum tricornutum*	Fatty Acid Composition	Multiple	Plant Science Sweden and BASF	Sweden	BCH
Maize	*Zea mays*	CM	O/Q	Phytase production 3-phytase enzyme from *Aspergillus niger*	Increased breakdown of plant phytases which bind phosphorus	Multiple	BASF	Long term	ISAAA
Maize	*Zea mays*	CM	O/Q	Phytase production (phyA2) from *Aspergillus niger*	Increased breakdown of plant phytases which bind phosphorus	BVLA430101	Origin Agritech	Short/medium term. Authorised for cultivation in China (2009)	ISAAA
Maize	*Zea mays*	CM	O/Q	Phytase	Increased breakdown of plant phytases which bind phosphorus	NutriDense	BASF	Possible commercialisation 2015	JRC
Maize	*Zea mays*	CM	O/Q	cordapA gene dihydrodipicolinate synthase from *Corynebacterium glutamicum*	High Lysine (grain)	Mavera Maize: LY038	Monsanto	USA (2005)	CERA
Maize	*Zea mays*	CM	O/Q	cordoaA, Cry1Ab	High Lysine (grain) and Insect Resistance (Eurpoean cork borer)	YieldGuard Maize: MON810 X LY038	Monsanto	Short/medium term: Japan (2007)	CERA
Maize	*Zea mays*	DM	O/Q	Gibberelin 20 Oxidase 1 gene from *Arabidopsis thaliana*	Altered Growth	GA20OX1	Departement Planten Systeembiologie	Short/medium term	BCH
Melon	*Cucumis melo L.*	CM	O/Q	S-adenosylmethionine hydrolase (*E.coli* bacteriophde T3) (sam-k), neomycin phosphotransferase II	Delayed ripening by introduction of gene resulting in degradation of ethylene precursor	Cantaloupe A,B	Agritope Inc.	USA (1999)	CERA

OUTPUT TRAITS

Crop	Name	Trait Type*	I/A or O/Q †	Genes Added	Effect	Event Name	Breeder	Approval Status	Source
Oilseed rape	*Brassica napus*	CM	O/Q	phyA to produce a fungal 3-phytase	Increased breakdown of plant phytates which bind phosphorous - improved phosphorous availability	Multiple	BASF	Long term. Risk assessed in the USA for import/use (2004)	BCH
Oilseed rape	*Brassica napus*	CM	O/Q	Acyl-acyl carrier thioesterase (CIFatB4)	Modifed fatty acid content	NBM99-CIFatB4	BAZ, Institut für landwirtschaftliche Kulturen	Long term. Risk assessed Germany, environmental (2010)	BCH
Oilseed rape	*Brassica napus*	CM	O/Q	Stilbene synthase	Synthesis of antioxidant/flavnoids - resveratrol (antifungal agent)	pPSty5	FINAB	Long term. Risk assessed Germany, environmental (2011)	BCH
Oilseed rape	*Brassica napus*	CM	O/Q	UPD-glucose:sinapate glucosyltransferase	Reduced concentration of sinapine (an antinutritive agent)	pLH-BnSGT-GUS	FINAB	Long term. Risk assessed Germany, environmental (2011)	BCH
Oilseed rape	*Brassica napus*	CM	O/Q	Thioesterase (*Ulbellularia californica*), neomycin phosphotransferase (*E. coli*)	Modified seed fatty acid content (high laurate levels and myristic acid production)	23-18-17, 23-198	Monsanto	USA (1994)	CERA
Oilseed rape	*Brassica napus*	CM	O/Q	Fatty acid desaturase FAD*2*	Modified seed fatty acid content (high oleic acid, low linolenic acid)	45A37, 46A40, 46A12, 46A16	Pioneer Hi-Bred	Canada (1996)	CERA
Pea	*Pisum sativum*	CM	O/Q	Vfaap1 gene from *Vicia faba* for amino acid permease	Protein content	Not classified	Institute for Plant Genetics and Crop Plant Research, Germany	Short/medium term	BCH
Potato	*Solanum tuberosum*	CM		Cell wall invertase inhibitor	Reduction in cold-induced sweetening	Nt-Inhh, ilR-INV	n/a (India)	Short/medium term	BCH
Potato	*Solanum tuberosum*	CM	O/Q	Granule bound starch synthase gene (gbss), acetohydroxy acid synthase	Altered Composition	Multiple	BASF	Long term. Environmental risk assessment in Czech Republic (2012) and Germany (2011)	BCH
Potato	*Solanum tuberosum*	CM	O/Q	Reduced expresison of Zeaxanthin epoxidase	Altered colour, accumulation of zeaxanthin secondary	Clone SR 47/00#18	TU Munchen	Germany risk assessment	BCH
Potato	*Solanum tuberosum*	CM	O/Q	Granule-bound starch synthase (*gbss*)	Altered starch composition, increased amylopectin to	BPS-25271-9	BASF Plant Science	Authorised in the EU for food and feed (2010)	CERA
Potato	*Solanum tuberosum*	CM	O/Q	Granule-bound starch synthase (*gbss*) antisense	Altered Composition	AM04-1020	BASF	Nearing application	GMO Compass
Potato	*Solanum tuberosum*	CM	O/Q	Granule-bound starch synthase genes	Starch content	EH92-527-1	BASF	Nearing application	GMO Compass
Potato	*Solanum tuberosum*	CM	O/Q	Granule-bound starch synthase gene in anti-sense	Strach content; reduction in amylose fraction	AV43-6-G7	BASF	Nearing application - risk assessment in Netherlands	BCH

