CUMULATIVE LONG-TERM EFFECTS OF GENETICALLY MODIFIED (GM) CROPS ON HUMAN/ANIMAL HEALTH AND THE ENVIRONMENT: RISK ASSESSMENT METHODOLOGIES REFERENCE: NO 07-0402/2005/414455/MAR/B4

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EXECUTIVE SUMMARY

1. The scientific evidence for long-term and cumulative effects of GM plant cultivation was reviewed. Currently available risk assessment methodologies were also reviewed and gaps in knowledge identified. Tools and techniques to fill these gaps have been identified, drawing on experience of risk assessment in other domains. Case studies were used to test and develop the risk assessment approaches, and to develop examples of monitoring and management measures.

2. Some areas of potential long-term risks and benefits were identified from the scientific literature but in fact there have been few comprehensive studies carried out in the field over the long-term that directly address long-term and cumulative effects.

3. Risk assessment methodologies for release of GMOs generally follow a case-by-case approach based on a scientific framework of assessment of hazards, potential for exposure, consequences of exposure and options for risk management. Many of these risk assessment frameworks do require that cumulative long-term effects are considered, however these assessments naturally tend to focus on identifying and monitoring effects of the GMO that can be anticipated, based on scientific evidence. Risk assessment methodologies currently in use probably do not easily lend themselves to the much broader, conceptual requirements of assessing unanticipated, long-term cumulative effects of the release of GM crops.

4. A workshop was held at CSL on 31 October 2005. A group of specialists attended the workshop and scoped different potential scenarios for long-term and cumulative risks from GM crops. Not all the risks identified in the workshop will necessarily be significant in practice, neither will they all be relevant to any particular release. Rather, together with the findings of the literature review, they show the range of possible risk types that assessment methodologies will need to be able to identify and evaluate.

5. Current approaches to risk assessment were identified and reviewed by contact with those involved in assessment of GM releases in the EU. A questionnaire was circulated to the competent authorities (CAs) of the Member States and other contacts supplied by DG Environment. A summary of their responses is given in the report, illustrating the diversity of approaches across the EU.

6. A risk assessment framework was developed and tested using case studies taken from GM plants in the pipeline for EU approval. The case studies were also used to develop examples of risk management strategies for monitoring and mitigation.

7. The risk assessment framework has been designed to be used by the CAs and to be made available to applicants. Consideration will need to be given to ensuring that the effort that it requires is proportionate to the effort required for the current environmental risk assessment (e.r.a), and to the level of risk identified. GM plants present inherently lower risk scenarios than, for example, medical products. GM crops, their products and derived products are traded commodities therefore, acceptability to the European Commission, WTO etc, will also need to be considered. If companies are asked to undertake a very lengthy risk assessment process for cumulative long-term risks, it may

not gain acceptance by them or by CAs and run the risk of not being addressed at all. If the long-term risk assessments were to be undertaken at a higher EU level on a more generic basis, then consideration of balancing these costs may become less important. Also, some thought will be needed as to how the framework will fit with current 'short-term' e.r.a.s, and any implications for these as the framework proposed in this report is more detailed than the guidance currently given. More work will therefore be required on the framework and its application before it can be used within the current EU regulatory system.

8. Areas where further work will be needed to improve the framework or fill gaps in our knowledge are as follows:

- **Obtaining Baseline Data.** There is a need for good baseline data if monitoring is to be able to detect changes. A study is required to advise on the best indicators of long-term/ cumulative changes. There are also institutional and other practical aspects of implementation to be considered. For example, who should carry out the baselining, and how would it relate to ecological baselining for other purposes?

- **Ranking the Risks of Generic Crop-Trait combinations.** It could be more cost-effective, for society as a whole, if some risk assessment work were to be done at a higher level than the release-specific ERAs. If certain crop-trait combinations (or other types of scenario) could be ranked in broad order of inherent risk, this would help both regulator and industry to ensure proportionate levels of depth and detail in risk assessments. It might also lead regulators to issue guidance indicating any broad classes of combinations that would be unlikely to be authorised, preventing wasted effort by all parties. The assessment framework could be applied in order to establish whether certain crop-trait combinations are inherently riskier than others.

- **Introgression into and Ecology of wild relatives.** Currently, the mechanisms of introgression into wild relatives are not understood in sufficient detail to enable the likelihood of introgression to be determined with confidence. Neither is there, in general, a good enough understanding of the ecology of wild relatives, and hence of how undesirable traits might spread in the environment. Research is required to improve the understanding of introgression and of the ecology of wild relatives, in sufficient detail for risk assessment.

- **Improved hazard identification for long-term and cumulative effects.** It is difficult to identify hazards that are fundamentally different from those that can occur in the short-term. In most cases, the long-term and cumulative hazards identified in this study were the same as the short-term ones, although the degree of risk associated with the hazard could be greater - given a longer time or more widespread planting, the likelihood and extent of undesirable effects may increase. Nevertheless, some genuinely different hazards can be identified. These tend to arise from rather subtle and complex combinations of factors, which would only be manifested in the long-term or as a result of widespread releases and plantings. The process of identifying long-term and cumulative risks could be made more efficient and effective if standard hazard-identification techniques (brainstorming, failure analyses, HHA etc) could be enhanced to focus more quickly on such genuinely long-term and cumulative effects.

- **Tolerability Criteria.** The absence of explicit criteria in the Directive for judging whether a risk is tolerable, and the apparent variations in practice between Competent Authorities, point to a need to develop some more explicit guidance on criteria that will encourage well-founded, consistent and fair decisions. There is a need to develop justifiable criteria for tolerable risk, and guidance on their application.

- **Tracking, Combining and Monitoring Cumulative Risks.** In considering cumulative risks, the EC will need mechanisms to track and combine the risk assessments from multiple, and in some cases apparently unrelated, releases. There is a need to explore the scientific and institutional issues involved in tracking multiple release assessments and consents and to develop guidance for regulators. The study could consider both scientific aspects (how to identify any potential for cumulative risk) and institutional aspects (processes and procedures, IT issues)**.**

- **Stakeholder Engagement in Risk Assessment.** In other controversial debates about risk and the environment, there has been a growing realisation of the importance of considering social and ethical aspects. Stakeholder participation in framing the question and defining assessment methods is being given serious consideration and has occurred in some cases, in addition to the more traditional model, in which stakeholders review and challenge assessment results and assertions. Risk assessment should be used to provide a structured framework for discourse and decision-making under irreducible uncertainty, rather than as a technical and scientific exercise alone. Deliberative processes have been used in other domains, and this experience could be adapted and applied to the GM domain.

CONTENTS

1. Introduction

1.1. Background to the Study

Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms (GMOs) entered into force on 17th April 2001. The deadline for transposition of the Directive into national laws was 17th October 2002, ensuring full applicability of its provisions.

According to the Directive, each application for deliberate release of a GMO into the environment for research purposes (Part B releases) or for placing on the market (Part C releases) must contain a comprehensive environmental risk assessment (e.r.a.). The e.r.a. must be performed according to the principles as laid down under Annex II of the Directive.

The objective of the e.r.a. is, on a case by case basis, to identify and evaluate potential adverse effects, either direct or indirect, immediate or delayed, relating to the release of a GMO in terms of human health and the environment. The e.r.a. should be conducted with a view to identifying if there is a need for risk management to be associated with the release and if so, the most appropriate measures to be used. A general principle of the e.r.a is that analysis of the 'cumulative long-term effects' should also be carried out.

On 24th July 2002 the Commission adopted a Decision establishing guidance notes supplementing Annex II of the Directive 2001/18/EC. EFSA published its draft guidance document on risk assessment in April 2004.

The guidance provides Competent Authorities and applicants with general principles to be followed with respect to the objectives, elements, general principles and methodology of the e.r.a. General guidance has been provided by the competent authorities, e.g. UK Defra guidance provided by ACRE in 2001. However, the guidance to date has concentrated on the concept of 10 year consents and not examined the longer-term problems which may arise if GM crops are grown on a large scale basis in many EU countries over many decades, or if many different types of Part B releases occur over a long period of time in one area. More specific guidance on risk assessment, and potential risk control measures is now required with regard to the potential cumulative long-term effects arising from GM crops on human /animal health and the environment.

'Cumulative long-term effects' refer to the accumulated effects of consents on human/animal health and the environment, including *inter alia* flora and fauna, soil fertility, soil degradation of organic material, the feed/food chain, biological diversity, animal health and resistance problems in relation to antibiotics. The availability of GM crops may also cause effects on all the above due to wider agricultural, land use and socio-economic changes.

'Cumulative' could be interpreted in terms of (a) the effect of many genes/traits in the crop proposed for release, (b) the cumulative effect on health or the environment of multiple releases over time, and/or (c) the effect of extremely large scale cultivation of GM crops across many EU countries.

Long-term effects cannot always be anticipated /identified in the e.r.a. Therefore, appropriate measures such as case-specific and/or general surveillance monitoring plans can help in detecting these effects. There is also a requirement to be able to reverse any effects detected by management of the risks identified.

1. 2. Objectives

- To collect and collate existing information/studies demonstrating the existence or potential for long-term effects of GM crops.

- To assess and document the adequacy of existing risk assessment methodologies/ protocols to account for the above effects from different crops and to identify possible gaps/lack of knowledge and where further research is required.

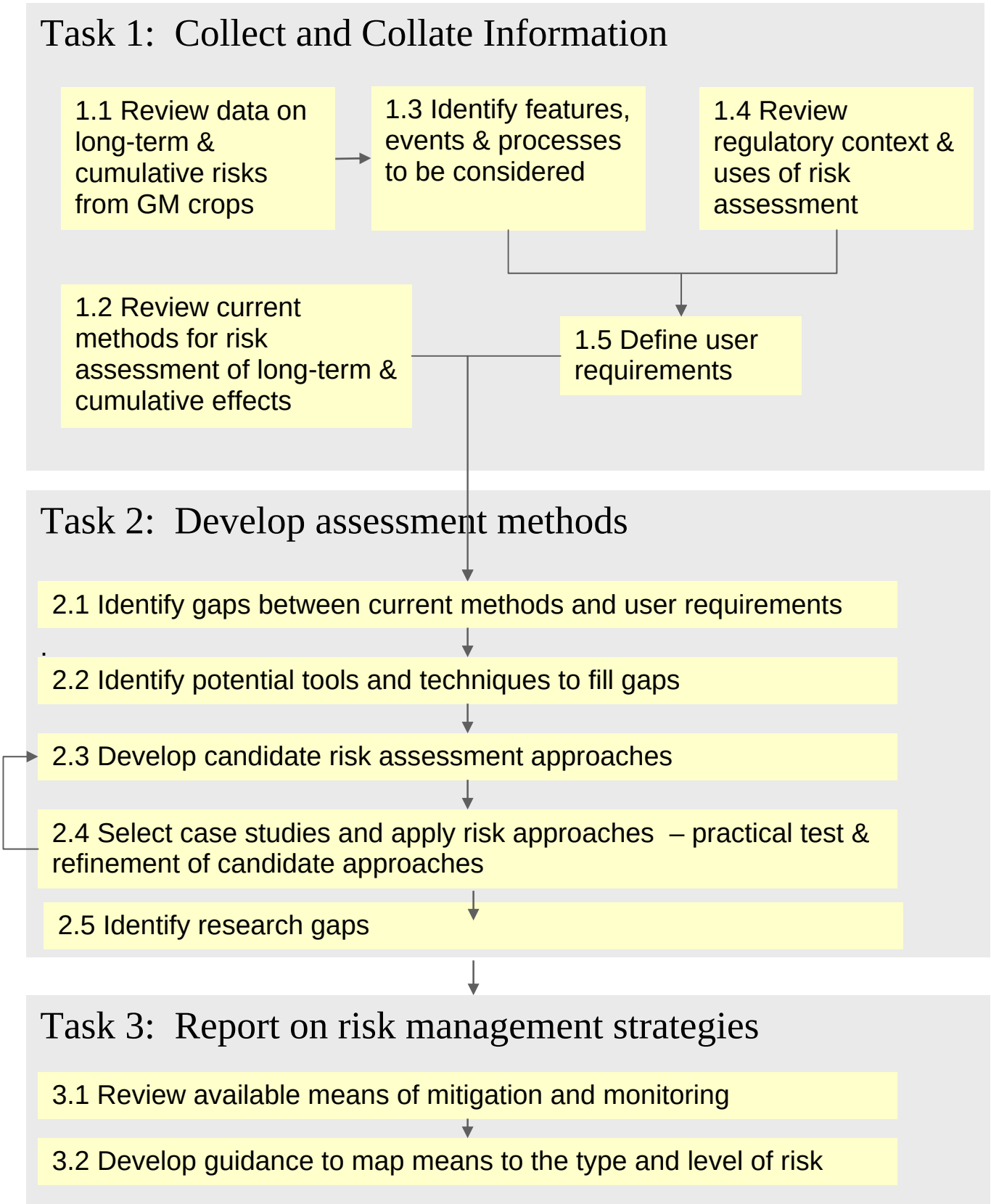
- To develop specific methodology in terms of risk assessment, with associated risk assessment criteria (e.g. specific indicators, etc), for the potential cumulative long-term effects from individual groups of GM crops (e.g. species) and for different transgenic phenotypes (e.g. herbicide tolerance, insect tolerance).

- To indicate, as appropriate, the types and extent of risk management measures (including monitoring activities) required to address potential long-term effects.

1.3. Methodology

Figure 1 provides an overview of the project methodology. The project will review the scientific evidence for long-term cumulative effects of GM plant cultivation. Currently available risk assessment methodologies will be reviewed and any gaps in knowledge will be identified. Tools and techniques to fill these gaps will be identified, drawing on experience of (long-term) risk assessment in other domains, and approaches that are appropriate to the GM crops domain will be developed. Case studies will be used to test and develop the risk assessment approaches, and methods to define monitoring and management measures. Risk management plans will be outlined to cope with the risks from a range of current and likely future GM releases within the Community.

Figure 1. Overview of Methodology



2. Collection of Existing Information on Cumulative Long-term Effects of GM Plants

Field releases of genetically modified organisms (GMOs) have grown enormously since the first field trial was held in 1986 (OECD, 1993). Field trial approvals almost doubled every year between 1988 and 1994 and reached a peak in 1998 at 2,312. During the nine-year period 1996 to 2005, the global area of biotech crops increased more than 47 fold, from 1.7 million hectares in 1996 to 90.0 million hectares in 2005 (ISAAA, 2005). Table 1 below shows global plantings of GM crops worldwide.

Table 1: Global plantings of GM crops in 2004 and 2005 (Source: ISAAA)

GM crop plantings (million ha)			
Country	**2004**	**% change**	**2005**
USA	47.6	+4.6	49.8
Brazil	162	+5.6	17.1
Canada	5.0	+88.0	9.4
China	5.4	+7.4	5.8
Paraguay	3.7	-10.8	3.3
India	1.2	+50.00	1.8
South Africa	0.5	>100	1.3
Uruguay	0.5	0	0.5
Australia	0.3	0	0.3
Mexico	0.2	+50.0	0.3
Romania	0.1	0	0.1
Philippines	0.1	0	0.1
Spain	0.1	0	0.1
Colombia	<0.1	0	0.1
Iran	-	-	<0.1
Honduras	<0.1-	-	<0.1
Portugal	-	-	<0.1
Germany	-<0.1	-	<0.1
France	-	-	<0.1
Czech Republic	-	-	<0.1
Total	81.0	+11.1	90.0

To date, the vast majority of releases involve GM plants; bacteria, fungi, viruses and animals account for only 1.0%, 0.1%, 0.3% and 0.2% of releases respectively (OECD, 2000a). Worldwide, the principal GM crops are soybean, maize and cotton, most of these contain a single transgene that modifies the plant for herbicide tolerance and or insect resistance, although plantings of twin trait (herbicide resistant and insect tolerant) cotton and maize was reported in 2004 (James, 2004). GM plants with traits that influence virus resistance, crop quality, male sterility and disease resistance are less common, but it is reasonable to expect increasing developments in these areas in the future.

2.1. Risk assessment: definition and requirements

Environmental risk assessment is the practice of determining the nature and likelihood of effects of human actions on animals, plants and the natural environment; it requires estimating the probability of harm to plant and animal life, and to ecosystem integrity (ERMA, 2004). Many environmental risks fall into the low probability and high consequence category which means that they cannot easily be compared with other types of risk; environmental risks may also have long lead times between cause and effect, with the possibility of irreversible outcomes. Risk assessment of GMOs aims to identify and assess all risks that could result in harm to human health or the environment due to the proposed dealings with the GMO.

Risk assessment comprises three main steps, firstly hazard identification, which involves analysis of what, how, where and when something could go wrong and the causal pathway leading to that adverse outcome. Hazard identification is arguably the most important component of any risk assessment; failure to correctly identify hazards will be carried through the assessment process with the likely outcome that risk will be underestimated. Tools and techniques are available to assist in identification of hazards, these are mostly deductive with the most commonly cited approaches being comparison with previous experience, brainstorming and checklists, however there is interest in the use of inductive hazard identification techniques in connection with ecological risk assessment (e.g. Hayes 2004). Hazard identification is followed by consideration of the likelihood of an adverse outcome and the severity of that outcome, or the consequences – if it happened would it be a problem. In making these assessment, aspects such as the possible severity of the hazard, the likely spatial and temporal extent, reversibility and possible cumulative impacts must be taken into consideration. The final stage is risk estimation in which the probability that the potential harm would be realized is determined; the risk estimate is a combination of the likelihood and consequences of an adverse outcome and assessment of likely interactions between the two, it should also incorporate consideration of uncertainty. Risk estimation is considered in the context of the ease with which management and mitigation options may be implemented and how effective they would be.

Risk estimations can be qualitative, semi-quantitative or quantitative, and if completed in a systematic and rigorous manner should be a valuable aid to decision making. There is no universally applicable procedure for conducting ecological risk assessment, indeed a multiplicity of techniques and methods are available (Hayes, 2003). This is due in part to the relative immaturity of ecological risk assessment as a discipline, but also in part to the complexity of environmental management issues and the variety of possible stressors and measurement endpoints, and thus the widely different types of assessment that are required. In the years since GM crops have been entered into trials and, more recently, placed on the market, there has been an increasing recognition of the importance of a robust, transparent and statistically sound approach to risk assessment that can be applied to a range of crop / environment scenarios, and which enables decision makers to assess GM crops or products in the context of prevailing policy, consumer and economic considerations. To achieve this, the scope and context of the risk assessment must be clearly defined, including the characteristics of the receiving environment. In addition, appropriate baselines against which to compare the properties and characteristics of the GM crop and the unmodified equivalent must be established.

Uncertainty analysis

Regardless of whether qualitative or quantitative risk assessment is used, it must be based on evidence. Biological systems are complex and environmental risk assessments will inevitably be faced with considerable uncertainty both in terms of variability of data and lack of information. Analysis of these uncertainties is a critical component of ecological risk assessment; it distinguishes risk assessment from impact assessment and promotes transparency and credibility, and leads to improved decision-making (Hayes 2003). In qualitative analysis, 'linguistic uncertainty' is particularly prominent since terms such as "low risk" for example are routinely used without reference to exposure, but with sufficient time or number of "trials", low risk events may be more or less certain. Contextual uncertainty can occur in the spatial and temporal components of the risk assessment and in its scope, resolution and boundaries. Linguistic and contextual uncertainty can be reduced by carefully defining the terms of reference and language in the risk assessment, but ultimately they can only be eliminated mathematically. Epistemic uncertainty reflects our limited knowledge of ecological systems; it occurs as measurement error – random (resulting from imperfect measuring devices) and systematic (resulting from bias), natural variation, model error, subjective judgement and ignorance. Ignorance, model error and measurement error are often collectively known as incertitude because they can be reduced with empirical effort. Random measurement error is minimised by taking additional measurements; systematic measurement error is minimised by careful experimental design and instrument calibration; natural variability cannot be reduced with empirical effort but can be described mathematically. Risk assessment models will also introduce uncertainty (Ferson and Ginzberg, 1996). When considering cumulative long-term impacts of GM crops our level of uncertainty is further increased, hence increasing the importance of transparent uncertainty analysis.

Cumulative and long-term effects

The potential long-term effects of GM crops on (agro)-biodiversity are often considered a major concern. However, a central problem is to define the term 'long-term' (van der Meer, 1993); it may vary from months to decades or centuries, depending on the organisms, environments and genes involved. To assess any potential unintended negative impact, information from over 10 to 100 generations of a species may be required (Kasanmoentalib, 1996). An authorisation to place a GM crop on the market is generally given for 10 years; however Conner *et al* (2003) estimate that, based on available data to date, it will take decades for current GM crops to have appreciable ecological consequences, if any, on a single agricultural site, and that it will take centuries for any appreciable ecological consequences, if they exist, to occur on a more global scale. Potential cumulative effects might be expected to occur more rapidly if the same GM crop is grow repeatedly within a specified area, however, identification of cumulative effects at an early stage will be dependent on monitoring programmes, and in the absence of these, growers'; observations. Hill *et al,* 2003 suggested that with GMOs (as with chemicals), poor data and confounding factors generally make it difficult to detect ecologically meaningful relationships, even where they do exist, and that the possibility of missing any effects is large because the statistical power to detect significant relationships is often low. Clearly, the availability of statistically useful relevant baseline data for use as a comparator for detection of changes in ecological trends will be a key requirement for interpretation of long-term monitoring data. In the USA where GM crops are most widely grown, there is no such requirement for deliberate monitoring of GM crops grown on a commercial scale, although any adverse or unanticipated effects must be reported to the regulatory authority. In Europe the requirement and framework for post market monitoring is

in place, but as the scale of cultivation of GM crops is limited to date, very little data has been gathered and it is unclear how monitoring arrangements will work in practice.

2.2. A review of evidence available from the scientific literature

The scientific literature was reviewed to identify any evidence that (1) effects are known to occur as a result of the insertion of a GM construct into plants, (2) effects have been recorded as the result of current long-term, large scale releases, (3) any unanticipated potential effects have been identified. The review concentrated on environmental effects, allergenicity effects of GM plants on the human population and adverse effects on animal health resulting from the use of antibiotic resistance genes in constructs.

Effects can result from:
Direct impacts (What the crop is): impacts which arise as a result of the modifications to the crop itself. These may include the effects on human health, gene flow, unintended effects on non-target species, and impacts on soils.
Indirect impacts (How the crop is grown): impacts arising from the effects of GM crops management on the environment. These include changes to farming inputs, farm management practices such as tillage, and broader impacts on the water resources and pollution, and farming landscape.
What the crop is used for: impacts arising from the development of new crops which may grown to replace non-renewable energy sources; have health benefits (such as enhanced vitamin content); or be used to make pharmaceuticals.

Summary of scientific evidence for risks associated with GM crops
A large number of papers have been published reviewing the anticipated effects of GM crops. Some experimental studies have been carried out in the laboratory or glasshouse. These are useful in providing indicators of possible risks but are often criticised as being artificial and not resembling conditions encountered in the field.