OUTPUT TRAITS

Crop	Name	Trait Type*	I/A or O/Q †	Genes Added	Effect	Event Name	Breeder	Approval Status	Source
Potato	*Solanum tuberosum*	CM	O/Q	Granule bound starch synthase gene	Increased levels of amylopectin	Multiple	BASF	Germany risk assessment	BCH
Potato	*Solanum tuberosum*	CM	O/Q	Granule bound starch synthase gene in antisense	Starch content: amylose free	Multiple	BASF	Germany risk assessment	BCH
Potato	*Solanum tuberosum*	CM	O/Q	Branching enzyme 1 and 2	Altered starch composition, increased amylopectin to amylose ratio	pHAS3	BASF	Germany risk assessment	BCH
Potato	*Solanum tuberosum*	CM	O/Q	Granule-bound stach synthase- truncated antisense RNA interference	Increased amylopectin to amylose ratio	EH92-527-1	BASF	Long term	BCH
Potato	*Solanum tuberosum*	CM	O/Q	RNAi of the apyrase gene	ß-carotene content	B33-apy1-RNAi 1331	Max-Plank Institue of Molecular Plant Physiology	Long term	BCH
Rice	*Oryza sativa*	CM	O/Q	psy (phytoene synthase) from *Narcissus pseudonarcissus* , crtI from *Erwinia uredovora* , lyc (lycopene cyclase)	ß-carotene content	Golden Rice 1	IRRI (Phillipines)	Long term	JRC
Rice	*Oryza sativa*	CM	O/Q	psy (phytoene synthase) from *Narcissus pseudonarcissus* , crtI from *Erwinia uredovora*	ß-carotene content	Golden Rice 2	IRRI (Phillipines)	Long term	JRC
Rice	*Oryza sativa*	CM	O/Q	Cryj I and Cryk II (cedar pollen antigen proteins)	Antigens for cerdar pollen allergy	7Crp10	National Institute of Agrobiological Sciences (NIAS)	Long term. Risk assessed Japan (2008)	BCH
Sorghum	*Sorghum bicolor*	CM	O/Q	rbcS transit peptide, Alcohol dehydrogenase, phytoene synthase 1, low phytic acid 1, phytoene desaturase 1 (fragments or full genes)	Vitamin A (beta-carotene), zinc and iron modification	ABS188	Pioneer Hi-Bred	Long term. Risk assessed in Burkina Fasso (2012)	BCH
Soybean	*Glycine max*	CM	O/Q	Segments of endogenous FAD2-1A gene	Modified fatty acid profile	MON 87705	Monsanto	Long term. Risk assessed in the Republic of Korea (2013)	BCH
Soybean	*Glycine max*	CM	O/Q	Delta(12)-fatty acid dehydrogenase, acetolactate synthase	Modified seed fatty acid content (high oleic acid, low linolenic acid)	DP-305423-1	Pioneer Hi-Bred	USA (2009)	CERA
Soybean	*Glycine max*	CM	O/Q	Deltra(12)-fatty acid dehydrogenase *(GmFad2*), beta-D-glucuronidase (*gus*), beta lactamase (*bla*)	Modified seed fatty acid content (high oleic acid)	DD-02005-3	DuPont, Canada	USA (1997)	CERA
Soybean	*Glycine max*	CM	O/Q	fan1	Modified seed fatty acid content (low linolenic acid)	OT96-15	Agriculture & Agrifood Canada	Canada (2001)	CERA
Soybean	*Glycine max*	CM	O/Q	FAD3 from red mould *Neurospora crassa*	Altered fatty acid profile	MON 87769	Monsanto	Short/medium term. Risk assessed in Australia, Canada, Mexico, New Zealand and the USA for food (2011) (and feed/cultivation in some cases)	ISAAA
Soybean	*Glycine max*	CM	O/Q	Delta(12)-fatty acid dehydrogenase	Modified seed fatty acid content (high oleic acid)	260-05 (G94-1, G94-19, G198), 305423	Pioneer Hi-Bred and DuPont	Australia, Japan, USA	BCH