The number of intensive studies carried out under field conditions has been small and they have generally been relatively short –term. Even the UK Farm Scale Evaluation (FSE) trials were only carried out over 3-4 years (possibly 6 years if the post release monitoring period is included). The largest scale releases of GM plants have been carried out in countries outside the EU, for example, the USA and China. However, there have only been few cases where these releases have been systematically and scientifically monitored and where the results of this have been published in the scientific press.

During the course of this review of the literature we have identified the following as areas where some potential long-term/ cumulative effects of GM crops have been scientifically verified:

Effects of the use of herbicides with herbicide tolerant (HT) GM crops on weed populations and seed banks: The UK FSE trials identified effects of GM crops on the number and diversity of weed seeds left in the soil after the crops had been grown. The number of weed seeds were reduced after GM crops had been grown, presumably as a result of the herbicide application. The effects on weed seed levels persisted through the second season after the GM crops had been removed. It is anticipated that this will affect the number of seeds available as food for insects and birds. Effects were more substantial with the crops of GM HT sugar beet and oilseed rape than with GM HT maize. Studies of

long-term GM HT crop releases in the USA have shown that the weed populations within crops shift to those which are naturally herbicide tolerant and that herbicide tolerant forms of common weeds emerge in some cases.

Effects of Bt crops on non-target insect populations: Most of the studies confirm that non-target insect populations remain at similar levels under GM crops. However, there are some studies, which suggest that fewer natural predators are present and that the population may be more 'unstable'.

Development of resistance to Bt: Monitoring of Bt crops to date has confirmed that the Bt resistance is holding up. However, laboratory experiments have suggested that it is quite feasible that resistance will develop in the long-term if crops are grown widely.

Effects of GM crops on soil decomposition: there are some well documented examples of effects of GM crops on soil decomposition and soil organisms. These are mainly from laboratory studies and effects appear to be transient - it is therefore not known whether these will contribute to long-term effects.

Gene flow to wild relatives: There are some studies where gene flow from GM or conventional crops to wild relatives has been substantiated in the field. Most authors agree that this will occur in the field at low levels where crops are grown in close proximity to compatible relatives, however, there is no evidence to show whether GM genes are likely to become established in the population. Only genes which confer a selective advantage are likely to become fixed in the wild population.

Lower usage of pesticide sprays: A number of studies have identified a reduction in the number of pesticide sprays applied compared to conventional crops, particularly for Bt crops. This could lead to better human health and less effect on non-target insects.

2.3. Existing plans and methodologies used for GMO risk assessment

Most developed nations producing or releasing transgenic products have put in place regulatory systems aimed at assessing and managing risks associated with products of biotechnology developments to ensure protection of human and animal health and the environment. For example, the United States, Canada, New Zealand, Australia, Argentina and the European Union have individually enacted legislation that requires assessment of the ecological and human-health risks associated with the contained use and deliberate release of GMOs. On a much wider scale, the parties to the Convention of Biological Diversity adopted the Cartagena Protocol on Biosafety in January 2000, providing an international regulatory framework to reconcile the respective needs of trade and environmental protection in the context of the rapidly growing global biotechnology industry. The Cartagena protocol also bases assessments of movements of living modified organisms, (as GMOs are termed in the protocol) on principles of risk assessment and risk management. Similarly, international organisations such as the Organisation for Economic Co-operation and Development (OECD), the Food and Agriculture Organisation of the United Nations (F AO), the United Nations Environment Programme (UNEP), the International Plant Protection Convention (IPPC), the Association of Southeast Asian Nations (ASEAN) to name a few, have all considered developments in plant biotechnology and developed frameworks for assessment of the risks associated with release and movement of genetically modified crops.

Two general concepts have been proposed to guide ecological risk assessment in regulatory and associated procedures; these are the concept of familiarity and the precautionary principle. The concept of familiarity is based on the fact that most genetically modified

organisms are developed from organisms such as crop plants whose biology is well understood (OECD, 1993a). Familiarity allows the risk assessor to draw upon previous

knowledge and experience with the introduction of similar crops, including GM crops, into the environment, and to use the non-GM crop as the comparator to the GM crop in order to highlight differences associated with the genetic modification and the subsequent management of the GM crop. Some consider this concept to be too loosely defined to be very useful for risk assessments (Levidow et al., 1996; Regal, 1999; Torgersen, 1996), nevertheless it is still widely used in GMO risk assessment, particularly for assessment of food products.

The precautionary principle was first introduced in the Rio Declaration of the Convention of Biological Diversity (CBD), stating: ''where there is a threat of significant reduction or loss of biological diversity, lack of full scientific certainty should not be used as a reason for postponing measures to avoid or minimise such a threat'' (CBD, 1992). It has since seen many different and much more generalised forms (Goklany, 2000). The Cartagena Protocol on Biosafety (SCBD, 2000) and the EU GMO regulatory framework are both based on the precautionary principle.

We have reviewed many risk assessment frameworks for which information is publicly available, paying particular attention to guidance given with respect to cumulative long-term effects. A number of similar reviews have been undertaken in recent years and we have used these as a starting point. Summaries have been drawn from the "A*d hoc* expert group on risk assessment" established under the Cartagena Protocol on Biosafety[1], and a review by K. R. Hayes (CSIRO, Australia, 2003) entitled "Robust methodologies for risk assessment; best practice and current practice in ecological risk assessment for GMOs". Each methodology was reviewed in terms of its scientific principles and framework, approaches to hazard identification, likelihood and consequence assessment, uncertainty analysis, monitoring and reviewing, and finally whether cumulative long-term effects must be considered.

Details of twenty eight publicly available approaches to GMO risk assessment were reviewed, although many more could have been found. The key framework in use by member states of the EU is EU Directive 2001/18/EC (specifically Annex II)[2] and associated guidance documents, also guidance produced by the European Food Safety Authority (EFSA) in connection with regulation 1829/2003[3] on genetically modified food and feed. Within the EU member states, we have also reviewed national guidance developed by the UK and Italy. Internationally, we identified 10 organisations presenting approaches specifically for GM risk assessment, these range from being extremely detailed frameworks such as that developed by the Australian Office of the Gene Technology Regulator (OGTR) to much broader guidelines such as those published by the Association of South East Asian Nations (ASEAN). In USA, New Zealand and Switzerland GMO risk assessment is undertaken in the broader context of releasing ecological 'stressors' such as new organisms or hazardous substances. Risks associated with plant pests and pathogens and non-native species have parallels with release

[1] http://www.biodiv.org/biosafety/cop-mop/result.aspx?id=10787
[2] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC - Commission Declaration. *OJ L 106 , 17/04/2001 p.1 – 39).*
[3] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (Text with EEA relevance) (*OJ L 268, 18/10/2003 p.1 – 23).*

of GMOs and risk assessment methodologies for these were also reviewed. To provide conceptual thoughts on the approach to long-term cumulative risks, we identified the US

Environmental Protection Agency (EPA) framework for assessment of cumulative risks. Reviews of the risk assessment methodologies are in Annex 2, table 2 below summarises the key features of the (thirteen) most detailed frameworks.

Table 2: Summary of detailed risk assessment methodologies for the release of genetically modified organisms

Method	**Specific to GMOS?**	***Does the method take into account these issues and are clear guidelines provided***					
		Scientific principles	**Hazard identification**	**Likelihood & consequence assessment**	**Uncertainty analysis**	**Monitor & review**	**Long-term / cumulative effects**
EU Council Directive 2001/18/EC	✓	Precautionary principle; 6-step ERA framework. Case-by-case	General guidelines, no specific techniques; checklists	General guidelines, no specific techniques	Uncertainties must be documented	General surveillance monitoring required in all cases	Must be considered in context of monitoring
EFSA guidance for the risk assessment of GM plants and derived food and feed 2005	✓	Precautionary principle; 6-step ERA framework. Case-by-case	General guidelines, no specific techniques; checklists	General guidelines, no specific techniques	Uncertainties must be documented	General surveillance monitoring required in all cases	Must be considered in context of monitoring
UK (Defra) guidance on principles of risk assessment and monitoring for the release of GMOs	✓	Precautionary principle; 6-step ERA framework. Case-by-case.	General guidelines, no specific techniques; checklists	General guidelines, no specific techniques	Uncertainties must be documented	General surveillance monitoring required in all cases	Must be considered in context of monitoring
Italian Ministry of Agriculture	✓	Model-driven approach based on risk analysis and risk management. Science-based and case-by-case.	Hazard identification s based on assessment of source, diffusion factors, migration routes and receptors	Likelihood and consequence is calculated by the model, based on answers to electronic questionnaire	Not mentioned in the preliminary document we have seen	Not mentioned in the preliminary document we have seen.	So far applied to experimental releases only, so long-term cumulative risks do not appear to have been considered yet.
CBD: The Cartagena Protocol	✓	Precautionary principle. Best practice using recognised RA techniques should be used. Case-by-case.	No specific hazard identification techniques recommended	No specific techniques recommended. Receiving environment is key.	Uncertainty to be managed by obtaining more information	Monitoring is a means of managing uncertainty.	Not specifically mentioned

UNEP International technical guidelines for safety in biotechnology	✓	Familiarity is key concept. 3-step assessment, but not a strict framework.	No specific hazard identification techniques recommended	No specific techniques recommended. Forecasting and quantitative approaches may be appropriate	Not referred to	To verify assumptions of risk assessment & evaluate efficacy of risk management.	Not specifically mentioned
US EPA: Guidelines for ecological risk assessment (1998)	×	ERA is applicable for chemical, physical and biological stressors. 3-phase framework - problem formulation, analysis and risk characterisation	Conceptual model for use by risk assessors is provided	Conceptual model for use by risk assessors is provided	Discussed in detail; techniques for addressing uncertainty discussed.	Not discussed specifically, but implicit through document	No specific requirement, but rigorous model should make extrapolation possible
Canadian FIA: Assessment criteria for determining environmental safety of plants with novel traits (2004)	✓	5-stage framework; familiarity with unmodified equivalent is key feature. Can extend ERA to group of similar GMOs.	No specific hazard identification techniques recommended	Based on practical experience. Reference made to seed dormancy model	Not discussed	Post-release programme is required for unintended &/or unexpected effects.	Not specifically mentioned
Australia: Office of the Gene Technology Regulator Risk Analysis Framework (2005)	✓	3-stage framework based on best scientific practice. Case-by-case. Unmodified organisms as the comparator	Techniques to be used are not specified, but checklists and techniques that could be used are provided	Risk estimation matrix is provided with guidance on scale and terminology.	Discussed in detail and is required as part of the ERA. Advice is given on how this might be approached	Risk management component is required as part of framework	Must be considered and management options presented.

New Zealand ERRMA: Identifying Risks (1999)	×	5-step risk assessment framework. Case-by-case. Environmental baseline is comparator.	Must demonstrate thorough consideration of hazards. Comprehensive list of tools & techniques provided.	No specific techniques recommended. Qualitative, quantitative and semi-quantitative approaches discussed, reference to NZ standards made.	Discussed in detail. Focuses on consideration of significance of variability. Data used must be checked for statistical competence & have been peer-reviewed	Explicitly required in ERA framework. No guidance on this is given	Implicit throughout the document but no specific guidelines
IPPC International Standard for Phytosanitary Measures #11, 2004 (ISPM 11)	× Plant health	3-step risk assessment framework. Case-by-case. Focused on potential for entry, establishment and spread of non-native pests	Techniques to be used are not specified, but comprehensive checklists and techniques that could be used are provided	Detailed guidance provided on assessment of probability and consequence, includes economic consequences.	Must be considered and documented where main areas of uncertainty lie. No techniques recommended	Conclusion of the PRA is whether risk management is required and what needs to be used.	Assessment of potential long-term consequences of pest entry is implicit in PRA
EPPO Framework for Pest Risk Analysis (DATE?)	× Plant health	3-step risk assessment framework. Case-by-case. Focused on potential for entry, establishment and spread of non-native pests	Techniques to be used are not specified, but comprehensive checklists and techniques that could be used are provided	Detailed guidance provided on assessment of probability and consequence, includes economic consequences.	Must be considered and documented where main areas of uncertainty lie. No techniques recommended	Conclusion of the PRA is whether risk management is required and what needs to be used.	Assessment of potential long-term consequences of pest entry is implicit in PRA

UK (Defra) Standard Methodology to assess the risks from non-native species considered possible problems to the environment (2005)	✖ Non-native species	6-step risk assessment framework. Case-by-case	Comprehensive step-by-step guidance provided including scores (unlikely through to very likely). Electronic template available	Comprehensive step-by-step guidance provided including scores (unlikely through to very likely). Electronic template available	Must be taken into account; mathematical approach to calculation of conditional probability presented. Worksheets are provided. Options for further work in this area discussed	Final module of PRA is dedicated to risk management options and monitoring of efficacy.	Assessment of potential long-term consequences of pest entry is implicit in PRA

Risk assessment methodologies - summary

There is no universally adopted approach for undertaking environmental risk assessments for the release of GMOs. All the risk assessment methodologies reviewed did, however, follow a similar approach based on a case-by-case scientific framework aimed at assessment of hazards, potential for exposure and consequences of exposure, followed by consideration of risk management. The EU framework follows this basic approach in a six-step analysis; the guidance document is detailed but does not describe any tools that may be used at the various stages; brief guidance is provided on consideration of uncertainty, but again little guidance on how assessments could be made. Outcomes of the ERA need not be quantified, but should be relative, e.g. to a non-GM comparator; the overall evaluation of risk of the GMO must take into consideration strategies employed to manage risks.

The Australian OGTR 'Risk Analysis Framework' is the most recent publication and probably provides the greatest level of accessible guidance for completing all stages of the risk assessment. The model used is clearly explained, clear terminology for likelihood and consequence assessments are provided together with matrices for assessment of risks; it also devotes quite a few pages to an easily understandable discussion of the role of uncertainty in assessment. Assessment of long-term cumulative risks is required in the context of consequence assessment and is not a separate consideration *per se* within the framework. The guidance is focussed on GMOs and is presented very clearly, including details of the regulatory process for GMOs in Australia and the responsibilities of the OGTR. The New Zealand framework is similarly detailed (although not focussed specifically on GMOs), and again considers long-term cumulative risks in the context of hazard and consequence assessment as part of the overall process, it also asks the applicant to consider more socially-oriented questions, for example in relation to Maori values, and future generations, which have relevance for longer term effects. The US EPA has developed an extremely complex generic framework in which terminology is clearly defined and flow diagrams illustrate the process, but it may be somewhat unwieldy and daunting to the prospective risk assessor. While the Australian, New Zealand and USA frameworks are very much written from the perspective of the risk assessor, the EU framework, although comparable in concept, in presentation is far more focussed on science and consideration of real scenarios that may arise as a result of release of the GMO. Synthesis of the two approaches might prove powerful.

Throughout the documents reviewed the most common approaches to hazard assessment involve brainstorming and use of checklists; there is limited reference to structured tools and techniques which support deeper questioning of "what happens if…?", although the New Zealand guidance for identifying risks explores techniques in greater depth. Hayes (2004) considers uncertainty analysis to be the very rationale of risk assessment, fundamental to ensuring that "we don't have any regrets about releasing particular GMOs", yet, apart from the Australian and USA approaches, there is limited attention paid to uncertainty analysis. With GMOs, as with chemicals, Hill *et al,* 2003 suggested that poor data and confounding factors generally make it difficult to detect ecologically meaningful relationships, even where they do exist, and that the possibility of missing any effects is large because the statistical power to detect significant relationships is often low.

Most of the frameworks do not specify criteria for acceptability, or otherwise, of risks. This is understandable when we think that such decisions must be taken in the context of shifting policy goals and, in Europe, of national regulatory frameworks. The European Environment Agency reported in 1999 that "differences in acceptability of risks are evident across the

different member states of Europe where acceptable and unacceptable risks vary widely between member states, leading to differences in interpretation of the deliberate release directive", some evidence of this was found in this study when we undertook to assess competent authority(CA) requirements of the risk assessment process, this is discussed in more detail later in the report.

Consideration of cumulative long-term effects is implicit in risk assessment frameworks presented by the USA, Australian and New Zealand models, but mainly in the context of anticipated adverse effects of the GMO under consideration. The frameworks do not specifically provide guidance for assessment of effects that might be expected to arise in the longer term, or cumulatively due to large-scale release of a particular GMO, or interaction with other GMOs in the environment. Models developed for pest risk assessment or non-native species assessment focus on longer-term probabilities and include more detailed analysis of uncertainty, it is possible that some elements used in these assessments may also be useful for consideration of the release of GMOs. Consideration of the broader, unanticipated effects of the commercial release of GM crops is covered in the European legislation but no specific framework for assessment is suggested, nor is clear guidance provided on the key issues that must be considered, the questions that should be asked. We found only one reference relating specifically to assessment of cumulative long-term risks; in 2003 the United States Environmental Protection Agency published details of an initiative to 'develop a simple, flexible structure for conducting and evaluating cumulative risk assessment' (summarised in Appendix 2). The approach is essentially similar to ecological risk assessment but differs fundamentally in focusing on assessing the combined effects of more than one agent or stressor (leading to greater need for assessment of interactions and uncertainty), and there is increased focus on the populations potentially affected. This framework is unlikely to be applicable to the release of GMOs, but may prove conceptually useful as it evolves.

2.4. A review of the regulatory context and needs.

Formal scientific risk assessment is normally only part of a wider process of decision-making and risk management. We have attempted to establish what regulators operating within the EU regime could appropriately expect from risk assessment in the case of long-term and cumulative effects of GM crops. We directed a set of questions at EU member states' competent authorities to determine the extent to which they rely upon risk assessments in their decision making, and the variations in the scope of what the risk assessment is expected to cover.

Questionnaire responses

The questions that were directed at the competent authorities and the replies that we received are listed in Table 3 below. Of the 29 individuals approached, formal replies were received from twelve competent authorities, ten of which are summarised below and nine full replies are provided in Appendix 3 of the report. Some member states did not feel qualified to answer the questions, in particular some of the new member states because they have limited experience of the authorisation procedure. We are very grateful to those who were able to respond for providing such comprehensive answers. Replies were provided from Scandinavian, Mediterranean, western, central and eastern regions of from EU-15 and some of the newer member states, we think this has provided a reasonably good representation of

views. It is important to note, however, that none of the respondents are currently cultivating GM crops.

A key point to emerge was that the understanding of 'long-term' depends on the context of the release, for example perennial crops have very different timeframes from fruit or forest trees and therefore long-term must be a variable factor set on a case-by-case basis. There was general consensus that long-term is the period of time beyond the ten years of the consent, and can be anything up to 100 years. Cumulative effects were generally considered as those due to repeated release of the same GMO or trait, or the effects of release of different GMOs in one area. Many member states were unwilling to be drawn on what long-term or cumulative effects may occur, but most comments related to potential for gene flow leading to impacts on biodiversity, effects of changes in agricultural practice and allergenicity effects in humans. One member state commented that effects that might be anticipated as the result of cultivation of a GM crop could be just as likely to occur due to the introduction of a new conventional crop or change in management practice. In most cases member states have national environmental monitoring schemes in place and anticipate that these will be reviewed and adapted to enable monitoring of any potential effects of the release of GMOs; proving cause and effect may prove difficult. In many cases respondents thought that current monitoring programmes would not be sufficient to identify long-term or cumulative effects; for this aspect of the review it would have been interesting to have received responses from those countries currently cultivating GM crops.

Responses regarding the EU risk assessment framework and whether it adequately enables applicants to address potential long-term and/or cumulative effects varied, some suggested that clearer guidance to applicants from the European Commission would be beneficial, while others thought that monitoring aspects of applications generally needed to be improved. None of the respondents had developed their own criteria for environmental impacts against which they assess the acceptability of the GM releases; some respondents base their judgements on the framework provided by directive 2001/18 (Annex II) while others are currently developing their own criteria. A number of member states commented that where a crop offers a clear environmental benefit and/or a benefit to society, it was likely to be assessed less stringently than a crop that offered no clear benefits. No models for risk assessment were proposed for consideration within the European context.

Table 3: Summary of responses to questionnaire, by respondent in no particular order. Full (anonymous) responses are provided in Appendix 3.

Questions:

- What do you understand by 'long-term' - 10 years, 50 years, 100 years or other?
- What sort of effects do you think might emerge after this period of time? What are your reasons for thinking this?
- What monitoring arrangements do you have in place that would support identification of such effects, should they occur? Do you envisage that it will be possible to prove cause and effect given your current monitoring arrangements?
- What do you understand by cumulative effects?
- What sort of cumulative effects do you think might emerge after what you have defined as the long-term in Q2? What are your reasons for thinking this?
- Do you think the monitoring arrangements in place in your member state are adequate to identify these effects, should they occur?
- Do you think the current EU risk assessment framework enables applicants to address adequately the potential long-term cumulative effects of GMOs? Do applicants generally pay sufficient attention to this aspect of applications? What do you think would improve this aspect of the authorisation process?
- Do you have specific criteria for deciding what are acceptable and unacceptable risks? If so, can you let us know what these are and why you have identified these?
- Are there any models used by other regulatory authorities worldwide that you would like to be incorporated into the European approach that might provide for a better assessment of long-term cumulative risks?
- Are you aware of any reports of unexpected effects of the release of a GMO?

	Question									
	1	2	3	4	5	6	7	8	9	10
1	Not fixed, but exceeds the time period of the consent of a specific product. Will be defined on a case-by-case basis – e.g. timeframe for tree is different to perennial plant. Intensity of usage also determines what is 'long-term'	Dissemination of GMO into different habitats (similar to experiences with invasive non-native species). Gene stacking, multiple resistances, long-term change in agricultural practice due to intensification, soil alteration, Bt resistant pest species, HR weeds, long-term loss/change of natural habitats, subtle elimination of wild species by cultivated GMO, continuous contamination of crops	Concept for national biodiversity monitoring currently being developed – will be very difficult to design a monitoring programme that will foresee unknown effects. Good statistical design will be essential	Cumulative effects are effects that arise from i) placing on market of several different GMOs; ii) introduction of same trait in several GMOs (e.g. Bt, HR), iii) intensification of agricultural practice due to the GMO	Soil alterations due to cumulative use certain herbicides or GMOs; multiple herbicide resistance in weeds; accelerated selection of Bt resistant pest spp; transgene contamination in non-GMO crop varieties; change/loss of natural habitats &/or biodiversity due to intensification of agricultural production.	No –not at the moment	Applicants generally do not address long-term or cumulative risks adequately; monitoring plans are in most cases insufficient; the current procedure only identifies short-term effects. Effects of GMOs in combination with agricultural practice should be analysed	No specific criteria at the moment for environmental aspects. Broadly speaking criteria are protectionist & consensus in society. For human health effects, assessment of toxicology, allergy & substantial equivalence are key criteria		Stem split of GM soybean under stress conditions; loss of unripe cotton balls on GMO cotton; change of fat composition in cow milk upon feeding with GMO soybean, gene stacking of herbicide tolerance traits in Canada. Plus 1 reference provided
2	10 years or more	Public health effects (allergies & toxicity) &/or environmental effects (GMO crossing with non-	No specific monitoring for GMOs at present	Cumulative effects are indirect & long-term due to intended	Could be both environmental & human health effects, e.g. escape of		General surveillance plans need to be more detailed & prescriptive, & provide detail of	No specific criteria	No model. But feeding studies should be more	Unexpected release of GMO products destined for

		GMO). Under National law, permit will only be granted for 5 years		release.	GMO plants to the wild, affecting wild flora; allergies or toxicity ling after being placed on the market		preventive measures. Further assurances for proper implementation should also be sought. The issue of unintended release should be considered more thoroughly. Needs to be improvements in accuracy & precision of analytical methods, e.g. for compound feeds.		prolonged; need more thorough evaluation of substantial equivalence & need further investigation into long-term consequences of GMO products on fauna & flora	feed use on Japan
3	20-50 years	Effects caused by characteristic of the organism &the modification; effects emerging fro the change in agricultural practice. Specific effects may be on biodiversity, gene transfer to wild relatives, resistant pest populations, & changes in use of herbicides. May see positive effects, e.g. reduced soil erosion, reduced use of chemical insecticides. Should be further studies to look at effects on soil	Multi-year studies of GM crops in place, but not aimed at systematic long-term monitoring of effects of GM crops. Monitoring network for conventional crops been in place >20 years, looking at agronomic performance & effects on pests, diseases, biogeochemist	Effects occurring at same time & same place or area. May be positive or negative	As in Q3. Will also be connected with increasing number cultivated GM crops & spectrum of new traits. Effects very difficult to anticipate. Effects are most likely to emerge from changes in agricultural practice rather than the genetic	Current monitoring arrangements are not sufficient. Authorities & notifier should monitor for these effects. Methodology should react to new traits & changes in agricultural practice	Framework is very general, especially for long-term & cumulative effects. Notifications are often too brief in these areas. Existing framework should be accompanied by specific guidelines & case studies focused on identification of possible long-term effects. Improvement of this aspect of the authorisation process is important	Risk criteria used are those given in 2001/18/EC. Acceptable risks are those lower than or on the same level as the risks of the non-modified crop variety. A risk-benefit analysis should be carried out.	No	No

		organisms	ry & soil microorganisms. GM crops will be included in this network		modification itself.					
4	20-50 years	More cases of contaminated grain; increase in stacked events, which cannot be detected. Hybridisation of GM OSR in southern Mediterranean. Possibility of food-related allergies developing	Current monitoring would not prove cause & effect for events in (Q2).	Cumulative = events generated over time by a combination of factors	Cannot predict	Current monitoring is not sufficient to detect cumulative effects	Current EU regulatory framework does not require applicants to determine long-term or cumulative effects. Applicants tend to focus on why they do not need to present information	Currently developing risk criteria	No	Starlink
5	Any effect occurring after 10 years or longer	Not wise to start guessing about possible effects, need to stick with case-by-case assessment. Only thorough risk assessment could identify possible long-term effects of a particular GMO.	Many different networks exist that could be applied to the observation of long-term, unforeseen effects of GMOs. Systems exist for monitoring plant diseases, amphibians, plants, birds, marine life, & so on. Where specific	Effects that pile up	Emergence of cumulative effects will depend on the GMO/s in question – not possible to predict them as we don't know what sort of GMOs will be on the market in the future	Current monitoring arrangements will possibly lead to detection of cumulative effects	Current risk assessment framework is adequate to address potential long-term effects of GMOs. Current application process works well for these aspects of risk assessment	We use the criteria given in Directive 2001/18/EC to decide whether risks are acceptable or not – this Directive provides the framework for such decisions	No	No

			monitoring requirements are identified, this will be specified in the permit for release. Any discussion on cause & effect is purely theoretical – not possible to predict whether proving cause & effect will be possible; it will probably be possible							
6	Long-term depends on the context – species, environment, human effects etc. Consider 10 years to be adequate but e.g. for forest trees 10 years would be inadequate.	Easier to focus on immediate effects & health effects, allergies may be expected in longer term. Environmental effects have much longer time frame anyway, e.g. herbicide resistance effects could take much longer than 10 years to present, so we much think longer term anyway. Different crops have different lifecycles &	Generic schemes are in place that could pick up effects of growing GM crops, but these have not been properly evaluated yet. Monitoring networks will need clear instruction/education as to what should be monitored	Cumulative = sum of factors which individually do not have an effect but when combined, may have an effect. These scenarios become increasingly complex.	Refer to answer given for Q3; again comes down to consideration on a case-by-case basis.	None in place at the, moment, CA will consider it when it becomes necessary.	No, applicants do not generally pay sufficient attention to long-term risks. Very little peer-reviewed data is provided, it is often poorly presented & with poor hypotheses, which makes its validity questionable. Data is also presented from biased perspective. Makes assessment very difficult. Not	No specific indicators identified yet. Would be less critical in assessment of a crop that demonstrates clear benefits for the country & society.	No	[Referred to a couple, awaiting information]

		rotations anyway, so risk assessment must be considered on a case-by-case basis. Greatest effects are expected to be gene flow & effects on seedbanks.	when GM crops become a more realistic prospect. National agricultural community quite opposed to GM crops at present so prospects for cultivation are limited.				necessarily the applicants fault as 2001/18/EC does not provide guidance on data collection, analysis & presentation. CAs could be more exacting in their requirements Clear guidance is needed, but this would require EU-wide agreement. Independent research in this area would be very valuable.			
7	Depends on the crop – long time for a perennial crop is different to that for a forest crop. For forest trees even 100 years would not be unreasonable. Ecological timeframes are different from agricultural timeframes	All / any compartments of the environment might be affected.	See answer to Q7.	The effects of having more than one GMO, leading to a change in potential risks; this also affects the long-term risks	All / any compartments of the environment might be affected.	Monitoring programmes are in place for conventional grazing & forestry. Intensive agriculture is monitored less.	Tools for monitoring are available, lead CAs should use these & put pressure on companies to use them. Applicants do generally pay sufficient attention to these & do a good job, but too little effort is put into monitoring.	No specific criteria. CA weighs advantages & disadvantages. Acceptability of risks is a national, internal value for each CA, not an economic consideration & varies crop to crop. Would be prepared to	No	No

								accept greater risks for a crop that demonstrated real advantages for the environment or general public.		
8	10 years or more. Magnitude, likelihood & timing of long-term effect can change depending on receiving environment – could change in 10-20 years.	Loss of biodiversity due to gene flow; changes in insect community structure & function; changes in soil microbial profile, diversity or community structure	Monitoring specifically for GMOs id only at the regional level for research purposes - to identify bio-indicators & to establish baselines. National environmental monitoring schemes could be adapted to monitor for effects of GMOs	Cumulative effects – from repeated exposure or intake of non-target expression of toxins, allergens, carcinogens. Synergistic effects - combinations of GMOs released in neighbouring sites give an adverse effect, or characteristics of single GMOs act together to produce a harmful effect	Undetected toxins, allergens & carcinogens enter the food chain & accumulate; decrease in insect diversity by repeated exposure of target species to a toxin. Reduction in biodiversity of agricultural landscape	Not at present because of lack of databases concerning environmental baselines, lack of indicators; lack of GMO monitoring activities at National level	EU framework does not enable applicants to address long-term/cumulative risks. Applicants do not pay sufficient attention to this aspect of application. Could be improved by standard ERA methodology; R&D on gene technology, ecological interactions & genomic, proteomic & population dynamics	No	The government has developed a standard methodology which has been implemented for Part B releases only so far.	No

9	A change that is not reversible within 15 years, and / or a small change that is not easily detected, but is in the same direction for 10 years+ leading to significant environmental change, not reversible in the short-term	Answer is limited to crops that are currently available in EU or have pending applications. Effects can be good, bad, neutral; effects may include landscape scale effects, e.g. cultivation patters, management practices, could in turn affect farmland biodiversity. Similar effects may be seen with any new or different agricultural practice. Emergence of agricultural weeds with herbicide tolerance is a possibility. Could see volunteer crop plants, gene flow into sexually compatible wild relatives.	No specific arrangements in place for GM crops, as none currently grown. Other monitoring programmes are carried out for certain indicators. Difficult to envisage linking and effect to a GM crop, would need to tested experimentall y	Combined inputs that accrue over time & space from cultivation of GM crops. Might be interactive or incremental due to increases in scale.	Difficult to predict. Possibly evolution of herbicide tolerant agricultural weeds – e.g. through herbicide use or gene flow. Gene stacking leading to multiple herbicide resistance is a possibility - this could also arise from conventional crops.	Yes – if they have adverse effect on environment relative to non-GM equivalent	Current framework is adequate. LTC effects will have to be dealt with through general surveillance as we don't know what they will be. CS monitoring may be applicable in some circumstances; clearer guidance on principles of CS & GS monitoring from EU would be useful.	Risks are assessed in accordance with criteria in Annex II of 2001/18. Comparator is non-GM equivalent & conventional management practices associated with it.	No	Not aware of any unsubstanti ated reports.
10	Long-term is not defined, is subject to interpretation by expert assessors	Will mainly see socio-economic effects in long-term, linked with agrochemical applications & IP.					Environmental & health risks will be addressed adequately by the EU regulatory regime			

2.5. Lessons learnt from long-term risk assessment in other domains

This section considers what lessons can be learned from the assessment and management of long-term and cumulative risks in other domains.

The main area in which research and practice have been developed is that of the disposal of radioactive wastes. Most EU States, and others with nuclear programmes, have developed policies that require assessment of the long-term risks before authorisation can be granted. In these cases, 'long-term' typically refers to timescales of thousands or even millions of years – these being the timescales over which activity is expected to emerge from (deep) underground repositories to the accessible environment. These long timescales also reflect the need to address concerns about the loss of institutional control (security of the site, monitoring etc).

Although these timescales are much greater than those which most CAs would currently interpret as 'long-term' for GM crops, and the decision contexts and nature of the risks are also very different, there are several lessons that are relevant. These lessons relate to two main, inter-connected areas: the principle of how far into the future a risk assessment should look, and the treatment of uncertainty within long-term assessment. The radioactive waste field has less to offer in terms of identifying hazards, since the hazard – radioactivity – is already known. It may appear in different forms, according to the radioactive decay chains, and cause harm by different routes (ingestion, inhalation etc) but is not so variable as the very wide range of environmental consequences that can arise from the release and cultivation of a crop. The potentially useful lessons learned are listed below:

Some radioactive waste regulators set, or allow the developer of the facility to define and justify, a cut-off on the timescale to be considered. This is justifiable in the radioactive waste domain because the inherent risk – the level of activity – generally decays over time, such that it is possible to evaluate when the peak risk is likely to have passed. This is not possible for GM crops – once released into the environment there is a possibility, at least, that the risk will increase – for example if an invasive species is given an additional selective advantage. It is much more difficult to understand and forecast what human or ecological mechanisms might eventually control it. The lesson for the GM crop domain is that regulators should not set an arbitrary cut-off time on assessments – it should be demonstrated on a case-by-case basis how the risk is expected to change over time and when the peak risk will have passed.

Over very long timescales, the effects of natural climatic cycles (as well as more immediate, anthropogenic climate change) will need to be considered, as well as radical changes in land use and human activity. It is recognised that, as time progresses, uncertainty increases, such that the value of the risk assessment, as a tool for quantitative prediction diminishes and may ultimately become meaningless. However, this is not to be taken as a justification for truncating the assessment period (see (1) above). Rather, it shifts the emphasis away from quantitative predictions about what is expected to happen towards a broader, qualitative consideration of 'what-if' scenarios. The proponent needs to demonstrate that, for a range of credible future scenarios, the environmental mechanisms and barriers to risk will continue to provide adequate assurance of safety. It is not expected that the assessment should 'predict the future' in detail, with regard to habitats or human behaviour, but rather that safety should be demonstrated for a range of stylised scenarios, spanning the factors that affect risk. Given that most GM risks are difficult to quantify even in the short-term, this would seem an appropriate model for assessing long-term GM risks – the emphasis should be on considering

the crop within the whole environmental system, and the effectiveness of barriers to harm, rather than detailed modelling of those aspects that can be quantified.

In predicting the dispersion of radionuclides through the geosphere, computational modelling approaches have been complemented by work on natural analogues – the observational study of the migration of activity, over geological timescales, from naturally occurring radioactive rocks. There could be scope for parallel approaches in the GM crop domain, considering the historic or palaeontological evidence for the spread and impacts of new species

Very sophisticated techniques have been developed for probabilistic (stochastic) analysis, enhancing the basic Monte Carlo methods to allow more complex representations of future scenarios to be assessed, for example by allowing correlations between input parameters, and better statistical analysis and presentations of the outputs – for example in sensitivity analysis and identification of critical parameters. These techniques are potentially applicable to GM crops, although the aspects of radioactive waste disposal that have been subjected to this kind of analysis – typically the hydrogeological dispersion and uptake of the activity in the geosphere and biosphere - are generally more amenable to quantitative analysis than most aspects of GM crop risks.

Following on from (4) above, there is increasing recognition of the need for a balance between quantitative and qualitative approaches – neither is sufficient by itself.

The difficult of finding any politically acceptable sites in many States has led to a growing realisation of the importance of considering social aspects of risk. There has been much research on the social construction of risk and risk perceptions. Stakeholder participation in framing the question and defining methods is being given serious consideration and has occurred in some cases (Smith & Collier, 2005) in addition to the traditional model of stakeholder review of results. These developments underline the importance of developing risk assessment as a structured framework for discourse and decision-making under irreducible uncertainty, rather than as a technical and scientific exercise alone.

Decisions about long-term disposal of waste, as against the main alternative of continued surface storage under controlled conditions, raise ethical issues of the responsibility of current generations to those in the future and of choice and consent. Continued surface storage imposes a burden on future generations, in that the costs of storage and the search for and implementations of a longer-term solution are handed on to them. On the other hand, it allows them a greater degree of control, and keeps open the possibility that better techniques will be developed. There are parallels in the GM domain in deciding the balance between imposing a risk on future generations and denying them potential benefits. The GM crop case is different, however, in that radioactive waste already exists, and a solution has to be found, whatever decisions are made about the future use of nuclear energy. There is no such unavoidable imperative to commercialise GM crops. These ethical issues require exploration, both at the policy level and in their implementation with regard to specific releases.

3. A Framework for Risk Assessment

This section describes the development of a generic framework for the assessment of long-term and cumulative risk. Section 3.1 describes the high-level principles and user requirements adopted in designing and developing the framework. Section 3.2 outlines how the framework was developed, including how the evolving framework was tested, refined and demonstrated by means of an initial workshop and iterative application to case studies. Section 3.3 outlines the proposed framework so far as it has been possible to develop it, outlining tools and techniques that can support it and providing guidance on its use. Section 3.4 reviews how well the framework satisfies the principles and requirements, and highlights the specific challenges that users will need to consider in applying it. Section 3.5 identifies the research gaps - areas in which further work would be needed to develop a fully coherent and workable approach.

3.1. Guiding Principles and User Requirements

In order to meet regulatory needs and comply with best practice in risk assessment, the following principles and user requirements have been adopted in the design of the framework:

Scientifically sound. The framework must be conceptually sound and theoretically rigorous, making the best use of available science.

Human-centred. Risk assessment is more than just technical analysis – it should encourage, structure and inform discourse between stakeholders. The process of using the framework can therefore be as important as its products. The guidance presented here includes consideration of the ways in which the framework can be used (e.g. in whether in group sessions or by an individual analyst) as well as the more technical aspects.

Usable. The user of the framework must remain in control of the process, and be aware of its limitations. Conceptual models of the environment and of risk should be as easy to understand as possible. The framework must be as easy to learn and use as possible, given the inevitably complex nature of the decisions it is intended to support. It should be flexible, offering a variety of tools appropriate to different cases. Note that 'Users' here includes all the various stakeholders in risk assessment: both those who will actually gather data and perform analyses, and those who will interpret and base decisions on the results.

Transparent. The framework should help the user to identify and document the reasoning and assumptions behind their judgements and any limitations.

Proportionate. Trade-offs often have to be made between the above principles. For example, a compromise is often necessary between usability and scientific rigour. The appropriate balance between conflicting principles will vary with each application, so the framework should be applicable at various levels of depth and detail, as appropriate to the decision stakes and the data available.

3.2. Development of the Framework

The development of the framework took as a starting point a generic risk assessment approach that will be familiar from other domains. In brief, it is based around the identification of hazards – things that might go wrong – and the evaluation of the likelihoods and severities of consequences of those hazards. At this level, the approach is relatively standard – the more

complex and difficult issues arise in applying it to the specific challenges posed by long-term and cumulative risks from GM crops.

The framework was first tested in an initial workshop, described in detail in Appendix 4. The workshop involved participants with experience in various scientific domains related to GM crops, the environment and human/ animal health, as well as risk assessment specialists. It identified potential scenarios for long-term/ cumulative risks from GM crops, chosen to show the range of possible risk types that the framework will need to be able to address.

The framework was then applied to a number of case studies of releases currently in, or likely to be in the regulatory pipeline within the EU. The case studies were used to test and refine the approaches in the previous sections. In addition they were used to illustrate the type and extent of risk monitoring and mitigation measures that may be required for the commercial release of GM crops (Section 4). Details of the case study assessments are given in Appendix 6. It is stressed that the purpose of these case studies was to help with the development of the framework. They were carried out within a very limited time, and in many areas large assumptions had to be made rather than seeking real data. The findings are therefore indicative of what might emerge from a real regulatory assessment, but must not be relied upon as such. They also help to indicate the range of possible risk types that the eventual assessment methodology framework will need to be able to identify and evaluate.

Lessons learned from the workshop and case studies have been incorporated in the latest version of the framework, as described in Section 3.3 following

3.3. The Proposed Framework

The framework is intended to define a coherent, but flexible process of assessment. It does not prescribe particular tools or explain their theory and use in detail, but suggests a range of tools that may be helpful. It is recognised that every case will be different, and that the most appropriate tools will depend on:

- the nature of the proposed release and its environment;
- the specific decision context;
- the potential level of risk – the breadth and depth of assessment reflecting the decision stakes in accordance with the principle of proportionality; and
- the extent and quality of data available.

It is also recognised that many users will already have experience in using certain tools for some of the steps in the framework, that they may prefer to continue using.

A (non-exhaustive) catalogue of the tools and techniques that can be applied at the various stages of the framework is provided in Appendix 4. It summarises the uses, limitations and requirements of each method and provides references from which more detailed information can be found.

Overview of the Process

The steps in a generic risk assessment process can be represented as shown in Table 4 following.

Table 4. The generic risk assessment process.

STEP		AIM/ QUESTION TO BE ANSWERED
1	Frame the Question	Define the question in risk terms, in a way that best informs the decision to be made. Define what is meant by risk - the problem boundaries, types of risk measures, etc
2	Define Criteria	Define how it is to be decided whether the risk is tolerable
3	Hazard Identification and Analysis	Identify what could go wrong - intended or unintended events associated with the release that could lead to harm / adverse effects.
4	Hazard Analysis	Identify and understand the casual and contributory factors for each Hazard, and its potential effects, and analyse the relationships between and within causes, Hazards and effects.
5	Assess Likelihoods Consequences and Risks	Assess how likely it is that each hazard will occur. Identify the various possible outcomes of each hazard. For each outcome, assess its severity (how bad it would be) and the probability of that outcome actually occurring (given that the hazard itself has occurred) Assess the level of risk – this will be a function of the likelihood of the hazard and the severities and probabilities of its consequences
6	Compare Risk against Criteria	Is the predicted level of risk tolerable, against the criteria devised in step 2?
7	Consider Uncertainties	What are the main uncertainties in the assessment models, data or assumptions? How might they change the conclusions? In view of these uncertainties, is there sufficient confidence to make a decision? If not, what could be done to reduce the uncertainties?
8	Identification of Mitigation & Monitoring Methods	What could/ should be done to reduce the risk? (return to Step 3 and re-assess as appropriate – the mitigation/ monitoring methods may change the hazards)

The following sections describe each step in more detail, as applied to long-term and cumulative risks from GM crops.

Step 1: Framing the Question

Real regulatory decisions and questions are not always framed in terms directly amenable to risk assessment. The critical, and often challenging, first step in risk assessment is therefore to frame the initial question in a way that informs the decisions to be made and that is amenable to practical risk assessment.

At the highest level, the decision question about GM crops can usually be posed in terms such as:

"Is it acceptable to release [crop] modified with [gene, trait]?

This can be fairly readily restated in risk terms as:

"What are the risks associated with the release of the modified crop, and how do these compare with the agreed tolerability criteria

But in order to apply risk assessment tools it will be necessary to have a more precise definition of the question and of the risks to be assessed. For example:

- What is the decision context – an internal, commercial decision by the industry, or a regulatory one? Risk assessments may be used to inform regulatory policy or strategy, as well as release-specific decisions.
- What is the geographic scale of the decision to be made - local, State, EU or global?
- What types of risk are considered to be within the scope of the assessment? The present study focuses on human/ animal health and the environment, but 'environment' is a very broad term that is not specifically defined in Directive 2001/18. There are, for example, overlaps between environmental risks and agro-economic, social and ethical ones.
- What measures of risk are to be used? The measure will, in broad terms, usually be the likelihood of the various potential harmful consequences. But is it the risk per release that is of concern, or the aggregate over an (assumed) number of releases? Is it the risk to individuals or the collective risk to society?
- What is to be assumed about other GMO releases or changes in the environment over the assessment timescale? This is a particular issue for the study of long-term and cumulative effects.
- How it is it to be decided what level of risk is tolerable?

The answers to all these questions will strongly affect the results. For example, the 'right' decision from a regional point of view may be different from that for an international decision. Framing of the question is critical, and the questions above should be thoroughly discussed in order to define the question appropriately and precisely. In practice, the framing step is usually iterative with later stages of assessment - it is not until the more detailed assessment questions are asked that the subtleties of the overall question become apparent.

Long-term and cumulative risks are the specific subject of this assessment, but these are not always easy to distinguish from shorter term ones. In many cases, the hazards are the same, but differ in degree and in the greater level of uncertainty.

Step 2: Defining Tolerability Criteria

In order to decide whether a particular release should be allowed (at all, or with additional mitigation or monitoring measure) criteria must be defined for what level of risk is considered tolerable.

Directive 2001/18 does not provide any explicit, risk-based criteria such as limits on the frequency of occurrence of particular effects. Current practice (see Section 4.1.4-

Questionnaire Responses) seems to be largely based on a comparison between the risks associated with the GM crop and those for its unmodified equivalent. Most Competent Authorities (CAs) that responded to the questionnaire had no more specific criteria, but one required risks to be 'lower than or the same level as' those of the non-modified crop. It would seem extremely difficult ever to demonstrate this robustly for long-term and cumulative risks - given the complexity and interconnectedness of the environment it is almost always possible to postulate some potential negative effect. However this CA did also say that 'a risk-benefit analysis should be carried out' opening the possibility of trading off advantages and disadvantages. Another CA stated that risks were considered unacceptable if 'significant' adverse effects were revealed by the comparison, but the threshold of significance was not defined.