OUTPUT TRAITS

Crop	Name	Trait Type*	I/A or O/Q †	Genes Added	Effect	Event Name	Breeder	Approval Status	Source
Soybean	*Glycine max*	CM	O/Q	Additional copies of portion of omega-6-desaturase encoding gene FAD2-1, therefore silencing of omega-6 desaturase gene	Modified seed fatty acid content (high oleic acid)	DP-305423	Pioneer Hi-Bred	Risk assessed in Australia, Canada, Mexico and the USA (upto 2010)	CERA
Soybean	*Glycine max*	CM	O/Q	Additional copy of omega-6-desaturase encoding gene FAD2-1, therefore silencing of omega-6 desaturase gene	Modified seed fatty acid content (high oleic acid)	G94-1, G94-19, G168	DuPont Canada	Short/medium term. Risk assessed in Canada, Australia, Japan and the USA (upto 2000)	CERA
Tomato	*Lycopersicon esculentum*	DM	O/Q	Aminocyclopropane cyclase (ACC), neomycin phosphotransferase II	Increased shelf life (delayed ripening) - reduced ethylene accumulationthrough truncates aminocyclopropane cyclase	1345-4	DNA Plant Technology Corporation	USA (1994)	CERA
Tomato	*Lycopersicon esculentum*	DM	O/Q	S-adenosylmethionine hydrolase (*E.coli* bacteriophde T3) (sam-k), neomycin phosphotransferase II	Delayed ripening by introduction of gene resulting in degradation of ethylene precursor	35 1 N	Agritope Inc.	USA (1996)	CERA
Tomato	*Lycopersicon esculentum*	DM	O/Q	polygalacturonase (PG), neomycin phosphotransferase II	Delayed softening through suppression of polygalacturonase activity	B, Da, F	Zeneca Seeds	USA (1994)	CERA
Tomato	*Lycopersicon esculentum*	DM	O/Q	polygalacturonase (PG), neomycin phosphotransferase II	Delayed softening through suppression of polygalacturonase activity	FLAVR SAVR; CGN-89564-2	Monsanto	USA (1994)	CERA
Tomato	*Lycopersicon esculentum*	DM	O/Q	1-amino-cyclopropane-1-caboxylic acid daminase (ACCd) : 1-amino-cyclopropane-1-caboxylic acid daminase (ACCd), hydrolyses ethylene precursor	Delayed ripening	8338	Monsanto	USA (1994)	CERA
Wheat	*Triticum aestivum*	CM	O/Q	Endosperm-specific expression of sucrose transporter HvSUT1	Protein content	HOSUT wheat	Institut für Pflanzengenetik und Kulturpflanzenforschung	Germany	BCH
Wheat	*Triticum aestivum*	CM	O/Q	Endosperm specific expression of amino acid permease 1 VfAAp1 (likely to exhibit early flowering)	Protein content	XAP Wheat	Institut für Pflanzengenetik und Kulturpflanzenforschung	Germany	BCH
Wheat	*Triticum aestivum*	CM	O/Q	Endosperm specific expression or amino acid permease VfAAp1 (likely to exhibit early flowering)	Protein content and herbicide tolerance	SUTAP60	Institut für Pflanzengenetik und Kulturpflanzenforschung	Germany	BCH