The absence of explicit criteria, and the apparent variations in practice between CAs, point to a need to develop more explicit guidance on criteria, in order to encourage well-founded, consistent and fair decisions. It may not be possible, or indeed desirable, to devise any very rigid criteria – the risks and benefits of GM crops are probably too complex to be encapsulated in, for example, simple quantitative thresholds. And Member States will wish to retain some flexibility to reflect national and local concerns, as indeed Article (9) of Directive 2001/18 explicitly acknowledges, with regard to ethical concerns. Rather, it is important that guidance should ensure that all relevant factors are taken into account, and provide a systematic framework for the discussion about risk.

Whatever criteria are adopted need to be related to the framing of the question (Step 1) – i.e. to the decision context and the specific definitions of risk. The development of tolerability criteria is usually iterative with the identification of hazards – it may be more practical to set, for example, a limit on the likelihood or extent of specific hazards arising than to define generic limits on some aggregate measure of risk

The main questions and areas for further development are noted below:

- What benchmarks or standards can be adopted? For example tolerable levels of risk could be defined by reference to a comparison with the risks from the unmodified crop in the current environment, or against the risks from the current crop allowing for normal bounds of variability and mutation in that crop and its environment.
- Is there a need for a formal requirement to minimise risks, such as the As Low As Reasonably Practicable (ALARP) principle? What should be the bias in favour of safety? How is this to be related to the implementation of the precautionary principle?
- Should the assessment take account of the benefits as well as risks? The necessarily rather risk-averse nature of a regulator's function, and traditional, hazard-based approaches to risk assessment have lead to an emphasis on potential negative aspects.
- As well as considering what levels of risk may be considered tolerable, an aspect of particular importance when considering long-term and cumulative risk is the level of confidence in the assessment that is required in order to make a decision. Long-term and cumulative effects are by their nature uncertain, and some of the uncertainty will be irreducible, but quite possibly large. What are the appropriate rules for decision-making under uncertainty?
- Any decisions about tolerability inevitably involve ethical considerations of environmental and social justice, the bounds of discourse and value judgements about the

weight given to various benefits and risks. For long-term and cumulative risks additional ethical concerns of responsibility and choice arise from the potential for decisions taken now to affect future generations.

Step 3: Hazard Identification

Hazard identification is the process of identifying 'what could go wrong'. It should be as comprehensive as possible – GM crops have a very wide range of potential effects, not all of which will be obvious, especially where long-term and cumulative aspects are concerned.

Hazard identification techniques

Hazard identification can be conducted against a standard checklist – for example the general types of potentially harmful characteristics and adverse effects listed in Annex II of Directive 2001/18. However, such approaches are 'closed' - comparison against non-exhaustive lists does not prompt consideration of what else might occur. It is better to complement them with a more open-ended brainstorming approach, and/ or with individual techniques that encourage more inductive thinking (see, for example, Hayes 2006)

Brainstorming should be structured, and led by an experienced facilitator, to ensure that it remains on track and covers all the aspects. Initially the aim should be simply to identify potential hazards, resisting the (time-consuming) temptation to begin to assess their likelihood or consequences. Analysis can be carried out later, in Step 4, by an individual or smaller group of assessors, when all the results have been collated and appropriate data sources can be sought.

The brainstorming participants should have experience covering all the potentially relevant disciplines, such as genetics, ecology, agriculture, veterinary science and human health.

Note that a 'hazard' is to some extent arbitrary – it can be defined at any level of detail, and at any stage along the chain or network of causes and effects that leads from the release of the GM crop to the harm. In brainstorming sessions, participants will usually mention 'hazards' at many different levels of detail and stages, such that some hazards will be subsets of others, and hazards will have interactions. If time permits, these relationships should be explored, but detailed analysis of dependencies is usually better performed in Step 4.

It is however important during the brainstorming session to obtain as clear an understanding as possible of the hazards that participants have in mind. Hazards should ideally be stated in terms of specific events that either do or do not occur, rather than generic processes. So for example, it is better to define hazards in terms such as 'introgression into wild relative, giving herbicide-resistance ' than simply as 'introgression of genes'.

Prompting hazard identification using Themes

It is suggested that first stage in brainstorming should be designed to broaden the scope of what is considered. Some forms of potential harm may be relatively obvious, such as the potential for a herbicide-resistant gene to enter a related weed species, conferring a selective advantage that allows it to become a problematic weed. But less obvious, indirect or

secondary effects may be particularly important when long-term and cumulative effects are considered, and these are harder to identify.

To encouraging thinking beyond the immediate and obvious it is recommended that the group should begin by considering whether the release can have any effect (positive or negative) on a wide-ranging list of environmental 'Themes'. Themes express the environmental goods and values that regulators aim to protect. Those in the Table 5 following, for example, were developed using as a starting point the Themes from the UK Environment Agency's draft corporate Vision (Environment Agency 2005), modifying them to be more specific to the present study.

Table 5. Example Environmental Themes

Theme	Description
Human health	Freedom from toxic or allergenic characteristics, from antibiotic resistance or other compromising of therapeutic techniques and from adverse nutritional effects
Animal heath	Freedom from toxic or allergenic characteristics, from antibiotic resistance or other compromising of therapeutic techniques and from adverse nutritional effects
Quality of life	Knowledge of living in a healthy environment, rich in wildlife and natural diversity, that can be cared for, used, appreciated and enjoyed. This Theme could also be extended to include socio-economic dimensions of environmental risk, such as the well-being of farming and rural communities.
Agricultural sustainability	Agricultural systems that maintain productivity, sustainability and resilience and ensure fair markets.
Environment for wildlife	Habitats improved in their extent and quality to sustainable levels for the benefit of all species.
Air quality	Clean air – levels of pollutants below the level at which they can do significant harm.
Water quality	Clean water - levels of pollutants below the level at which they can do significant harm. Water bodies sustain diverse and healthy ecosystems, fisheries, sports and recreational activities.
Land use and soils	Restored, protected land with healthy soils and biogeochemical cycles, supporting a wide range of uses, including production of healthy, nutritious food and other crops. Contaminated and damaged land restored and protected. Appropriate land uses – balancing conflicting demands to make best use of the limited resource.
Greener business	Sustainable business practices
Sustainable resource use	Wiser, sustainable use of natural resources, minimising waste production, reusing and recycling products and materials and making efficient use of energy and materials.

Theme	Description
Limit and adapt to climate change	Reductions in the emission of greenhouse gases and preparedness for the effects of changes in climate.
Regulatory effectiveness	Ability to regulate effectively, efficiently and fairly – good relations with those regulated and all stakeholders

It is recognised that the selection of Themes involves a number of (value) judgements about what constitutes a health or environmental theme, as opposed to an economic, social or ethical ones. The Themes used might vary according to the specific remit of the CA in each Member States, and the list above is intended only as an example. It should also be stressed that the aim of using the list here is simply to broaden discussion, not to set out specific criteria.

The participants in the brainstorming session should be asked to consider whether there could be a risk or benefit to each Theme, scoring Yes, Maybe or No for each. At this level, there is no need for a metric (scale) of risk or indeed any formal definition of risk. The aim is to broaden the thinking for later stages of hazard identification and risk assessment rather than to inform a decision directly.

In pilot studies and workshops, we found that it was often possible to think of some very indirect or unlikely mechanism by which the system being considered could potentially present a risk or benefit to any of the Themes. It is therefore suggested that some sensible cut-off should be set. One way to do this is to impose a time limit on discussion – for example that if no-one can identify an affect on the theme after two minutes' discussion, the score should be 'No'. While this seems to go against the general spirit of open-ended brainstorming, in which the aim is to identify risks as comprehensively as possible, it should be recalled that the aim is to widen the discussion by identifying broad classes of effects that could merit further analysis. It is not required at this stage to identify detailed combinations of failures, errors and circumstances that may lead to an effect.

Prompting hazard identification using FEP lists

It has already been noted that long-term and cumulative risks are not always easy to distinguish from shorter term ones. In many cases, the hazards are the same, but they differ in degree and in the greater level of uncertainty. To encourage participants to identify specific long-term and cumulative effects that would not occur in the short-term, some specific prompts may be helpful.

For example, in the workshop of Oct 2005 (Appendix 4) we asked participants to consider the various elements of a Source – Pathway - Receptor framework, and applied two sets of prompt words, as shown in the Table below, to help participants identify Hazards from changes in the source, pathway or receptor.

Step 4: Hazard Analysis

As already noted, hazard identification tends to produce lists of hazards at varying levels of detail, and that may overlap or interact to varying degrees. The task in Step 4 is to collate

these hazards on a consistent basis and understand the relationships and dependencies between them.

This analysis can often be most effectively performed using a graphical technique, such as the construction of a fault tree, event tree, influence diagram or other systematic representation of the network of causes and effects that is required for the release itself to lead to actual harm to the health and environmental Themes. Examples of such presentations are provided in the Case Studies.

The construction of such diagrams has been found to be very valuable in its own right, in forcing clarity of thinking about how potential harm can be realised and helping the assessor to identify the main pathways or contributors to risk. And, by showing what events, errors and circumstances have to conspire and what barriers have to fail for harm to occur, it provides a systematic basis for considering where risk can be mitigated in Step 8.

Table 6. Prompts for Identifying Long-term and Cumulative Hazards

FEPS Features Events & Processes that may change over time and affect Source, Pathway or Receptor	**Deviations** ways in which the FEPs may change
recessive genes dormant/ latent traits climate land use, soils habitats agricultural practices food consumption human activity population distribution regulation social, organisational and institutional structures and practices availability of information	More Than/ Less than Earlier/later Not Done Other Than Used Beyond Intent

Step 5: Assessing Likelihoods, Consequences and Risks

In general, risk can be expressed as a combination of the likelihood of some adverse effect, and the severity of that effect. For example, it could be expressed in terms of the probability of occurrence of certain defined hazards, or as the sum, over all hazards, of the expectation value of the severity of the consequences of those hazards. The specific definition and measures(s) of risk, and hence the ways in which it is evaluated in any particular assessment will have been decided in Step 1 and may also be determined by how the hazard analysis (Step 4) has been constructed.

Where adequate quantitative data are available, fault trees, event trees and other types of cause-consequence diagram as developed in Step 4 can be used to quantify likelihoods, consequences and risks. For example, in a fault tree, events combine through logical AND or OR gates to lead to the next level up the tree. (AND means that all events have to occur to pass to the next level, OR means that only one event has to occur.) The probability of an event can be derived from the probabilities of lower-level events using standard rules of probability and Boolean logic. For example, the probability of both event A and event B occurring, P(A AND B) can be evaluated as P(A) x P(B) (provided P(A) and P(B) are small).

If the probabilities of the 'base events' are known, or can be estimated, the probability of the top event can be derived.

In principle one could evaluate the risk to each Theme as the top event of a separate tree, but this would be very time-consuming, and there remains the problem of whether and how to combine the risks to each Theme into some kind of aggregate measure. It may often be better to use the Themes simply to broaden the consideration of potential hazards at the early stages, rather than necessarily carrying them through all the detailed analytical and quantification stages..

In most cases, a compromise has to be made between rigour and complexity. If hazards and their outcomes are explored in great detail, the analysis becomes complex and there is a 'combinatorial explosion' of data to be obtained and processed. On the other hand, too simplistic a representation is likely to miss important features. There are no easy rules to help set the balance at an appropriate point – usually it is a matter of starting with a broad-brush assessment and iteratively refining the analysis as the critical aspects become apparent.

In a field with as short a history, and as many uncertainties, as GMOs, it is unlikely that robust quantitative data will exist for many elements of the risk calculation. Assessors will therefore have to rely on subjective estimates in many cases, and use should be made of the many techniques available for ensuring that subjective data are elicited from individual experts or groups in ways that minimise, or at least reveal, uncertainty and bias.

Step 6: Assess Uncertainties

Assessors should give ranges of uncertainty, or other appropriate descriptions of the uncertainty in their risk estimates. As a minimum, the main areas of uncertainty in data, assumptions and models should be highlighted, and their potential to affect the evaluation discussed.

The use of sensitivity tests, best-estimate and worst-case scoping calculations should be considered.

If a quantitative analysis has been undertaken, formal techniques such as probabilistic or stochastic modelling (e.g. Monte Carlo analysis) can be applied, in which input data are specified using statistical distributions, rather than single point estimates, and the corresponding distribution of outputs (risk levels) is calculated.

Step 7: Compare Risk against Criteria

The risk as assessed in Step 6 should be compared against the criteria defined in Step 2, taking account of the discussion of uncertainties in Step 6.

The release may then be categorised as either acceptable as proposed, unacceptable, or requiring further work – such as a more detailed analysis, or further evaluation of mitigation and monitoring methods.

There are special issues arise in making decisions about cumulative risks - the regulator will need a mechanism to track and combine the risk assessments from multiple, and in some cases apparently unrelated, releases.

Step 8: Identification and Evaluate of Mitigation & Monitoring Methods

Dependent on the outcome of Step 7, further mitigation and monitoring methods may need to be identified to ensure that the risk is tolerable.

Methods may include those that reduce the likelihood of the hazards, and/ or that reduce the probability and / or severity of their consequences. Diagrammatic representations of how risks may be realised, such as fault trees (Step 4), can be helpful in providing a systematic framework for identifying and evaluating potential mitigations.

For each identified method, its likely effectiveness (including whether it may have any negative effects) and its practicability should be assessed. This can be carried out:

by simple subjective ranking, using a matrix of effectiveness and practicability;

by reference to fault trees etc – it may be possible to see which mitigations control the most critical links in the chains of cause and effect; or

by re-running the assessment. Note that it is necessary to return to the hazard identification step (Step 3) or even earlier, not just the later, analytical steps, since mitigations can introduce new hazards. For example, a reactive mitigation such as control of the problem GM variant using a more powerful herbicide is likely to have adverse effects on other flora.

4. Approaches to monitoring and management of risk

EU Directive 2001/18/EC requires all applicants seeking authorisation to release a GMO within the EU to elaborate their plans for monitoring and reporting statements made in their e.r.a. in a post-market monitoring (PMM) plan. This has two purposes, firstly to confirm assertions made in the ERA regarding the occurrence and impact of potential adverse effects of the GMO or its use, so called 'case-specific monitoring', and secondly to identify the occurrence of completely unanticipated effects of the GMO or its use on human health or the environment, termed 'general surveillance'. When consent to place a GMO on the market is granted, the consent holder has a legal obligation to ensure that the post market monitoring plan and reporting of its findings are carried out as specified. Whereas case-specific monitoring is only necessary if a specific potential risk has been described, commercial release of all GMOs must be accompanied by a general surveillance plan that extends over the full life of the consent to release the GMO.

4.1 Case-specific monitoring

Case-specific monitoring serves to confirm that any assumption made in the ERA regarding potential adverse effects arising from a GMO and/or its use are correct, for example to confirm that a GM insect resistant crop has no different effect on non-target insects than the conventional crop. Case-specific monitoring plans are therefore devised with specific objectives, parameters and timescales in mind and specific data will be collected to assess the potential impact/s.

4.2 General surveillance monitoring

While it is possible to predict that certain effects may occur based on risk assessments and scientific data, the prediction of unanticipated (direct or indirect) long-term and/or cumulative effects does not lend itself to the formulation of clear scientific hypotheses. General surveillance therefore has its basis in long-term observation of the wider receiving environment, often making use of existing monitoring and stewardship programmes that will detect significant changes at the field, farm and landscape levels that may be related to specific GM releases. This may include observing the agricultural/rural environment and assessing biodiversity of weed and feral crop populations, vertebrate and invertebrate populations, changes in behaviour of natural populations (e.g. flowering and seed set, breeding patterns), changes in the soil ecosystem, changes in plant pest and disease incidence, changes in pesticide usage and changes to general land management. Although general surveillance takes a much wider view than case-specific monitoring, it is still undertaken in the context of what might be seen in the ten years duration of the marketing consent.

Company plans for surveillance tend to focus on utilising data gathered by existing monitoring programmes and networks, and engaging those who work in the agricultural environment such as farmers, agricultural consultants and grain handlers, asking them to note any unusual or unexpected occurrences when growing or handling GM crops. In designing the monitoring plan, the extent to which geographical areas around the GM crops must be included in the monitoring is also a key consideration.

General surveillance will also be useful for monitoring effects of potential interactions between two genetically modified crop lines. While these can to some degree be anticipated, commercial companies cannot predict which varieties of GM crops may be grown in close proximity to each other and what effects might arise from their interactions. Again, this would only continue for the duration of the consent.

4.3. Data gathering for post market monitoring

The key to identifying and evaluating any changes in the receiving environment as a result of cultivating a GM crop is to establish the baseline status of the receiving environment for the component being studied. Ideally this should be established well before the GMO has been placed on the market. The consent holder must establish which networks and other routes they will utilise and enter into arrangements with these bodies for gathering the necessary data; they must then critically analyse the findings and report their conclusions to the competent authorities on an annual basis. To be of value the data must be relevant to the question asked, of good quality, and in sufficient quantity to provide adequate statistical power for meaningful assessments. Ideally, the data should be appropriately described by metadata and be accessible in a format suitable for direct input to statistical software.

4.4. Environmental monitoring data in existence

A vast quantity of environmental data currently exists, much of which is available via the internet. However, the sheer volume of data and the range of methods of access can be barriers to efficient use of data. The information available via the internet can be broadly divided into four categories, although the distinctions between these are not clear-cut and some sites will fall into more than one category:

- Web based data portals that enable users to search for and obtain data.
- Metadata gateways that have information and links to external sources of data, but no data provision.
- Access to data held on the site.
- Information about data owned by the organisation, but no access to datasets.

The form in which data is presented is enormously varied and it may be freely available, or only accessed for a fee. Sites that present information only rather than datasets may be of limited value, but may be important in terms of highlighting the existence of data which may be either available elsewhere or not freely available. Many organisations make their own data available either on their own websites or held as part of larger collections of data. There are also datasets that are gathered as part of ongoing research projects and for which little internet-based information is available. Given the range of monitoring programmes being undertaken by different bodies, and the range of components that could be studied, it is easy to see that competent authorities will receive a varied collection of data and analyses from consent holders.

4.5. Review of current means of monitoring and mitigation

Methodology
Monitoring plans submitted with applications to release GT73 oilseed rape, Bt11 maize and EH92-527-1 potato under EU Directive 2001/18/EC were reviewed. Based on the applicants' conclusions in the ERA, we speculated as to what monitoring might be undertaken to identify

long-term cumulative effects[4]. Monitoring plans were reviewed according to guidance notes to 2001/18[5], which state: "Potential adverse effects of GMOs will vary from case to case, and may include:

- disease to humans including allergenic or toxic effects,
- disease to animals and plants including toxic, and where appropriate, allergenic effects,
- effects on the dynamics of populations of species in the receiving environment and the genetic diversity of each of these populations,
- altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or creating new reservoirs or vectors,
- compromising prophylactic or therapeutic medical, veterinary, or plant protection treatments, for example by transfer of genes
- conferring resistance to antibiotics used in human or veterinary medicine,
- effects on biogeochemistry (biogeochemical cycles), particularly carbon and nitrogen recycling through changes in soil decomposition of organic material".

C/NL/98/11 Roundup Ready oilseed rape, event GT73 (Monsanto – updated version July 2003).
Trait: Tolerance to the herbicide glyphosate ('Roundup Ready').
Scope: The notification covers the importation and storage of GT73 oilseed rape and its use as feed as well as in the processing for feed, and its industrial uses as or in products.
Current status: European Commission decision 31 August 2005[6]; consent not yet issued.
Note: Originally submitted in 1998 under Directive 90/220/EC, which did not require elucidation of monitoring plans, these have been added in subsequent submissions to the lead CA.

Proposals for case-specific monitoring
The notifier claims that the results of the e.r.a. show effectively zero overall risk arising from the placing on the market of GT73, therefore case-specific monitoring is not considered applicable to the placing on the market of this product.

Several Member States commented that the prevention of seed spillage and the consequences of it happening have not been sufficiently taken into account, and more information is needed. European Commission document 2005/637/EC[7] addressed these concerns by advising: "specific technical guidelines should be added to the decision to place GT73 oilseed rape on the market to prevent any damage to the environment in case of accidental spillage of the product".

Proposals for general surveillance
The notifier will ensure that awareness of the GM crop is made widely available by providing key information, for example:

- The notifier will provide international traders with the necessary information to comply with statutory requirements relating to the placing on the market of the crop;
- Product briefings to selected networks;

[4] It was not within the scope or resources of this project to critically assess the ERAs, or to evaluate speculative suggestions for monitoring against scientific evidence.
[5] Document number 2002/623/EC. OJ L200 pp 22-33 30/07/2002.
[6] Document number 2005/635/EC. OJ L228 pp 11-13 03/09/2005. Section 4.2.1. Step 1: Identification of characteristics which may cause adverse effects
[7] OJ L228 pp 19-20 3/9/2005.

- Technical literature (e.g. product-specific information, company contact details).

Further information and relevant legislation will be available from a number of sources, including industry and government websites, official registers and government publications.

Surveillance information will be collected from 1) feedback from selected networks, and 2) reported potential adverse effects and other relevant information received via direct contacts with the notifier. Where unanticipated adverse effects are confirmed they will be investigated to establish if there is a correlation between the observed effect and the GM crop, and if so appropriate remedial action will be carried out.

Suitability of monitoring plan for GT73 for identifying short and long-term cumulative risks:

- Short-term risks: consistent with other authorisations for import and processing.
- Long-term cumulative: key risks are associated with unmitigated spillage of GT73 rapeseed along roadsides, railways, waterways, leading to establishment of localised feral populations of GT73 and possibly gene transfer into sexually compatible species. Risks of reduced biodiversity in areas where this persists, and possible economic risks to commercial oilseed rape crops.
- Consent holder will be required to monitor spillage, and to encourage reporting of unexpected adverse effects as part of the general surveillance plan. How effectively this will work in practice away from the port environments is yet to be tested. Monitoring will only operate for the duration of the release.

Table 7. Summary of ERA/Proposed monitoring and possible monitoring for GT73 0SR (C/NL/98/11)

Risk component	**In ERA?**	**Monitor?**	**Proposed monitoring method**	**What could be monitored to detect/mitigate long-term cumulative risks?**
General surveillance monitoring only				
Spillage of GT73 oilseeds	✓	✓	Specific technical guidelines to be issued regarding prevention of spillage. HACCP[8] guidelines should ensure spillages are correctly cleared up.	Surveys of OSR populations along roadsides, railways, waterways. Monitor for presence of GT73. Monitor extent of feral populations and monitor presence of GT73 in nearby weedy communities. For conventional OSR crops – monitor compliance with labelling thresholds, and link spatially with known spillages.
Direct/indirect and anticipated and unanticipated effects:				
Human toxicity and allergenicity	✓	×	Traders and bulk processors requested to inform relevant authorities of any adverse effects on the environment or human health	Monitor for chronic toxicity &/or allergenicity through prolonged operator exposure, e.g. at ports and crushing plants.
Potential to compromise human therapy	✓	×	None	No antibiotic resistance in GT73, so unlikely to be an issue.
Potential beneficial effect to humans	✓	×	None	EU quality of life indicators.

[8] HACCP - Hazard Analysis at Critical Control Point.

Animal toxicity and allergenicity	✓	×	Traders and bulk processors requested to inform relevant authorities of any adverse effects on the environment or human or animal health	Monitor for chronic toxicity &/or allergenicity through feeding studies; questionnaire attached with feed containing GT73 meal.
Compromised animal therapy	✓	×	None	No antibiotic resistance in GT73, so unlikely to be an issue.
Potential beneficial effect to animals	✓	×	None	Would be identified by traders and bulk processors if have monitoring scheme in place.
Disease to plants	✓	×	None	GT73 rapeseed must only be transported in leak-proof lorries/containers. Monitor closely for spillage events and control of volunteers at ports, along roadsides, railways and waterways to prevent feral populations establishing. Penalties on operators if they do not comply.
Target organisms (incl. predators and parasitoids)	✓	×	None	Ditto above
Effects on non-target organisms	✓	×	None	Ditto above
Effects of gene-transfer (incl. to soil organisms)	✓	×	None	Ditto above
Potential for increased weediness	✓	×	None	Ditto above
Altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or new reservoirs or vectors	✓	×	None	Ditto above
Horizontal gene transfer	✓	×	None	Ditto above
Effects on biogeochemistry, particularly carbon and nitrogen recycling through changes in soil decomposition	✓	×	None	Ditto above

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C/FR/96/05/10 - Bt11 maize (field or sweet maize) (Syngenta Seeds SAS)
Trait: Resistance to corn borers *Ostrinia nubilalis* and *Sesamia nonagrioides* using the Cry1Ab gene.

Scope: For use as any other maize, including cultivation in the EU. Bt11 also possesses herbicide tolerance but the scope of the notification has been limited to use without the application of glufosinate ammonium herbicides.
Current status: pending.

Case-specific monitoring
Based on the e.r.a. the notifier concluded that no case-specific monitoring was necessary; however, in order to delay insect resistance to Bt11, an Insect Resistance Management plan would accompany Bt11. The monitoring plan was divided into four parts:

- Maintenance of refuges (20% in the case of holdings with over 5 ha of maize);
- Monitoring of Bt resistance;
- Action plan in the event of resistance being detected;
- Training for producers.

Some Member States have commented that case-specific monitoring should also be undertaken to consider the impact of insect resistance on non-target insects

General surveillance
The proposed general surveillance plan includes an assessment of the impact of Bt11 on non-target arthropods and the impact of tolerance to the herbicide, but its implementation in practice has still to be finalized.

The original proposal for general surveillance was revised in May 2004. The revised proposal includes more detailed plans for surveillance 'modules' operating within existing surveillance 'nodes' in agriculture, the environment (agro-environment and wider environment) and the supply chain for living Bt11 maize grains. A detailed farmer questionnaire has been developed to collect relevant information from farmers relating to environmental and agronomic aspects of the GM crop (questions specifically cover agricultural practices, general observations of the crop and corn borer infestation). Farmer data will be collected from a structured subset of farmers within a representative geographical area and participant numbers will be sufficiently large to allow for meaningful data analysis. A questionnaire will also be sent to farmers in the year following cultivation on Bt11 maize. The exact network will be defined in advance of commercialisation of Bt11 maize in specific regions, Syngenta will coordinate and report the monitoring; all data will be compiled in a 'general surveillance database'. It is not clear how the general surveillance monitoring will work in practice and how the various networks involved will be compelled to participate.

Suitability of monitoring plan for Bt11 maize for identifying short and long-term cumulative risks:

- Short-term risks: currently proposals will only identify development of resistance in target organisms. Will not identify any indirect effects on non-target populations. Farmer questionnaire will provide information regarding on-farm effects.
- Long-term cumulative risks: key risks are likely to be associated with target and non-target organisms; on-farm monitoring may extend for 1 year beyond the life of the consent in the form of farmer questionnaire.

Table 8. Summary of ERA/Proposed monitoring and possible monitoring for Bt11 (C/FR/96/05/10)

Risk component	In ERA?	Monitor?	Proposed monitoring method	What could be monitored to detect/mitigate long-term cumulative risks?
Case-specific monitoring				
Development of insect resistance	✓	✓	Unknown. Target insect resistance to Bt toxin will be monitored as part of Insect Resistance Management Plan, but monitoring procedures not specified	Measure resistance in populations of target insect (*O. nubilalis* and *S. nonagrioides*) in areas of Bt11 cultivation
Effect on non-target organisms	✓	×	Still to be finalised. Predators and parasitoids are specifically mentioned in ERA but monitoring methods are not specified	Monitor altered predator-prey interactions; altered population dynamics (e.g. altered fecundity, altered behaviour). Monitor populations of nominated indicator species in areas of Bt11 cultivation
Geneflow to wild relatives (incl. production of 'superweeds'	✓	×	None – there are no sexually compatible native species in Europe	Not applicable
Problem volunteers	✓	×	None – maize volunteers are not considered a problem in Europe	Monitor volunteer numbers/density; monitor susceptibility of volunteers to herbicides
General surveillance				
Human toxicity	✓	×	None	Assumed to be safe. Will be monitored by CAs.
Human allergenicity	✓	×	None	Monitor for chronic allergenicity e.g. in farmer workers, processing operators etc. Should be identified by farmer questionnaire
Human nutrition		×	None	Assumed to be equivalent to conventional maize. Will be monitored by CAs.
Antibiotic resistance – compromised human therapy	✓	×	None	Will be monitored by CAs.
Benefits to humans	✓	×	None	EU quality of life indicators
Animal allergenicity	✓	×	None	Grower and veterinary observation/records
Animal toxicity	✓	×	None	Mortality or morbidity of farm animals; animal pathology. Could extend questionnaire to feed merchants
Animal nutrition	✓	×	None	Milk yields; live weights; reproductive capacity; produce (e.g. milk or meat) quality. Could extend questionnaire to feed merchants
Antibiotic resistance - compromised animal therapy	✓	×	None	Veterinary observation/records
Benefits to animals	✓	×	None	Extend questionnaire to feed merchants

Plant pathogenicity	✓	×	None – but farmer questionnaire could be extended to cover this	Note: Two components used to generate the transformation event are derived from plant pathogenic sequences (tNos derived from *Agrobacterium tumefaciens*; p35s derived from Cauliflower Mosaic virus)
Altered plant susceptibility to pathogens	✓	×	Farmer questionnaire (reduced *Fusarium* infection expected)	Fungicide usage records; Grower/agronomist observation & records
Altered plant physiology/ physiognomy		×	Farmer questionnaire	Altered growth habit; altered flowering time; altered seed viability; possible effect of gene recombination on GM gene/endogenous gene interaction
Potential beneficial effect to plants	✓	×	None	Could be identified by farmer questionnaire
Development of insect resistance	✓	✓	Case-specific monitoring (detailed proposals not available)	Sampling and LD50 testing of *O. nubilalis* and *S. nonagrioides*
Effect on target organisms	✓	✓	Case-specific monitoring (detailed proposals not available)	Sampling and LD50 testing of non-target feeding Lepidoptera (especially any that feed on maize or other Gramineae)
Effect on non-target organisms	✓	×	None	See case specific monitoring above
Potential for horizontal gene transfer	✓	✓	None	Sampling and testing soil micro-organisms
Potential for increased weediness	✓	✓	Farmer questionnaire?	Monitoring of flora in fields and surrounding areas; grower / agronomist observation & records
Effects on biogeochemical processes (incl. carbon and nitrogen cycling)	✓	×	None	Effect of Cry proteins from breakdown of plant parts on soil biota; effect of Cry proteins on decomposition rates; effects of herbicide on soils (soil biota, nutrient cycling, soil structure). Composition of runoff to water courses
Management effects	×	×	Farmer questionnaire?	Effect of possible monocultures/continuous same species cropping on wildlife; altered pesticide regimes; soil compaction
Demographic changes	×	×	EU-wide data	Altered consumer trends; changes in employment characteristics

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C/SE/96/3501 Potato line EH92-527-1 (Amylogene HB)

Trait: Modified starch content.

Scope: Cultivation of potato clone EH92-527-1 and use for the extraction of starch for industrial uses, and the use of the by-products of starch extraction in animal feed.

Current status: Pending.

Case-specific monitoring
The case-specific monitoring will focus on (1) any significant detrimental changes in the composition of the tubers and (2) a change in the presence of the bleomycin resistant protein associated with ORF4. Monitoring will comprise verification of the following assumptions of the risk assessment:

- the genes of interest remain stably inserted,
- the ORF 4 is not expressed at the protein level, and
- the starch composition and the glycoalkaloid content are stable.

The assessment report of the Swedish CA states that the notifier has developed methods and accounted for sampling and analysis. The suggested case-specific monitoring will continue for five years, after which the monitoring plan will be evaluated and may be extended or altered. The Swedish Board of Agriculture (SBA) concluded that in particular it is important to study how the glycoalkaloid content may vary depending on the place of cultivation or weather conditions. In addition, the SBA state there is very little reason to expect that the possible expression of a protein from ORF4 would result in negative health effects in animals that will eat the by-products of the potato. The SBA conclude that it is reasonable that monitoring is designed to fulfil the most important criterion in this context, that is to detect and avoid the unlikely scenario that ORF 4 causes a protein to occur in a significant share of the potatoes.

General surveillance
The general surveillance plan considers areas related to (1) growth characteristics of the plant (including time to flowering, abortion of flowers, and time to maturity), (2) general characteristics of the plants (including height, shape and colour of the foliage, size and shape of the plant), (3) susceptibility to disease and pests and (4) any changes in animals within and in proximity to the fields. Farmers who cultivate and/or use by-products of EH92-527-1 in feed will be required to note all possible unexpected effects. The notifier will supply the appropriate forms for this purpose. General surveillance will also monitor the spread of potatoes outside the field. Management of volunteer potatoes will be carried out according to standard agricultural practice. The general surveillance plan will run throughout the 10-year period of the consent. The base-line information consists of data that the notifier has gathered during previous years of cultivation and analysis. A general surveillance questionnaire to all growers and handlers of the potatoes has not been proposed.

Sampling plan
The applicant has a strategy for introduction of EH92 potatoes and has developed a clear plan for areas of production of seed potatoes and a sampling strategy at sites of production. Sampling strategies must ensure homogeneity and will be undertaken according to European (CEN) standards, further advice will be taken from the 'Ecological Monitoring and Assessment Network', and e.g. ISTA and the EC's Joint Research Centre.

EH92 post market monitoring sampling plan:

Object	**Parameters**
Seed potatoes (80 pooled samples)	PCR- identity Absence of ORF4
Production potatoes (20 locations, 4 pooled samples each)	Starch composition Glycoalkaloid level

	Carbohydrates Protein
Seed potato production sites (5 per year)	Plant characteristics Susceptibility to pests and disease
Production sites (20 new sites per year, cumulative)	Persistence in field Persistence outside of the managed field
Production sites (all sites, verification by the growers)	Plant characteristics

Suitability of monitoring plan for EH92 for identifying short and long-term cumulative risks:

- The scope of the notification includes cultivation and use for industrial purposes, and feeding of the by-products of processing to cattle. It is not intended for direct human consumption, but it cannot be completely excluded that humans will consume the potatoes. The post market monitoring plan includes a case-specific element to confirm the stability of the inserted genes (*nptII* and non-functional ORF 4) and to ensure that the content of starches and glycoalkaloids remain stable, general surveillance will include monitoring growth characteristics and pests and disease susceptibility. The notification has received favourable assessments for being very thorough and for the applicant's commitment to post market monitoring. The applicant has collated 9 years of baseline data regarding the performance of EH92 relative to the parental variety.
- The biological characteristics of the potato and the relatively benign nature of the trait make it difficult to anticipate long-term or cumulative effects arising as a result of cultivation of this crop; the comprehensive post market monitoring plan is designed to identify any unanticipated effects of cultivation in the short-term. Use of a farmer questionnaire may be advisable to identify any changes in management practice associated with cultivation of EH92, such as altered use of herbicides or pesticides.
- The main risks possibly lie in the co-mingling of EH92 with food potatoes, for which it is not authorised, however the strict Identify Preservation scheme should ensure against this and enable rapid risk management if co-mingling should occur.
- Longer-term cumulative impacts may lie in economic benefits, for example farmers may be able to secure long-term production contacts, or the increased supply of high quality raw materials may boost local industries with knock-on benefits to the local economy. There may also be potential benefits from the slight reduction in glycoalkaloid levels, which may improve the quality of EH92 pulp for cattle.

Table 9. Summary of ERA/proposed monitoring and possible monitoring for potato clone EH92-527-1 (C/SE/96/3501).

Risk component	**In ERA?**	**Should monitor?**	**Proposed monitoring method**	**What could be monitored to detect/mitigate long-term cumulative risks?**
Case-specific monitoring				
The genes of interest remain stably inserted	✓	✓	Event specific identification of inserted sequences (Southern blot or PCR)	Continue to monitor for whole duration of consent.
ORF 4 is not expressed at the protein level	✓	✓	Western blot to confirm absence of expression of peptides derived from ORF4	Continue to monitor for whole duration of consent.

Starch composition is stable	✓	✓	Use of standard methods to monitor ratio amylose to amylopectin as indicator of quality and stability	Continue to monitor for whole duration of consent.
Glycoalkaloid (GA) content is stable.	✓	✓	Use of standard method to monitor GA level & ensure it remains within range documented for starch potatoes	Continue to monitor for whole duration of consent.
General surveillance				
Human toxicity	×	×	None	N/A EH92 will not be authorised for food use. Strict control over Identity Preservation scheme to ensure EH92 does not enter the food chain.
Human allergenicity	×	×	None	N/A EH92 will not be authorised for food use. Strict control over Identity Preservation scheme to ensure EH92 does not enter the food chain.
Human nutrition	×	?	None	Inadvertent use as food potato could affect insulin levels in diabetics if consumed over a long period. Strict control over Identity Preservation scheme to ensure EH92 does not enter the food chain.
Antibiotic resistance – compromised human therapy	×	×	None	EH92 is not intended for direct human consumption. Strict control over Identity Preservation scheme to ensure EH92 does not enter the food chain.
Benefits to humans	×	×	None	Possible economic benefits to farmers.
Animal allergenicity	×	✓	None	Inclusion of specific question in general surveillance programme about possible adverse effects on cattle fed with pulp derived from EH92.
Animal toxicity	×	✓	None	
Animal nutrition	×	✓	None	
Antibiotic resistance - compromised animal therapy	×	✓	None	
Benefits to animals	×	✓	None	Inclusion of specific question in general surveillance programme about possible beneficial effects on cattle fed with pulp derived from EH92.
Plant pathogenicity	×	×	None	Promoters are derived from *Agrobacterium tumefaciens;* no viral promoters are used.
Altered plant susceptibility to pathogens	✓	✓	Seed production sites (5 per year) and all production sites (growers to verify) will be monitored for susceptibility to pests and diseases	Could derive further information from a farmer questionnaire. Monitor for duration of the consent.

Altered plant physiology/ physiognomy	✓	✓	Seed production sites (5 per year) and all production sites (growers to verify) will be monitored for plant characteristics	Could derive further information from a farmer questionnaire. Monitor for duration of the consent.
Potential beneficial effect to plants	×	?	None	Any unanticipated beneficial effects to EH92 could be identified through farmer questionnaire.
Development of insect resistance	×	×	None	Trait is not related to insect tolerance so no need to monitor.
Effect on target organisms	×	×	None	There are no target organisms so no need to monitor.
Effect on non-target organisms	×	✓	None	Changes in glycoalkaloid levels may alter palatability to insects and other organisms (larvae, slugs, rodents etc), this may alter within-field populations in the long-term. Monitor populations of invertebrates and vertebrates feeding in and around crops of EH92.
Potential for horizontal gene transfer	×	×	None	Kanamycin resistance is ubiquitous in soil. Transfer of *gbss* gene to wild relatives or other cultivated potatoes considered highly unlikely in northern Europe. Widening of area of cultivation to southern Europe could lead to increased potential for horizontal transfer of *gbss* as potatoes can become weedy and wild relatives occur – may need to re-assess ERA and PMM plan in this case.
Potential for increased weediness	✓	✓	20 production sites will be monitored each year for persistence in the field (monitored for 4 years) and outside of the field (monitored for 2 years).	Monitoring is appropriate; the 20 sites must be fully representative of the areas in which the potatoes are being cultivated. Monitor for duration of the consent.
Effects on biogeochemical processes (incl. carbon and nitrogen cycling)	×	?	None	Potato does not have a substantial role in soil processes; altered starch composition may alter degradation profile in the long-term. Monitor soil biota.

Management effects	✓	✓	General surveillance programme.	Monitor herbicide use in fields where EH92 has been grown; monitor changes in cultivation practice. Possible scenario: increased numbers of smaller tubers may lead to increased numbers groundkeepers following harvest. This would necessitate increased use of herbicides where EH92 has been grown to control any volunteers. Potential for adverse impacts on plant populations in the field and organisms reliant on the plants as a food source. This scenario may also lead to increased cultivation of fields that have grown EH92, leading to disruption of soil integrity.
Demographic changes	×	×	None.	Map cultivation of EH92 relative to starch production factories to confirm the applicant's assumption that EH92 will only be grown in close proximity to these. Market forces will determine contracts for production. EU socio-economic data.

4.6. Summary

General surveillance aspects of post market monitoring plans are intended to identify unanticipated effects of release of a GM crop, success here will depend on establishment of effective mechanisms for collating and comparing baseline data for nominated key indicators over a period of time, and being able to identify any significant changes in baseline values, these can then be further investigated for causal linkages. Data would need to be available in a format that is spatially and temporally appropriate for statistical analyses. Current post market monitoring programmes are focussed on identification of adverse/beneficial effects that may occur within the 10-year period in which the crop is marketed, monitoring by the consent holder in connection with a specific release will substantially cease soon after the consent expires. The monitoring plans for GT73, Bt11 and EH92 confirm this; there is little scope for identification, by the consent holder, of any effects associated with a specific release once this period is completed. Monitoring for cumulative long-term effects is expected to encompass even broader themes than general surveillance; it is possible that collation and analysis of data for identification of these effects would be more effective if undertaken by, or on behalf of, competent authorities as part of higher-level national environmental surveillance initiatives.

5. Findings & Recommendations

5.1. The State of the Art

A review of the scientific literature on long-term and cumulative risk assessment has illustrated the paucity of scientific evidence in this area. Experimental evidence is largely based on laboratory and glasshouse experimentation, which can only tentatively be used to extrapolate to the field in the medium to long-term. Very few monitoring studies have been carried out over longer time periods.

Many risk assessment frameworks are currently available for risk assessment of the release of GM crops. These tend to follow a similar approach based on a case-by-case scientific framework aimed at assessment of hazards, potential for exposure and consequences of exposure, followed by consideration of risk management; very few of the frameworks reviewed specifically consider long-term and cumulative risks. The EU Directive states that these long-term and cumulative risks must be assessed, but gives limited guidance and there is limited evidence that it is actually done. The Australian Office of the Gene Technology Regulator (OGTR) 'Risk Analysis Framework' is the most recent publication and probably provides the greatest level of accessible guidance for completing all stages of the risk assessment. The New Zealand and USA approaches are also very detailed. However, they provide limited guidance specifically for consideration of cumulative long-term risks.

The European approach is noticeably focussed on the GMO and the underlying science, while other approaches focus on the risk assessment methodology. Methodologies for assessing risks of the impacts of plant pathogens or pests and non-native species tend to consider the longer-term cumulative impacts of the release or introduction of new species and these may provide some useful insight for GM crops.

Of the competent authorities that responded to the questionnaire, most considered the EU risk assessment framework to be adequate; most also had a clear view of the meaning of cumulative long-term effects and how this might be approached in practice, although none had developed a clear monitoring strategy. In this respect it was unfortunate that none of the countries that are currently growing GM crops could be contacted. Gaps in regulators' stated requirements were identified, particularly with respect to presentation of monitoring plans following the release of a GMO, however this was generally thought to be due to lack of precedent, and possibly not always the applicant's fault (i.e. better guidance is needed). None of the respondents had developed their own risk acceptability criteria and most abide closely by the criteria set in 2001/18 while also taking national policy goals into consideration.

5.2. The Proposed Risk Assessment Framework

A risk assessment framework has been developed, refined and demonstrated in a number of case studies. The steps in the framework follow a standard risk assessment methodology. It begins by framing the question: defining the boundaries of system to be assessed and the types and measures of risk to be considered. Criteria for judging the tolerability of the risk are then defined. Hazards are identified and analysed, and the risk evaluated and compared against the criteria. Additional risk mitigation and monitoring measures can then be identified and evaluated, if seen to be required. Tools and techniques have been identified that can support the various steps in the framework. These are available at different levels of depth

and detail, allowing users to select a method appropriate to the decision stakes and the data available.

The assessment structure and the tools and techniques suggested are thus firmly based on approaches that have been extensively used and proven in other domains. Experience with the case studies suggests that the proposed approach is intelligible and usable, given the inevitably complex nature of the risks it assesses, and that it does help to clarify discussions about long-term and cumulative risks.

The real value of the framework may be more in terms of *process* than *product*. The framework provides a structure for a systematic discussion between stakeholders that leads to better understanding and insight, in addition to its more obvious 'results' in terms of risk evaluations. It should be seen as an aid to decision-making under uncertainty rather than a clear-cut procedure to test a proposed release against prescribed criteria.

It is anticipated that the framework would be used by the CAs and made available to applicants, so that everyone is working to the same procedures, and that therefore all parties should come out with the same (or similar) assessment. Long-term & cumulative risk assessment is in effect only one aspect of the e.r.a., therefore, the effort that the risk assessment requires needs to be proportionate to the effort required for the preparation of the current e.r.a. and to the level of risk identified. Thought needs to be given to implications for the 'short-term' e.r.a. as the framework proposed in this report is more detailed than the existing, relatively general guidance on 'short-term' e.r.a.

5.3. Key Issues in Applying the Proposed Framework

Although elements of the framework have already been used in some GM risk assessments, it's the systematic application of the full approach is relatively new and uncommon. While the tools and techniques will be familiar to most risk assessment practitioners, their application to the GM domain does raise specific challenges, as follows, that will need to be kept in focus when applying it.

Once released into the environment there is a possibility that the risk from a GM crop could increase – for example if an invasive species is given an additional selective advantage. It is difficult to identify, understand or forecast what human or ecological mechanisms might eventually control the risk. Regulators should not set an arbitrary cut-off time on assessments – it should be demonstrated on a case-by-case basis how the risk is expected to change over time and when the peak risk will have passed.