Summary of Identified 'Non-Novel' GM Input Traits

Crop	Name	Trait Type	Specific Trait	Gene Added	Event Name	Breeder	Approval
Maize	*Zea mays*	Herbicide tolerance	Glyphosate tolerance	EPSPS gene	Roundup Ready; NK603	Dow AgroSciences, Pioneer Hi-Bred	50 approvals in 22 countries + EU-27
Maize	*Zea mays*	Insect resistance	Resistance to European corn borer	Cry1Ab gene	YieldGuard; MON810, NatureGard; Bt176	Monsanto	47 approvals in 22 countries + EU-27
Maize	*Zea mays*	Insect resistance	Resistance to European corn borer	Cry1F gene from *Bacillus thuringiensis*	Herculex I; TC1507	Dow AgroSciences, Pioneer Hi-Bred	
Maize	*Zea mays*	Insect resistance	Lepidoptera resistance	Cry1Ac gene from *Bacillus thuringiensis*	DBT418		
Maize	*Zea mays*	Insect resistance	Lepidoptera resistance	Cry9C gene from *Bacillus thuringiensis*	Starlink; CBH-351		
Maize	*Zea mays*	Resistance to corn root worm	Lepidoptera resistance	Cry3Bb1 gene from *Bacillus*	Rootworm; MON863	Monsanto	
Maize	*Zea mays*	Insect resistance	Lepidoptera resistance	Vegetative insecticidal protein 3Aa20 (Bt)	MIR162	Syngenta	43 approvals in 20 countries + EU-27
Oilseed rape	*Brassica napus*	Herbicide tolerance	Glyphosate tolerance	EPSPS gene *Arabidopsis tumefaciens*	ZSR500	Monsanto	

Crop	Name	Trait Type	Specific Trait	Gene Added	Event Name	Breeder	Approval
Oilseed rape	*Brassica napus*	Herbicide tolerance	Gluphosinate tolerance	phosphinothricin-N-acetyltransferase (PAT)	Falcon; GS20/90pHoe6/Ac		
Soybean	*Glycine max*	Herbicide tolerance	Gluphosinate tolerance	phosphinothricin-N-acetyltransferase (PAT)	W98/ Liberty Link	Bayer CropScience	
Soybean	*Glycine max*	Herbicide tolerance	Dicamba herbicide tolerance	dicamba monooxygenase gene	MON87708		
Soybean	*Glycine max*	Insect resistance	Lepidoptera resistance	Cry1Ac from *Bacillus thuringiensis*	MON87701	Monsanto	
Soybean	*Glycine max*	Herbicide tolerance	Glyphosate tolerance	5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)	GTS-40-3-2	Monsanto	48 approvals in 24 countries + EU-27
Rice	*Oryza sativa*	Herbicide tolerance	Gluphosinate tolerance	phosphinothricin-N-acetyltransferase (PAT)	Liberty Link		
Rice	*Oryza sativa*	Insect resistance	Lepidoptera resistance	Cry1A from *Bacillus thuringiensis*	Multiple	Centro de Ingenieria Genetica y Biotecnologia	

Crop	Name	Trait Type	Specific Trait	Gene Added	Event Name	Breeder	Approval
Potato	*Solanum tuberosum*	Insect resistance	Lepidoptera resistance	Cry3A from *Bacillus thuringiensis*	New Leaf; BT16	Monsanto	
Alfalfa	*Medicago sativa*	Herbicide tolerance	Glyphosate tolerance	EPSPS gene from *Arabidopsis tumefaciens*	Roundup Ready; J101	Monsanto	
Tomato	*Solanum lycopersicum*	Insect resistance	Lepidoptera resistance	Cry1A from *Bacillus thuringiensis*	5345	Monsanto	
Chicory	*Cichorium intybus*	Herbicide tolerance	Gluphosinate tolerance	phosphinothricin-N-acetyltransferase (PAT)	RM3-3	Bejo Zaden BV	
Sugar Beet	*Beta vulgaris L.*	Herbicide tolerance	Glyphosate tolerance	EPSPS gene from *Arabidopsis tumefaciens*	InVigor; GTSB77	Monsanto	
Wheat	*Triticum aestivum*	Herbicide tolerance	Glyphosate tolerance	EPSPS gene from *Arabidopsis tumefaciens*	Roundup Ready; MON-71800	Monsanto	
Linseed	*Linum usitatissimum*	Herbicide tolerance	Phosphinotricine resistance	Bacterial *bar* gene	Not classified	Agritec Plant Research	
Linseed	*Linum usitatissimum*	Insect resistance	Insect tolerance	SPI-2 gene encoding inhibitor proteases	Not classified	Agritec Plant Research	

Appendix D Summarising Identified 'Novel' GM traits including:

1. Alterations to concentration of storage compounds or nutritional content (e.g. starch in tubers/ sucrose in fruit/ fatty acid in seed);

2. Introduction of 'foreign' storage compound(s);

3. Physiological/ morphological change to plant, e.g. changes to protein or metabolite abundance to alter plant processes; and

4. Alterations in metabolite concentrations to enable plant to tolerate stresses such as frost, cold, salt etc. (This is not an exhaustive list.)

*Abiotic Stress Tolerance/Biotic Stress Tolerance/ Compositional Modification/ Developmental Modification (AST/BST/CM/DM)

† Input/Agricultural or Output/Quality