There is extreme uncertainty in the prediction of the nature, likelihood and severity of long-term and cumulative effects. The environment is a complex, highly interactive system and, almost by definition, we can have no direct means to verify the predictions about long-term and cumulative risks before a decision has been made. Tools such as stochastic analysis are available to manage and analyse the effects of such uncertainties within the confines of the risk assessment itself, but wider issues are raised regarding the use of risk assessment within decision-making.

Over very long timescales, uncertainty increases, such that the value of risk assessment as a tool for quantitative prediction diminishes and may ultimately become meaningless. This shifts the emphasis away from quantitative predictions about what is expected to happen towards a broader, more qualitative consideration of 'what-if' scenarios. The applicant

needs to demonstrate that, for a range of scenarios spanning the range of credible futures, the environmental mechanisms and barriers to risk will continue to provide adequate assurance of environmental safety. The emphasis should be on considering the crop within the whole environmental system, and the effectiveness of barriers to harm, rather than detailed modelling of those aspects that can be quantified.

5.4. Research Gaps and Possible Future Work

There are a number of significant outstanding issues that would make practical application of the assessment framework problematic. With the current state of knowledge, it is unlikely that risk assessments can be performed with sufficient confidence to support robust regulation and decision-making. A number of further work packages (WPs) have therefore been identified that would help to close these gaps, as follows:

WP1: Obtaining Baseline Data. There is a need for good baseline data if monitoring is to be able to detect changes. A study is required to advise on the best indicators of long-term/ cumulative changes. There are also institutional and other practical aspects of implementation to be considered. For example, who should carry out the baselining, and how would it relate to ecological baselining for other purposes?

WP2: Ranking the Risks of Generic Crop-Trait combinations It could be more cost-effective, for society as a whole, if some risk assessment work were to be done at a higher level than release-specific e.r.a.s. Ranking crop-trait combinations (or other types of scenario) in broad order of inherent risk, would help both regulator and industry to ensure proportionate levels of depth and detail in risk assessments. It might also lead regulators to issue guidance indicating any broad classes of combinations that would be unlikely to be authorised, preventing wasted effort by all parties. This WP would apply the assessment framework in order to establish whether certain crop-trait combinations are inherently riskier than others.

WP3: Introgression into and Ecology of Wild Relatives Currently, the mechanisms of introgression into wild relatives are not understood in sufficient detail to enable the likelihood of introgression to be determined with confidence. Neither is there, in general, a good enough understanding of the ecology of wild relatives, and hence of how undesirable traits might spread in the environment. Research is required to improve the understanding of introgression and of the ecology of wild relatives, in sufficient detail for risk assessment.

WP4: Improved hazard identification for long-term and cumulative effects. It is difficult to identify hazards that are fundamentally different from those that can occur in the short-term. In most cases, long-term and cumulative hazards identified in this study were the same as the short-term ones, although the degree of risk associated with the hazard could be greater - given a longer time or more widespread planting, the likelihood and extent of undesirable effects may increase. Nevertheless, some genuinely different hazards can be identified. These tend to arise from rather subtle and complex combinations of factors, which would only be manifested in the long-term or as a result of widespread releases and plantings. The process of identifying long-term and cumulative risks could be made more efficient and effective if standard hazard-identification techniques (brainstorming, failure analyses, HHA etc) could be enhanced to focus more quickly on such genuinely long-term and cumulative effects.

WP5: Tolerability Criteria. The absence of explicit criteria in the Directive for judging whether a risk is tolerable, and the apparent variations in practice between Competent Authorities, point to a need to develop some more explicit guidance on criteria that will encourage well-founded, consistent and fair decisions. The aim of this WP would be to develop justifiable criteria for tolerable risk, and guidance on their application.

WP6: Tracking, Combining and Monitoring Cumulative Risks In considering cumulative risks, the EC will need mechanisms to track and combine the risk assessments from multiple, and in some cases apparently unrelated, releases. There is a need to explore the scientific and institutional issues involved in tracking multiple release assessments and consents and to develop guidance. The study could consider both scientific aspects (how to identify any potential for cumulative risk) and institutional aspects (processes and procedures, IT issues)

WP7: Stakeholder Engagement in Risk Assessment In other controversial debates about risk and the environment, there has been a growing realisation of the importance of considering social and ethical aspects. Stakeholder participation in framing the question and defining assessment methods is being given serious consideration and has occurred in some cases, in addition to the more traditional model, in which stakeholders review and challenge assessment results and assertions. Risk assessment should be used to provide a structured framework for discourse and decision-making under irreducible uncertainty, rather than as a technical and scientific exercise alone. Deliberative processes have been used in other domains, and this experience could be adapted and applied to the GM domain.

Appendix 7 provides some further details for each of these WPs. For each, we have documented why it is needed, given an initial specification of the objectives and scope, indicated the tasks and outputs and provided indicative timescales and costs.

Many of the WPs suggested above would also have benefits in improving the quality of current, 'short-term' risk assessments – the same difficulties arise, although not always with such severity.

Decisions about these WPs and their priorities will need to be made in the light of wider considerations of EU policy and EC research programmes. However if the priority were, for example, to issue *some* guidance to industry and regulators as soon as possible (accepting that may scientific and risk management challenges remain to be solved), we would suggest that WP 4 and WP5 deal with the most urgent needs and would provide the greatest value in the short-term. WP4 would help to resolve the difficulty of identifying long-term and cumulative effects, which are often subtle and hidden. WP5 would address the current absence of any clear criteria for judging the tolerability of risks, and so encourage well-founded, consistent and fair decisions.

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List of figures

List of tables

References

Ayyub, B. M. (2001). Elicitation of Expert Opinions for Uncertainty and Risks. CRC Press

Cartagena Protocol on Biosafety to The Convention on Biological diversity (2000). http://www.biodiv.org/doc/legal/cartagena-protocol-en.pdf

Conner A.J., Glare T.R., Nap J.P.(2003). The release of genetically modified crops into the environment. Part II: Overview of ecological risk assessment. *The Plant Journal* 33(1) p. 19-46.

Convention on Biological Diversity. Official text published 1992 http://www.biodiv.org/doc/legal/cbd-un-en.pdf

Environmental risk management authority New Zealand (2004). Decision Making, A technical guide to identifying, assessing and evaluating risks, costs and benefits (ER-TG-01-1). http://www.ermanz.govt.nz/resources/publications/pdfs/ER-TG-05-01.pdf

European Environment Agency (1999). Environment in the European Union at the turn of the century. Environmental Assessment Report No. 2. Published by European Environment Agency.

Ferson S. and Ginzberg L. R. (1996). Different methods are needed to propagate ignorance and variability. *Reliability Engineering and System Safety*, 54: 133-144.

Goklany, Indur M., "Applying the Precautionary Principle to Genetically Modified Crops" (August 2000). Weidenbaum Center Working Paper No. PS 157. Available at SSRN: http://ssrn.com/abstract=246530 or DOI: 10.2139/ssrn.246530

Hayes K.R. (2003). Robust methodologies for ecological risk assessment. Best practice and current practice in ecological risk assessment for Genetically Modified Organisms. http://www.deh.gov.au/settlements/publications/biotechnology/pubs/summary-report.pdf

Hayes K.R. (2004). Identifying hazards in complex ecological systems. Part 3: Hierarchical holographic model for herbicide tolerant oilseed rape. *Environmental Biosafety Research* 3 p.109-128.

Hill, R.A. & Sendashonga, C. (2003). General principles for risk assessment of living modified organisms: Lessons from chemical risk assessment. *Environmental Biosafety Research* 2 p. 81-88

James, C (2004). ISAAA Brief 32. Global Status Of Commercialized Biotech/GM Crops: 2004. International Service for the Acquisition of Agri-Biotech Applications (ISAAA).

James, C (2005). ISAAA Brief 34. Global Status Of Commercialized Biotech/GM Crops: 2005. International Service for the Acquisition of Agri-Biotech Applications (ISAAA).

Kasanmoentalib, S. (1996) Deliberate release of genetically modified organisms: applying the precautionary principle. In Coping with Deliberate Release: the Limits of Risk Assessment (van Dommelen, A., ed.). Tilburg, the Netherlands: International Centre for Human and Public Affairs, pp. 137–146.

Levidow, L., Carr, S., von Schomberg, R. and Wield, D. (1996) Regulating agricultural biotechnology in Europe: harmonization difficulties, opportunities, dilemmas. *Science and Public Policy* 23, p. 135–157.

Organisation for Economic Cooperation and Development (1993). Field releases of transgenic plants, 1986-1992: An analysis. OECD, Paris, France, pp 39.
Organisation for Economic Cooperation and Development (2000a). Summary of Data from OECD's Database of Field Trials, http://www.oecd.org/ehs/summary.htm

Regal, P.J. (1999) Brief history of biotechnology risk debates and policies in the US. http://www.mindfully.org/GE/History-Biotech-Risk-Debates.htm

Smith G & Collier D. (2005). Community Stakeholder Involvement. Report for the SAFEGROUNDS Learning Network - available at www.safegrounds.com

Stolk H., Gates K. & Hanan J. (2003). Discovery of Emergent Natural Laws by Hierarchical Multi-Agent Systems. In IEEE/WIC International Conference on Intelligent Agent Technology, October 2003, pages 78-85, Halifax, Canada.

Stolk H.J. (2005). Emergent Models in Hierarchical and Distributed Simulation of Complex Systems. PhD Thesis, University of Queensland, Brisbane, Australia.

Torgersen, H. (1996) Ecological Impacts of Traditional Crop Plants – a useful basis for the assessment of transgenic plants? Vienna, Austria: Monograph 75 http://www.umweltbundesamt.at/fileadmin/site/publikationen/M075.pdf

Van der Meer P. (1993) Potential Long-Term Ecological Impact of Genetically Modified Organisms: a Survey of Literature, Guidelines and Legislation. Strasbourg, France: Council of Europe Press.

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Index

Uncertainty analysis	10-11,14-15,18,37
UNEP	13,16
USA	2,9,11-12,14,19-20, 55
Weeds	12,23,29,37,43,46,48-49,53
Wild relatives	3,13,24,29,48,53,57

Appendix 1

Literature Review

Contents

Weaver & Morris (2005) have presented a comprehensive annotated bibliography of peer-reviewed scientific research highlighting the human health, animal health and environmental risks associated with GM. They identified two potential risk categories:

Risks associated with expression of the gene e.g. Bt toxin.
Unpredictable effects of the GM construct on the plant genome

It is also clear that there are risks which are not related to the GM plant itself but are part of the management practice required for agricultural use of the GM crop.

1.1. Risks associated with expression of the transgene

1.1.1. GM plants containing Bt toxin

Insect resistance
GM crops containing the genes for *Bacillus thuringiensis* (Bt) toxin have been grown widely since 1996. The cumulative area of these crops grown over the years is now > 80 million hectares worldwide. Originally, the biggest concern was that insect resistance to Bt would appear as quickly in the field as it had done in laboratory experiments (Agi *et al*, 2001; Alyokhin *et al* 1999; Cannon 2000; Cao *et al* 1999). Insect resistance management (IRM) strategies were therefore put in place to try and mitigate this anticipated problem.

As part of their requirements, companies selling Bt plants are mandated by the US EPA to implement an annual resistance monitoring program, the goal of which is to detect changes in resistance levels in pest population. The most widely used method is diagnostic or discriminating dose incorporated into an artificial diet. Bates *et al* (2005) have reviewed the management of insect resistance over the last 8 years of Bt releases in the USA and have found no reports of increased insect resistance developing to the Bt crops in the field.

In their review, Bates *at al* (2005) suggested that IRM strategies can do much to delay the development of resistance to Bt plants but that the most effective use of Bt crops will be as a component of overall IPM programs. For example, the use of Bt crops in conjunction with cultural or biological methods, that have limited efficacy on their own, may help increase the feasibility of large refuges or help suppress local pest populations in the near or long term. Although treating Bt crops as a silver bullet for pest management will almost certainly hasten the evolution of resistance in the long term, incorporation of transgenic crops with traditional, integrated approaches to pest management should help ensure their long-term sustainability and maximize their environmental and human health benefits.

Tabashnik *et al* (2005) monitored pink bollworm (*Pectinophora gossypiella*) resistance levels over 8 years and 10-17 fields of Bt cotton annually, using bioassays, and found no evidence of increased resistance to Cry1Ac in populations. Wu & Guo (2005) report that no trend towards Bt resistance has been observed in cotton bollworm (*Helicoverpa armigera*) in Bt cotton crops in China. In their paper, Tabashnik *et al* comment that most common type of Bt resistance (‘Mode 1’) is characterized by recessive inheritance, 500-fold resistance to at least one Cry1A toxin, negligible cross-resistance to Cry1C, and reduced binding of Bt toxins to midgut membrane target sites. Mutations affecting a Cry1A binding midgut cadherin protein are linked to laboratory selected Mode 1 resistance in *Heliothis virescens* and *Pectinophora gossypiella*. Baxter *et al* (2005) have shown that field evolved Mode 1 resistance in the

diamondback moth, *Plutella xylostella*, has a different genetic basis, indicating that screening for resistance in the field should not be restricted to a previously proposed DNA-based search for cadherin mutations. Tabashnik *et al* (2005) tested this using a unique model system composed of broccoli plants transformed to express different Cry toxins (Cry1Ac, Cry1C, or both) and a synthetic population of the diamondback moth (*Plutella xylostella*) carrying genes for resistance to Cry1Ac and Cry1C at frequencies of 0.10 and 0.34, respectively. After 24–26 generations of selection in the greenhouse, the concurrent use of one- and two-gene plants resulted in control failure of both types of Bt plants. When only two-gene plants were used in the selection, no or few insects survived on one- or two-gene Bt plants, indicating that concurrent use of transgenic plants expressing a single and two Bt genes will select for resistance to two-gene plants more rapidly than the use of two-gene plants alone. The results of this experiment agree with the predictions of a Mendelian deterministic simulation model and have important implications for the regulation and deployment of pyramided Bt plants (Zhao et al, 2005). Thus there are still concerns that if the hectarage in Bt crops increases further resistance may yet develop.

Effects on non-target arthropods
Non-specificity is another issue of transgenic insecticidal crops, e.g. the Monarch butterfly study which demonstrated a toxic effect of GM pollen to the Monarch butterfly (Hansen *et al* 2000, Losey *et al* 1999, Sears *et al*, 2001, etc). Debate has raged on with a variety of rebuttals of these types of studies. Other non-target effects have also been documented on 2-spot ladybirds, green lacewing, etc. (see Weaver *et al* 2005 refs, p166). Many of these studies were carried out in the laboratory under artificial conditions and these are therefore open to interpretation.

An EPA report (USEPA 1999) reported that the toxin CryIAb caused significant mortality and reduced reproduction of the soil-dwelling collembolan, *Folsomia candida*. Despite its own finding of an adverse effect on a non-target species, the EPA concluded that there is a 200-fold "safety factor" in the levels of toxin present in the field. The meaning of this EPA safety factor is not entirely clear, though perhaps it signifies that the concentration of Bt toxin in corn residue or soil is less than $1/200^{th}$ of the concentration needed to kill Collembola (Obrycki, 2001).

Lozzia (1999) reported a two-year survey in two North Italian locations (near Pavia) during 1997-98, which revealed no significant differences in the abundance of non-target entomofauna in transgenic and isogenic corn crops. The authors concluded that transgenic Bt 176 maize (Novartis product) appeared to have no deleterious effects on non-target abundance or biodiversity, although they also suggested that longer term and larger scale field trials are needed to confirm these findings.

A study carried out in the USA in order to collect information on ecological risks, as part of the regulatory process, has been published by McKee *et al* (2003). The reported studies found no 'unacceptable' adverse effects of GM crops on a range of arthropod populations in plot or farmscale trials in the USA, including trials of Bt corn and Bt cotton.

Pons & Stary (2003) found no differences in aphid-parasitoid associations on *Bt* maize and isogenic maize for aphid species composition and associated parasitoid guild, in an irrigated area in Catalonia, Spain.

De la Poza *et al* (2005) found no detrimental effect on any predatory taxa or on the functional group as a whole in studies on Bt maize expressing the Cry1Ab toxin from *Bacillus*

thuringiensis. As part of a Spanish specific monitoring program for Bt maize, a farm-scale study was initiated in 2000 to assess the potential impacts of Bt maize on predatory arthropods. The trials were conducted at two maize growing areas (Lleida and Madrid) over 3 years. In each locality three treatments (each with 3–4 replicates of 0.4–0.7 ha) were arranged in a completely randomised block design, Bt-maize (cv. Compa CB) being compared with the isogenic cv. Dracma under conventional farm practices, with or without the insecticide (imidacloprid) seed treatment. Predator abundance was monitored from late May to mid September in Lleida and from mid June to mid September in Madrid by visual surveys and pitfall traps. Anthocoridae, Coccinellidae, and Araneae represented about 90% of the total number of predators recorded in visual samplings whereas Carabidae and Araneae were the two prevalent predator groups (85–90% of the total predators) collected in pitfall traps. Their abundance varied from year to year and between locations, but no clear tendencies related to Bt maize was recorded. Insecticide treatment reduced anthocorid numbers occasionally but no consistent effects were found for the rest of predators.

A set of comprehensive studies of the insect populations in Bt corn and Bt cotton in the USA and Australia have recently been published as a series in the journal *Environmental Entomology* during 2005. The study concluded that there are no substantial negative effects of Bt on non-target insects (Naranjo *et al* 2005). Studies were carried out over a minimum of three site years in either controlled moderate sized research plots or commercial fields and were subjected to typical grower production practices.

Studies have been carried out in China on Bt cotton e.g. Men *et al* (2003) who published a 3 year investigation in Henan Province which concluded that the use of Bt cotton can increase the diversity of arthropod communities and pest sub-communities but that natural enemy communities may be decreased. Similar results were obtained by Liu *et al* (1996) who were concerned that lower numbers of natural enemy arthropods under Bt cotton may mean that populations are unstable. Ma *et al* (2003) also suggest that the insect community under the GM crop is less stable and there is therefore more chance that minor insect pests could develop into major ones.

Men *et al* (2004) did find reductions in some groups of insects on Bt cotton in China due to sprays of pesticide. The effects of pesticide applications on pests (aphids and acarid mites) and predators (ladybeetles and spiders) were investigated in transgenic *Bt* cotton and nontransgenic cotton agroecosystems in 1999, 2000 and 2001. Although transgenic cotton did not cause changes in populations of acarids and did not reduce numbers of predators significantly; its effects on aphids were inconsistent. Although insecticides were not applied against the main pest – cotton bollworm – on transgenic cotton, the total number of insecticide applications in 3 years was no less than the total applied on nontransgenic cotton, because additional applications were required against sucking pests on transgenic *Bt* cotton. Pesticide applications decreased the numbers of aphids, acarids and predatory spiders significantly on both transgenic and nontransgenic cottons.

Effect of Bt toxin on soils

Stotsky (2000) reported that Bt toxin can bind to soil and therefore persist in the soil. They found evidence that the Bt corn biomass decomposed more slowly in soil when compared to its non-GM equivalent. However, they found no evidence of effects of the Cry1Ab protein on earthworms, nematodes, protozoa, fungi, bacteria and concluded this was an effect of lignin content in the GM crop not Bt toxin *per se*.

In a review by Liu *et al* (2005), they reported that CryIIA Bt cotton produce the protein at a level of 34 ug Cry11A protein/g of fresh weight of tissue prior to harvest. If the transgenic cotton plant matrix is uniformly incorporate into 7.6cm of soil, then the estimated maximum content of Cry11A protein is 1.6 ug/g dry soil (Sims and Ream, 1997). When purified Bt toxin was added to non-sterile soils, activity against the larvae of the tobacco hornworm (*Manduca sexta*) was still detected after 234 days. A high soil clay content and low soil pH increased the persistence of Bt toxin in soil (Saxena & Stotzky, 2000). These results suggest that the transgenic protein can remain in soil for a long time. It is still not known whether continuous planting of transgenic plants over many years will lead to increasing accumulation of transgenic proteins, or what impact this might have on soil microorganisms or the soil fauna.

Wu *et al* (2004) have reported that soil dehydrogenase activity in the soil amended with transgenic rice straw was significantly higher compared with the soil amended with non transgenic rice straw, but the difference disappeared after incubation for 63 days.

The overall general conclusion is that effects of Bt toxin on soils were minor compared to natural variations found between soils anyway. However, there could potentially be stronger effects with other types of GM plant.

Benefits

It is suggested that *Bt* crops can produce proportionately higher yield gains in developing countries due to a combination of high pest pressure and poor chemical control in traditional crops. Pest-related crop losses of 50% and higher are quoted. It is suggested that such crops could substantially reduce the current gaps between attainable and actual yields, especially in smallholder farming systems. Qaim & Zilberman (2003) carried out on-farm field trials with *Bt* cotton in seven different Indian states demonstrated substantial reduction in pest damage and yield increases (up to 80%). A total of 395 plants grew three adjacent 646 m^2 plots: 1) *Bt* cotton hybrid; 2) non-*Bt* cotton counterpart; and 3) local non-*Bt* hybrid. Farmers managed the trials according to customary practices. *Bt* crops were sprayed (especially against *H. armigera*) on average, three times less often than were the non-Bt counterparts; however there was no significant difference in the number of sprays applied against sucking pests (aphids, jassids and whitefly). Nevertheless, "insecticides amounts on Bt plots were reduced by almost 70%". A saving of about $US30 per ha. In terms of yields, the benefits were even more sizeable: 80-87%. The bollworm pressure in India was exceptionally high in 2001, but even over a 4-year period (1998-2001) Bt hybrids showed an average advantage of 60%. In other words, the yield is directly related to a reduction in the high pest damage (about 60%) caused by bollworms in the conventional plots.

Huang *et al* (2002) carried out a survey of the use of plant biotechnology in China. The list of GM plants being used included: rice, wheat, potatoes and peanuts. Poor farmers in China are cultivating more areas of GM plants than are small farmers in any other developing countries. A survey of agricultural producers demonstrated that *Bacillus thuringiensis* cotton adoption increases production efficiency and improves farmer health**.** Research on GM cotton began as a response to rising pesticide use and the emergence of pesticide-resistant bollworm populations in the late 1980s**.** *Bt* cotton farmers reduced pesticide use by an average of 13 spray applications (or c. 50 kg) per hectare per season, which reduced costs by $762 per ha. Total number of pesticide applications per ha decreased from c. 20 (without *Bt* cotton) to c. 7 with *Bt* cotton. Total production costs fell by $0.62c per kg.

The use of GM crops with Bt insect resistance is reported to lead to less application of insecticides to agricultural crops and therefore cause less impact on the environment and fewer direct effects on non-target species. There are documented reports of reduction in pesticide usage as a result of use of Bt crops, for example, Pray *et al* (2001) surveyed cotton farmers in Northern China in 1999. Farmers that used cotton engineered to produce the Bt toxin substantially reduced the use of pesticide without reducing the output/ha or quality of cotton. This resulted in substantial economic benefits for small farmers, also farmers using Bt cotton reported fewer pesticide poisonings than those using conventional cotton. Yang *et al* (2005) carried out a similar survey in 2002 and found that although farmers had adopted Bt crops in an effort to increase yields and reduce sprays and were willing to reduce sprays, lack of technical knowledge still led to some overspraying.

Gregory *et al* (2002) surveyed thus use of Bt cotton varieties expressing the Bollgard gene which were first commercialised in South Africa in 1998. Farmers in the Makhathini Flats region of KwaZulu Natal - where chemical control of pest infestations on cotton had proved inadequate – were early adopters. The product provides effective control of the main lepidopterous pests (*H. armigera*, Earias *spp.* and *Diparopsis castanea*). Cotton in this area is usually grown on 3-10 ha plots. Chemical control usually involves 6-10 applications of carbamate and pyrethroid insecticides per growing season. Theses spray applications also require considerable quantities of water, and it can take up to 20 km of walking to collect enough water to spray 1 ha using knapsack sprayers. The adoption of Bt cotton on these plots has reduced (by 5-8 sprays per season) the need to chemical pesticides and resulted in a 2-fold yield increase in some areas (1999/2000). Although little or no spraying is required to control bollworm pests on Bt cotton, jassids and other hemipterans (such as *Dysdercus*) can cause problems and need to be scouted for. One or two sprays with systemic compounds have proved effective against these secondary pests. Low sales of cotton have ensured that there are areas of conventional cotton in place to act as refugia.

Qaim *et al* (2003) reported that transgenic Bt cotton can halve pesticide application rates in Argentina while significantly increasing yields. Yield effects are bigger than in other countries due to the current low levels of insecticide use. Although smallholder farmers are not currently using the technology, gross benefits are predicted to be highest for them. Biological model simulations suggest that rapid resistance buildup in pest populations appears to be unlikely if minimum non-Bt refuge areas are maintained.

A more sceptical view is presented by the NW Science and Environmental Policy Centre (Idaho, USA) (Benbrook, 2001) where it is argued that *Bt* cotton has reduced insecticide use in several states, whereas *Bt* corn has had little if any impact on corn insecticide use (Cannon, 2000). The author argues that asking whether GM crops reduce pesticide use is not the important question, we should be "asking how biotechnology can lead toward prevention-based biointensive pest management systems that rest largely on low-impact ways to manage natural biocontrol processes and interactions". The greatest long-term management benefits from agbiotech may well be process- and management-based. Biotech will make it possible for farmers to subtly tip the competitive balance within agricultural systems towards beneficial organisms, at the expense of pests.

Phipps *et al* (2002) used published data to estimate the effects of GM crops on pesticide use on a global basis. They concluded that, overall, GM technology reduced pesticide use globally, with the size of the reduction varying between crops and the introduced trait. Estimates also indicated that if GM crops (maize, oil seed rape, sugar beet and cotton) were

grown on 50% GM basis in the EU, pesticide use would decrease by 14.5 m kg of formulated product (4.4. m kg a.i.) per annum.

1.1.2. GM herbicide tolerant plants

The second type of GM crop grown widely at present is herbicide tolerant (HT).

Effects of HT plants on biodiversity
Results of the UK Farm scale evaluation trials (Firbank *et al* 2005) have demonstrated that GM herbicide tolerant crops and their cropping systems could influence farmland biodiversity because of their effects on the weed biomass and seed production. Effects persisted at least two years after the crops were grown. Weed seed banks were significantly higher following GMHT maize and significantly lower following GMHT spring oilseed rape (SOSR).

Owen & Zelaya (2005) report that the widespread adoption of GM herbicide resistant crops in the USA has resulted in a change in the weed populations. Both weed population shifts and the evolution of herbicide resistant types has occurred. For example, *Commelina comminus* L.(Asiatic dayflower), *Chenopodium album* (common lambsquarters) and *Polygonum convolvulus* L (wild buckwheat) are reported as increasing in prominence in agricultural systems because of increased herbicide application. The evolution of herbicide resistant weed populations of horseweed (*Conyza canadensis* (L) Cronq) resistant to glyphosate was also identified.

Both of the above effects are likely to be due to the effect of the herbicide application directly not the GM plant *per se*.

Evidence for gene transfer from GM plants to wild relatives
The possibility that GM constructs from crop plants might be transferred into related wild plants or feral weed populations has been identified as a risk for some time. One of the major concerns is that herbicide tolerance will be transferred to weedy relatives making them more able to survive weed control practices than their non-tolerant forms and providing an invasive species, which could interfere with agricultural practices or invade the natural environment. For this to occur several stages need to take place, first an F1 hybrid must be formed, then there must be introgression of the gene into the population, then there must be spread to secondary populations.

The oilseed rape (OSR) (*Brassica napus*) to *Brassica rapa* (Bargeman's cabbage) model has been most intensively studied in the UK. Wilkinson *et al* (2003) measured and modelled introgression of OSR genes into *B.rapa* populations where these populations occurred together and estimated that around 32,000 hybrids occur between OSR and *B. rapa* per year. This gives some indication of the potential levels of transfer of GM genes likely to occur if GMHT OSR was grown in close proximity to *B. rapa*.

Daniels *et al* (2005) carried out a comprehensive screening of wild relatives around the UK Farm scale OSR crops over a three year period and identified one instance of gene flow from OSR to a wild relative, *Sinapsis arvensis.* This illustrates that gene flow to wild relatives can occur from GM crops at low levels in normal agricultural scenarios.

Hedge & Waines (2004) have reported that natural hybrids between GM herbicide resistant wheat and the weed *Aegilops triuncialis* can be created in the glasshouse but have not yet observed this in the field.

Bartsch & Schuphan (2002) have suggested that the transgenic form of sugar beet had no difference in competitiveness and winter hardiness and possibly a much safer performance due to its higher resistance to early pre-bolting than the isogenic control. Studies on *Beta* populations have shown that a century of gene flow from commercial beet (*Beta vulgaris* ssp. *vulgaris*) seed production has had little effect on the wild beet populations of *Beta vulgaris* ssp. *maritima* in Italy (Bartsch *et al*., 2003).

Betz (2005) reviewed gene transfer from cotton. Cotton has only two wild relatives that occur in the United States, *Gossypium thurberi* in Arizona and *G. tomentosum* in Hawaii, that could possibly outcross with commercial varieties of cotton (Fryxell, 1979; Stephens, 1964). Cultivated cotton is an allotetraploid, whereas *G. thurberi* is a diploid, so they are incompatible and would not produce fertile off-spring. *G. tomentosum* is morphologically and temporally incompatible with commercial cotton varieties. There is, therefore, no reasonable mechanism for out-crossing of the genes introduced into wild cotton relatives in the United States (Funchs *et al*., 1993). The US EPA has precluded the culture of Bt cotton in Hawaii for this reason. Unfortunately, the culture of non-modified cotton poses a threat to the biological diversity of this species and introgression of sequences from *G. barbadense* and *G. hirsutum* have likely occurred previously. As *G. tomentosum* may bloom at the same time as domestic cotton, there is no guarantee of either geographic or temporal isolation. For these reasons, EPA imposed stringent sales and distribution restrictions on the registration for cotton expressing the Cry1Ac delta-endotoxin grown in Hawaii. The Agency required the following labelling statement to mitigate the potential for the cry1Ac gene to move from cultivated cotton to *G. tomentosum*: "Not for commercial sale or use in Hawaii." (EPA, Biopesticides Regulation Action Document 15, 2001)

Hails & Morley (2005) have used mathematical models and integrated field data in an attempt to assess the relative fitness of hybrids. For example, to identify the key properties of *B.rapa* which if changed would make the plant more fit. The work has suggested that populations are restrained by competition with other vegetation. It then becomes more unlikely that even if the hybrid plants could produce more seed that they would be able to find a niche to germinate and grow in competition with the current flora.

It is therefore, generally accepted that gene flow will occur (although possibly at very low levels) but what effect this will have is more difficult to assess (Jenczewskski *et al* 2003). The levels of gene flow will depend on breeding systems, ploidy levels, etc and are relatively easy to assess for any plant/wild relative combination. More difficult to assess are the fitness costs of the transgene and benefits conferred to the wild relative, which will be key to its spread in the population. There is therefore a need to assess long-term establishment of the transgene in populations and this is limited at present by our lack of knowledge of the ecology and dynamics of weed and feral populations, how factors affect fitness and how they interact with each other and population genetics generally. The GM question has challenged some of the assumptions held in the past on introgression of genes into populations. Strategies to reduce gene flow from GM crops being developed will also have to be assessed in future.

GM introgression into landraces
Some examples of introgression of GM genes into traditional landraces have received a lot of attention for example, the Quist and Chapela (2001) report of introgression in Mexican corn

which has since been discredited both because of the non-specific test used and because only gene flow, not introgression , was shown (Metz, 2002). However, it is still perfectly feasible for this to occur although current awareness of the problem means that it is less likely to occur in practice.

Effects of GM HT plants on arthropod populations
McKee *et al* (2003) reported that no differences between GM HT soybean and conventional soybean crops were identified during 1-2 year trials at the plot or farmscale level.

The UK farm scale evaluation trials carried out a comprehensive assessment of the effects of growing HT forage maize, sugar beet and winter and spring oilseed rape under commercial practices on various insect groups. In beet crops, butterflies and bees were all less abundant in the HT crops than the conventional crop, whereas in general other groups were not consistently affected. In forage maize crops, there were few effects of the GM crop observed, although there were more ground dwelling detritivores and seed feeding carabids in the HT crops. Visits by honey bees were also consistently higher in the boundaries of HT maize crops. In spring oilseed rape there were few effects related to the GM crop, although butterflies were less frequent in HT crops and carabids were more frequent in conventional than HT crops. The results for winter oilseed rape were similar to spring oilseed rape although more springtails were recorded in the HT than in the conventional crop.

Effects of GM plants on soil microflora and soils
A useful review of the evidence to date on effects of GM plants on soil microorganisms has been produced by Liu *et al* (2005). GM plants have been found to have significant effects on soil populations of non-target bacteria and fungi, soil enzyme activities, colonisation of roots by mycorrhizal fungi and the structure of the soil microbial community. Some of these effects are transient, occurring only when viable plants are present, or only at certain periods of the plant lifecycle. In some cases no effect is seen at all. Some effects are clearly related to the type of GM construct, e.g. if the construct produces an anti-microbial protein, such as T4 lysozyme. There is some evidence that unintentional changes in the composition of root exudates may be responsible for some changes in microflora (e.g. Siciliano *et al* 1998).

Siciliano & Germida (1999) carried out a study on the effect of HT (EPSPS) oilseed rape on soil microorganisms. Approximately 2300 bacteria were isolated from roots of HT and non-transgenic OSR plants. According to the results, fewer *Bacillus*, *Micrococcus*, *Variovarax* isolates, and more *Flavobacterium* and *Pseudomonas* isolates were found on the roots of the transgenic cultivar compared with the non-transgenic cultivar. The bacterial root-endophytic community of the transgenic cultivar exhibited a lower diversity compared with the non-transgenic cultivar

A 2-year multiple site field study (transgenic canola and non-GM canola/ a conventional canola variety : six times sampling) was carried out by Dunfield & Germida (2003).The results showed that there were significant differences between the rhizosphere microbial communities associated with the two oilseed rape lines that were observed at several times throughout the growing season; however, there were no differences between microbial communities from field plots containing harvested transgenic line and field plots containing no oilseed rape during the field season after winter. This means that differences between the rhizosphere microbial communities associated with the transgenic plants were temporary and depended on the presence of the viable plant.

Possible inhibition of decomposition in GM tobacco expressing proteinase inhibitor 1 has been reported by Donegan *et al* (1997, 1999). Hopkins *et al* (2005) reported significant effects of lignin modified plants (e.g. reduced COMT lignin GM plants) on decomposition in laboratory studies with tobacco.

Evidence for horizontal gene transfer to bacteria
It is known that bacteria routinely exchange genetic material amongst themselves by conjugation, transduction and transformation (Panoff & Chuiton, 2004). De Vries & Wackernagel (2003) have identified a list of 87 bacterial species where natural transformation is possible.

Gay and Gillespie published a review of this area in 2005. As part of the safety assessment of genetically modified plants, a number of expert committees have examined whether the nptII gene in Calgene's FalvrSavr or the ampicillin resistance marker in Norvatis's Bt176 maize could be transferred from GM plants back to bacteria, thus becoming an additional source of antibiotic-resistance pathogens (Gay & Gillespie, 2005). Schubbert *et al.* (1998) showed that plasmid and bacteriophage DNA introduced into mice by oral gavage at very high concentrations was not totally degraded in the intestinal tract. Fragments of DNA of hundreds to thousands of base pairs, enough to include the open reading frame of an antibiotic resistance gene, were found in the intestine of the mice. Although surviving DNA represented less than 4% of that ingested, this was still a substantial amount of genetic material available for transfer to gut bacteria.

Gay and Gillespie concluded that whereas there is no evidence that antibiotic resistance from GM crops is being transferred to bacteria, this does not exclude the possibility that it might occur. However, the evidence suggests that, if it occurs at all, the contribution to the burden of antibiotic resistance from GM plants is low, and is dwarfed by inappropriate prescribing of antibiotics in medical practice and their use as animal growth promoters in agriculture. Antibiotic-resistance markers do not pose a substantial risk to human health because the contribution that recombinant bacteria might make--should the enormous barriers to transfer be overcome--is so small that any contribution to antibiotic resistance made by GM plants must be overwhelmed by the contribution made by antibiotic prescription in clinical practice.

In principal DNA from GM plants will be available for the transformation of bacteria but the likelihood that this could happen successfully is considered to be small.

1.2. Unpredictable effects of the GM construct on the plant genome

Many of the obvious effects of the GM construct on the plant are evident during the selection process and these lines are discarded. However, there are still concerns that the GM construct may interfere with plant genes and that this may not be identified early in the selection process. The need to demonstrate substantial equivalence in order to get products approved means that such plants would not be approved. However, there are a range of papers published which do demonstrate such effects (Weaver, 2005). There are no specific reports of field examples where this type of effect has been verified.

1.3. Human allergenicity

The health risk assessment of GM foods currently relies on the testing of the toxicity of single chemicals (Bskshi,2003). However, food is a complex mixture of thousands of chemicals.
According to the World Health Organization (1995), the safety of whole GM foods can be assessed by comparing the toxicity of the safety of whole GM foods to the food or food constituent from which is derived. From the standpoint of the FDA, the important thing for consumers to understand about these new foods is that they are likely to be as safe as the foods now on store shelves. All foods, whether traditionally bred or genetically engineered, must meet the provisions of the Federal Food, Drug, and Cosmetic Act. Decisions regarding safety should be based on the nature of the product, rather than on the method by which it was modified. It is important to bear in mind that many of the crop plants used contain natural toxins and allergens. The potential for human toxicity or allergenicity should be kept under scrutiny for any novel proteins produced in plants with the potential to become part of human food or animal feed.

Allergenic reactions to foods such as cereals, nuts, milk and eggs can be life-threatening in hypersensitive people. Both conventional and GM manipulations could result in production of more allergenic plants. It is also possible to use both techniques to reduce the allergenicity of plants, where sufficient scientific knowledge is available, for example gene silencing could be used to remove toxins. It is difficult to check for potential allergenicity to novel proteins, using current testing techniques, when the human population has not been exposed to these allergens to a large extent before. The science of prediction of allergenicity is in its infancy so there is no easy way to predict the likely effects of novel GM proteins.

WHO and FAO have described a hierarchical approach to evaluate the allergenicity of GM foods and crops. The three main approaches that can be utilized to identify allergen sources include 1) amino acid sequence characterization-that method would increase the number of allergenic sequences in the data bank, 2) identification of the amino acid sequences that define allergenic epitopes to develop more precise sequence-screening criteria; and 3) development of an animal model that can recognize food allergens in a manner similar to that which occurs in human disease (Bskshi, 2003).

One clearly documented case of increased allergenicity involving a GM plant is described by Nordlee *et al* (1996). Methionine rich 2S albumin from brazil nuts was transferred to soyabean to improve the nutritional quality of the soyabean. Allergenic effects were picked up during the testing regime and the product was withdrawn. In retrospect, this was a predictable scenario as Brazil nuts are known to be allergenic to humans.

A second example was the Starlink maize event when traces were found in taco shells in 2000. The FDA investigated 28 consumer complaints related to this, where consumers had complained of adverse reactions to the product. Analysis of sera from consumers, using ELISA to Cry9C–specific IgE antibody, found no trace of this suggesting that the allergenic responses were not specific to the GM product.

There is a significant potential for PR proteins to be human allergens as they comprise of the order of 25% of the proteins on the Official Allergen Database of the International Union of Immunological Societies (Hoffman–Sommergruber, 2002).

1.4. Other reported indirect effects of GM plants on human health

Wu *et al* (2004) suggest that decreased insect damage on Bt corn leads to lower levels of mycotoxins. For example, Munkvold & Hellmich (1999) carried out field studies on Bt11 and MON 810 and found that levels of fumonisin were lower in GM crops. This should provide some benefit for human health from growing GM crops.

Gay & Gillespie (2005) have analysed the likelihood of antibiotic resistance markers from GM plants becoming incorporated into human microbes, thus conferring resistance. They conclude the contribution of this is likely to be negligible compared to use of antibiotics in clinical therapy.

1.5. Animals and antibiotic resistance

The usefulness of antibiotics in the prevention of animal diseases needs to be preserved so that we don't need to use those currently used in human treatment. The use of antibiotic resistance genes as a selectable marker for GM crops has raised concerns about the potential for transfer of these genes to gut or soil bacteria, causing bacteria to become resistant to these antibiotics. The antibiotic resistance genes of most concern are the kanamycin resistance – *npt*II or hygromycin B resistance – *hpt* genes, normally driven by plant promoters, as well as the *aad*A genes (spectinomycin and streptomycin resistance) which are driven by bacterial promoters. This has led to the regulatory authorities recommending that the use of these markers be phased out, e.g. the US FDA has recommended that GM plant producers do not use valuable antibiotics in selections.

Reviews by Droge *et al* 1998; Neilsen *et al* 1998; FAO/WHO 2000, Smalla *et al* 2000 and Thompson 2000 have concluded that gene transfer from GM plants to bacteria is likely to be a rare occurrence.

More recently, Miki *et al* 2004 have concluded that the selectable markers commonly in use are not likely to increase the chance of resistance occurring naturally. Their rationale is that the genes are common in soil bacteria anyway so their use in plants is not likely to increase the available gene pool significantly. Van den Eede *et al* (2004) have also ranked *hpt*, *npt*II and *aad*A genes as being (1) already widely distributed in soil and enteric bacteria and, (2) conferring resistance to antibiotics which are of limited therapeutic value to animal and human medicine.

There appear to be few studies, if any, where this has actually been measured.

Plant-made Pharmaceuticals(PMP)

A recent more novel application of GM plants is in the production of vaccines. Transgenic plants are proving to be an ideal means by which to produce oral vaccines enabling intact antigen to reach the gut associated lymphoid tissue. For example, the Prodigene company has produced an edible vaccine for transmissible gastroenteritis virus in pigs which has been shown to be effective and has been patented. Plant-made vaccines have prevented the onset of disease in animal models and have proven to be safe in human clinical trials. One major issue is the risk of contaminating the general food supply. Few studies have been done in these types of GM plant to date, but this is likely to prove a controversial area for the future.

1.6. References

Agi, A., Mahaffey, J., Bradley, J. & van Dunn, J. (2001). Efficacy of seed mixes of Transgenic Bt and Non Transgenic cotton against bollworm, *Helicoverpa zea* Boddie. *Journal of Cotton Science* 5, p.74-80.

Alyokhin, A.V. & Ferro, D.N. (1999), Relative fitness of Colorado potato beetle (Coleoptera: Chrysomelidae) Resistant and susceptible to the *Bacillus thuringiensis* Cry3A toxin. Journal of Economic Entomology 92, p.510-515.

Bartsch, D., Cuguen, J., Biancardi, E. & Sweet, J. (2003). Environmental implications of gene flow from sugar beet to wild beet – current status and future research needs. *Environmental Safety Research* 2, p.105-115.

Bates, S. L., Zhao, J-Z., Roush, R.T. & Shelton, A.M. (2005). Insect resistance management in GM crops: past, present and future. *Nature Biotechnology* 23(1) p.57-62.

Baxter, S.W., Zhao, J.-Z., Gahan, L.J.& Shelton, A.M.(2005). Novel genetic basis of field-evolved resistance to Bt toxins in *Plutella xylostella. Insect Molecular Biology* 14(3), p.327-334.

Benbrook, C. M. (2001). Do GM crops mean less pesticide use? *Pesticide Outlook –October 2001*. Pp. 204-207. The Royal Society of Chemistry, UK.

Cannon, R.J.C., (2000). Bt transgenic crops: risks and benefits. *Integrated Past Management Reviews.* 5,p.151-173.

Cao, J., Tang, J.D., Strizhov, N., Shelton, A.M. & Earle, E.D. (1999). Transgenic broccoli with high levels of *Bacillus thuringiensis* Cry1C protein control diamondback moth larvae resistant to Cry1A or Cry1C. *Molecular Breeding* 5, p.131-141.

Daniels, R. Boffey, C., Mogg, R., Bond, J. & Clarke, R. (2005). The potential for dispersal of herbicide tolerance genes from Genetically-modified herbicide-tolerant oilseed rape crops to wild relatives. Defra report.

de la Poza, M., Pons, X., Farinós, G. P., López, C., Ortego, F., Eizaguirre, M., Castañera, P. & Albajes, R. (2005). Impact of farm-scale Bt maize on abundance of predatory arthropods in Spain. *Crop Protection* **24**, 677-684.

De Vries, J. & Wackernagel, W. (2004). Microbial gene transfer and the DNA release from transgenic crop plants. *Plant and Soil* 266, p. 91-104.

Droge, M., Puhler, A. & Selbitschka, W. (1998). Horizontal gene transfer as a biosafety issue: A natural phenomenon of public concern. *Journal of Biotechnology* 64, p.75-90.

Donegan, K.K., Seidler, R.J., Fieland, V.J., Schaller, D.L., Palm, C.J., Ganio, L.M., Cardwell, D.M. & Steinberger, Y. (1997). Decomposition of genetically engineered tobacco under field conditions: persistence of the proteinase inhibitor 1 product and effects on soil microbial respiration and protozoa, nematode and microarthropod populations. *Journal of Applied Ecology* 34, p.767-777.

Donegan, K.K. & Seidler, R.J. (1999). Effects of transgenic plants on soil and plant microorganisms. *Recent Research Developments in Microbiology* 3, p. 424.

Firbank, L.G., Rothery, P., May, M. J., Clark, S. J., Scott, R. J., Stuart, R. C., Boffey, C.W.H., Brooks, D.R., Champion, G.T., Haughton, A.J., Hawes, C., Heard, M.S., Dewar, A.M., Perry, J.N. & Squire, G.R. (2005). Efects of genetically modified herbicide tolerant cropping systems on weed seedbanks intwo years of following crops. Biology Letters, doi:10.1098/rsbl.2005.0390.

Gay, P.B. & Gillespie, S.H. (2005). Antibiotic resistance markers in genetically modifies plants: a risk to human health. *The Lancet Infectious Disease* 5, p. 637-646.

Gregory, P., Stewart, R. & Stavrou, S. (2002). Adoption of Bt cotton by small-scale farmers in South Africa. *Pesticide Outlook* – Feb 2002, pp. 31-34.

Hails, R. S. & Morley, K. (2005). Genes invading new populations: a risk assessment perspective. *Trends in Ecology and Evolution* 20(5), p. 245-252.

Hansen, L.C. & Obrycki, J.J. (2000). Field deposition of Bt transgenic corn pollen: lethal effects on the Monarch butterfly. *Oecologica* 125,p. 241-248.

Hegde, S.G. & Waines, J.G. (2004). Hibridisation and introgression between bread wheat and wild and weedy relatives in North America. *Crop Science* 44, p. 1145-1155.

Hoffman–Sommergruber, K. (2002). Pathogenesis-related (PR)-proteins identified as allergens. *Biochemical Society Transactions* 30(6), p.930-935.

Hopkins, D.W., Tilston, E.L., Webster, E.A., Marinari, S., Lapierre, C. & Halpin, C. (2005). Decay in soil of residues from plants with genetic modifications to lignin biosynthesis. Aspects of Applied Biology, 155-165.

Huang, J., Rozelle, S. Pray, C. & Wang, Q. (2002). Plant biotechnology in China. *Science* 295, 674-677.

Jenczewskski, E., Ronfort, J. & Chevre, A-M (2003). Crop-to-wild gene flow, introgression and possible fitness effects of transgenes. *Environmental Biosafety Research* 2, p.9-24.

Liu, L., Gu, G., Chen, J., Yang, G., Shao, X. (1996). Studies on the damage caused by the corn borer, *Ostrinia furnacalis* (Guenee) in the cotton field. Acta Entomol.Sin.39, p. 109-111.

Liu, B., Zeng, Q., Yan, F., Xu, H. & Xu, C. (2005). Effects of transgenic plants on soil microorganisms. *Plant and Soil* 271, p. 1-13.

Losey, J. E., Rayor, L. S. & Carter, M. E. (1999). Transgenic pollen harms Monarch larvae. *Nature* 399, p.214.

Lozzia, G. C. (1999). Biodiversity and structure of ground beetle assemblages (Coleoptera Carabidae) in Bt corn and its effects on non target insects. *Bull. Zool. agr. Bachic. Ser II* **31**, 37-58.

Ma, J., Gao, B., Wan, F. & Guo, J.(2003). Ecological risk of Bt transgenic cotton and its management strategy. *Ying Yong Sheng Tai Xue Bao* 14 (3),p. 443-446.

McKee, M.J., Fernandez, S., Nickson, T.E. & Head, G.P. (2003). An assessment of the environemental impact of genetically modified crops in the US. *BCPC International Congress Proceedings 2003*, p.1075-1085.

Metz, M. & Futterer, J. (2002). Suspect evidence of transgenic contamination. *Nature* 416, p. 600-601.

Men, X., Ge, F., Liu, X., Yardim, E. N.(2003). Diversity of arthropod communities in transgenic Bt cotton and non-transgenic cotton ecosystems. *Environmental Entomology*, 32,p. 270-275.

Men, X., Ge, F. Edwards, C. A. & Yardim, E. N. (2004). Influence of Pesticide Applications on Pest and Predatory Arthropods Associated with Transgenic *Bt* Cotton and Nontransgenic Cotton Plants. *Phytoparasitica* 32, 246-254

Miki, B. & McHugh, S. (2004). Selectable marker genes in transgenic plants: applications, alternatives and biosafety. *Journal of Biotechnology* 107(3), p.193-232.

Munkvold, G.P. & Hellmich, R. L. (1999). Comparison of fumonisin concentrations in kernels of transgenic Bt maize hybrids and non-transgenic hybrids. *Plant Disease*. 83(2), p.130-138.

Naranjo, S. E., Head G., Dively, G. P. (2005). Field Studies Assessing Arthropod Nontarget Effects in Bt Transgenic Crops: Introduction. *Environmental Entomology*, 34, 5, p.1178-1180 1181 and associated papers below:

Assessing the Effects of Pest Management on Nontarget Arthropods: The Influence of Plot Size and Isolation J. R. Prasifka; R. L. Hellmich; G. P. Dively; L. C. Lewis *Environmental Entomology*, Vol. 34, No. 5, October 2005 pp.1181-1192
1193

Steven E. Naranjo. Long-Term Assessment of the Effects of Transgenic *Bt* Cotton on the Abundance of Nontarget Arthropod Natural Enemies. *Environmental Entomology*, Vol. 34, No. 5, October 2005 pp.1193-1210 1211.

Long-Term Assessment of the Effects of Transgenic *Bt* Cotton on the Function of the Natural Enemy Community Steven E. Naranjo. *Environmental Entomology*, Vol. 34, No. 5, October 2005 pp.1211-1223 1224.

A Comparison of Arthropod Communities in Transgenic *Bt* and Conventional Cotton in Australia M. E. A. Whitehouse; L. J. Wilson; G. P. Fitt *Environmental Entomology*, Vol. 34, No. 5, October 2005 pp.1224-1241 1242.

Canopy- and Ground-Dwelling Predatory Arthropods in Commercial *Bt* and non-*Bt* Cotton Fields: Patterns and Mechanisms Jorge B. Torres; John R. Ruberson *Environmental Entomology*, Vol. 34, No. 5, October 2005 pp.1242-1256 1257.

A Multiyear, Large-Scale Comparison of Arthropod Populations on Commercially Managed *Bt* and Non-*Bt* Cotton Fields G. Head; W. Moar; M. Eubanks; B. Freeman; J. Ruberson; A. Hagerty; S. Turnipseed. *Environmental Entomology*, Vol. 34, No. 5, October 2005 pp.1257-1266 1267.

Impact of Transgenic VIP3A × Cry1Ab Lepidopteran-resistant Field Corn on the Nontarget Arthropod Community Galen P. Dively. *Environmental Entomology*, Vol. 34, No. 5, October 2005 pp.1267-1291 1292 .

Effect of *Bacillus thuringiensis* Transgenic Corn for Lepidopteran Control on Nontarget Arthropods Timothy Daly; G. David Buntin. *Environmental Entomology*, Vol. 34, No. 5, October 2005 pp.1292-1301 1302.

Impact of Transgenic *Bacillus thuringiensis* Corn and Crop Phenology on Five Nontarget Arthropods Clinton D. Pilcher; Marlin E. Rice; John J. Obrycki *Environmental Entomology*, Vol. 34, No. 5, October 2005 pp.1302-1316 1317.

Utility of Ground Beetle Species in Field Tests of Potential Nontarget Effects of Bt Crops Miriam D. Lopez; Jarrad R. Prasifka; Denny J. Bruck; Leslie C. Lewis *Environmental Entomology*, Vol. 34, No. 5, October 2005 pp.1317-1324 1325
.
Field Evaluation of the Impact of Corn Rootworm (Coleoptera: Chrysomelidae)-Protected *Bt* Corn on Ground-Dwelling Invertebrates Muhammad A. Bhatti; Jian Duan; Graham Head; Changjian Jiang; Michael J. McKee; Thomas E. Nickson; Carol L. Pilcher; Clinton D. Pilcher. *Environmental Entomology*, Vol. 34, No. 5, October 2005 pp.1325-1335 1336.

Field Evaluation of the Impact of Corn Rootworm (Coleoptera: Chrysomelidae)-Protected *Bt* Corn on Foliage-Dwelling Arthropods Muhammad A. Bhatti; Jian Duan; Graham P. Head; Changjian Jiang; Michael J. McKee; Thomas E. Nickson; Carol L. Pilcher; Clinton D. Pilcher. *Environmental Entomology*, Vol. 34, No. 5, October 2005 pp.1336-1345 1346.

Biodiversity and Community Structure of Epedaphic and Euedaphic Springtails (Collembola) in Transgenic Rootworm *Bt* Corn Royce J. Bitzer; Marlin E. Rice; Clinton D. Pilcher; Carol L. Pilcher; Wai-ki frankie Lam. *Environmental Entomology*, Vol. 34, No. 5, October 2005 pp.1346-1376.

Neilsen, K. M., Bones, A.M., Smalla, K. & van Elsas, J.D. (1998). Horizontal gene transfer from transgenic plants to terrestrial bacteria – a rare event? *FEMS Microbiology Reviews* 22, p. 79-103.

Nordlee, J.A., Taylor, S.A., Townsend, J.A., Thomas, L.A. & Bush, R.K. (1996). Identification of a Brazil-nut allergen in transgenic soybeans. *New England Journal of Medicine* 334, p.688-692.

Obrycki, J.J., Losey, J.E., Taylor, O.R. & Jesse, L.C.H. (2001). Transgenic insecticidal corn: beyond insecticidal toxicity to ecological complexity. Bioscience 51(5), p.1-19.

Owen, M. D.K. & Zelaya,I.A. (2005). Herbicide resistant crops and weed resistance to herbicides. *Pest Management Science* 61, p. 301-311.

Panoff, J-M. & Chuiton, C. (2004). Horizontal gene transfer: a universal phenomenon. *Human and Ecological Risk Assessment* 10, p. 939-943.

Phipps, R. H. & Park, J. R. (2002). Environmental benefits of genetically modified crops:global and European perspectives on their ability to reduce pesticide use. *Journal of Animal and Feed Sciences* 11, 1-8.

Pons, X. & Stary, P. (2003). Spring aphid-parasitoid (Hom., Aphididae, Hym., Braconidae) associations and interaction in a Mediterranean arable crop ecosystem, including *Bt* maize. *Anz. Schädlingskunde/J. Pest Science* **76**, 133-138.

Pray, C., Danmeng Ma, Jikun Huang and Fangbin Qiao (2001). Impact of Bt Cotton in China. *World Development* 29(5), 813-825.

Qaim, M., Cap , Eugenio J. and de Janvry, A. (2003) Agronomics and Sustainability of Transgenic Cotton in Argentina. *AgBioForum*, July 2003.

Qaim, M. & Zilberman, D. (2003). Yield effects of genetically modified crops in developing countries. *Science* 299, p. 900-902.

Quist, D. & Chapela, I.H. (2001). Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature* 29, p.541-543.

Saxena, D. & Stotzky, G. (2000). Insecticidal toxin from *Bacillus thuringiensis* is released from roots of transgenic Bt corn *in vitro* and *in situ*. FEMS Microbiological Ecology33, p. 35-39.

Schubbert, R., Hohlweg,U.,Renz,D. & Doerfler,W. (1998). On the fate of orally ingested foreign DNA in mice: chromosomal association and placental transmission to the fetus. *Molecular & General Genetics* 259, p. 569-576.

Sears, M.K., Hellmich, R.L., Stanley-Horn, D.E., Berhauser, K.S., Pleasants, J.M., Mattilla, H.R., Siegfried, B.D. & Dively, G.P. (2001). Impact of Bt corn pollen on Monarch butterfly populations: A risk assessment. *Proceedings of the National Academy of Sciences*, 98, p. 11937-11942.

Siciliano, S.D., Theoret, C.M., de Freitas, J.R., Hucl, P.J. & Germida, J.J. (1998). Differences in microbial communities associated with the roots of different cultivars of canola and wheat. *Canadian Journal of Microbiology* 44, p.844-851.

Sims, S. R. & Ream, J.E.(1997). Soil inactivation of the *Bacillus thuringiensis* subsp. *kurstaki* CryIIA insecticidal protein within cotton tissue: laboratory microcosm and field studies. *Journal of Agriculture and Food Chemistry*. 45, p.1502-1505.

Smalla, K., Borin, S., Heuer, H., Gebhard, F., Van Elsas, J.D. & Neilson, K. (2000). Horizontal transfer of antibiotic resistance genes from transgenic plants to bacteria. Are there new data to fuel the debate? In: Fairbairn,G., Scoles, G., McHughen, A. (Eds) Proceedings of the 6th International Symposium on the Biosafety of Microorganisms p. 146-154.

Stotsky,G. (2004). Persistence and biological activity in soil of the insecticidal proteins from *Bacillus thuringiensis*, especially from transgenic plants. *Plant and Soil*, 266,p. 77-89.

Tabashnik, B. E., Dennehey, T.J. & Carriere, Y. (2005). Delayed resistance to transgenic cotton in pink bollworm. *Proceedings of the National Academy of Sciences* 102(43), p.15389-15393.

Thompson, J. (2000). Topic 11: gene transfer - mechanism and food safety risks. Joint FAO/WHO Expert Consultation on Foods derived from Biotechnology, Geneva.

USEPA 1999: US EPA Office of pesticide programs 1999. Biopesticide fact sheet (2 Apr 2001:www.epa.gov/pesticide/biopesticides/factsheets/.

Van den Eede, G., Aarts, H., Buhk, H-J., Corthier, G., Flint, H.J., Hammes, W., Jacobsen, B., Midvedt, T., Van der Vossen,J., Von Wright, A., Wackernagel, W. & Wilks, A. *et al* (2004). The relevance of gene transfer to the safety of food and feed derived from genetically modified plants. *Food and Chemical Toxicology* 42, p.1127-1156.

Weaver, S.A. & Morris, M.C. (2005). Risks associated with genetic modification: an annotated bibliography of peer reviewed natural science publications. *Journal of Agricultural & Environmental Ethics* 18: 157-89.

Wilkinson, M.J., Elliott, L.J., Allainguillaume, J., Shaw, M.W., Norris, C., Welters, R., Alexander, M., Sweet, J. & Mason, D.C. (2003). Hybridisation between *Brassica napus* and *B.rapa* on a National Scale in the United Kingdom. *Science* 302, p. 457-459.

Wu, F., Miller, J. D. & Casman, E.A (2004). The economic impact of Bt corn resulting from mycotoxin reduction. *Journal of Toxicology : Toxin reviews.* 23(2&3), p. 397-424.

Wu, K.M. & Guo, Y.Y. (2005). The evolution of cotton pest management practices in China. *Annual Reviews Entomology* 50, p. 31-52.

Yang, P., Iles, M., Ya, S. & Jolliffe, F. (2005). Farmers' knowledge, perceptions and practices in transgenic Bt cotton in small producer systems in Northern China.

Zhao J.-Z., Cao, J., Collins, H.L., Bates, S.L., Roush, R.T., Earle, E.D. & Shelton, A.M. (2005).Concurrent use of transgenic plants expressing a single and two *Bacillus thuringiensis* genes speeds insect adaptation to pyramided plants. *Proceedings of the National Academy of Sciences of the USA* 102(24), p.8426-8430.

Appendix 2

Plans and methodologies used for risk assessment

2.1. EU regulatory framework and guidelines for use

2.1.1 EU Council Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms and the relevant annexes and guidance documents

Availability: http://europa.eu.int
This Directive repeals the earlier Council Directive 90/220. The scope of the directive is, *inter alia*, to provide a common European wide methodology for ecological risk assessment and common objectives for monitoring GMO releases to the environment. It requires an ecological risk assessment prior to any deliberate release of GMOs. Many of the countries that comprise the EU have ratified the Cartagena Biosafety Protocol, as such much effort has been expended in the EU to ensure that Directive 2001/18/EC is compliant with the Annex III of the protocol with respect to risk assessment.

With respect to risk assessment key parts of the directive or related documents are:
a) Annex II: Principles for the Environmental Risk Assessment. Describes principles and methodology, including specific information on risks to be considered for GM plants, and for GMOs other than plants. Note: superseded by European Food Safety Authority Guidance (discussed below).

b) Commission Decision of 24 July 2002 establishing guidance notes supplementing annex II of Directive 2001/18/EC (Decision 2002/623/EC), provides detailed information on general principles and methodology.

Scientific principles and framework of risk assessment under 2001/18/EC
In accordance with the precautionary principle, the potential direct and indirect (immediate and delayed) effects of GMOs are to be accurately assessed, on a case-by-case basis in the environmental risk assessment (ERA). A general principle of the ERA is also that an analysis of the cumulative long-term effects relevant to the release and the placing on the market is carried out. Releases are to be carried out in a stepwise fashion and must be field-tested in ecosystems that could be affected by their use. A differentiated procedure is permitted for GMOs that are well known and well characterised. The directive specifies a six-stage risk assessment process starting with hazard identification; followed by an evaluation of potential consequences and likelihood of each potential adverse effect, and an estimation of the risk. The estimation of risk is to be made as far as possible given the "state of the art". The fifth step identifies management options, followed by an evaluation of the overall risk taking management into account.

Hazard identification
The directive does not identify or recommend any inductive hazard assessment techniques. It notes that potential adverse effects will vary from case to case and lists five generic hazards such as diseases and toxic effects, impacts on population dynamics in the receiving environment with potential to affect biodiversity, altered susceptibility to pathogens and effects on biogeochemistry.

Likelihood and consequence assessment
The directive does not identify or discuss any specific techniques to assess the likelihood or consequences or adverse effects. It notes that the environment into which the GMO is released and the manner of the release are major factors. An estimation of the risk to human health or the environment posed by each identified characteristic of the GMO which has the potential to cause adverse effects should be made as far as possible given the state of the art, by

combining the likelihood of the adverse effect occurring and the magnitude of the consequences, if it occurs. The rationale for reaching estimation of the risk should be clearly presented and justified. Assessments of risk need not be quantitative but should be relative, e.g. to a non-GM reference.

Uncertainty analysis
The directive requires that the overall uncertainty for each identified risk should be described in relation to assumptions and extrapolations made throughout the ERA, different scientific assessments and viewpoints, uncertainties, the known limits of the mitigation measures; conclusions that can be derived from the data.

Monitor and review
The directive details the objectives, principles and design requirements of a monitoring plan. The objective of the plan is to confirm the assumptions made in the risk assessment and to identify the occurrence of adverse effects that were not anticipated in the assessment. The plan is to incorporate general surveillance for unanticipated effects as well as specific monitoring for those effects identified in the assessment. The latter must be continued for a sufficient period of time to identify delayed and indirect effects. The plan must be implemented in a systematic manner and consider mechanisms for identifying and confirming any observed effects.

Cumulative long term effects
The directive closely links the environmental risk assessment (era) and monitoring; monitoring requirements for placing on the market are intended to provide data for long-term potentially adverse effects of GMOs. Monitoring results can be used to confirm the ERA, or lead to its re-evaluation. The ERA must consider:

- long-term effects in terms of the long term interactions of the GMO and the receiving environment;
- the characteristics of the GMO which become important on a long-term basis; repeated deliberate releases or placings on the market over a long period of time; and
- the GMOs deliberately released or placed on the market in the past.

Further information may be required on long-term effects, e.g. multiple herbicide resistances, and there must be adequate research (partly within monitoring plans), which can provide data for assessing cumulative long-term effects. Guidance on monitoring plans for case specific monitoring and general surveillance have been developed, but general surveillance monitoring in particular is still only a very vaguely developed concept that has yet to be put widely into practice.

The following guidance documents have also been published with respect 2001/18/EC:

c) Annex VI (Guidelines for the Assessment Reports) to Directive 2001/18/EC, lists the information required by the Directive

d) Annex VII (Monitoring Plan) to Directive 2001/18/EC; describes aspects of the principles and design of monitoring plans

e) Council Decision of 3 October 2002 supplementing annex VII to Directive 2001/18/EC (Decision 2002/811/EC). Provides a detailed description of the objectives, general principles, strategy, methodology, and analysis of monitoring plans.

2.1.2 European Commission Guidance Document for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed (6-7 March 2003)

Prepared for the Scientific Steering Committee by the Joint Working Group on Novel Foods and GMOs
Availability: http://europa.eu.int/comm/food/fs/sc/ssc/out327_en.pdf
Key Characteristics: Scope is genetically modified plants and derived food and feed. The guidance provides a description of the comparative approach to risk assessment and discusses the detail that must be provided. This covers the molecular characteristics of the GMO and data on comparative analysis (choice of comparator, field trial data, agronomic traits and analysis of the products), together with a detailed description of specific information requirements related to the environmental risk assessment (geographical relevance of data, impact on wild plants, impact on non-modified crops, impact on organisms and ecological processes). The document does not discuss uncertainty or statistical analysis, nor does it consider how cumulative or long-term effects. This document has been superseded by the EFSA guidance for the risk assessment of GM plants and derived food and feed published in March 2005.

2.1.3 European Food Safety Authority

Guidance Document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed. Adopted 24 September 2004 (published March 2005).
Availability: http://www.efsa.eu.int/science/gmo/gmo_guidance/660_en.html
Scientific principles and framework
The scope of the guidance is commercial releases of genetically modified plants and derived food and feed submitted within the framework of EU Directive 2001/18/EC and Regulation (EC) 1829/2003 on GM food and feed. Risk assessment is described as "a process of evaluation including the identification of attendant uncertainties, of the likelihood and severity of an adverse effect(s)/event(s) occurring to man or the environment following exposure under defined conditions to a risk source(s)" (EC, 2000a). It provides a description of the comparative approach to risk assessment based on the principles of familiarity and substantial equivalence, and consideration of intended and unintended effects. A formal framework for risk assessment is not provided or recommended. Risk assessments must be demonstrably based on good scientific data and it must be clear the assumptions that have been made during the risk assessment process and what are the boundaries of the assessment.

Hazard identification
No hazard assessment techniques are identified or recommended, but a detailed description of information required to undertake the risk assessment is provided.

Likelihood and consequence assessment
A comprehensive risk characterisation should be carried out, based on data gathered from hazard identification and exposure assessment. Where there is an absence of data this should be discussed and taken into account in the final risk analysis. Assumptions and extrapolations should be clearly stated. Risk estimation may be qualitative and where possible, quantitative; terms for definition of risks and associated uncertainties are not prescribed.

Uncertainty analysis
Any uncertainties inherent in the different steps of the risk assessment should be highlighted and quantified as much as is possible. The guidance requires that estimation of uncertainties in experimental data should be handled by proper statistical analysis; probabilistic methods can be used. Quantification of uncertainties in extrapolations from laboratory or experimental studies should be highlighted. . Where possible, statements in essentially qualitative risk assessments such 'low risk', medium risk' etc should be quantified in terms of probability of exposure and/or occurrence of adverse effects.

Monitor and review
The document defines monitoring as "the systematic measurement of variables and processes over time and assumes that there are specific reasons to collect such data, for example, to ensure certain standards or conditions are being met or to examine potential changes with respect to certain baselines". Guidance on developing case specific and general surveillance monitoring plans is provided; EFSA has also issued a separate guidance document for general surveillance of GM crops.

Long term and cumulative effects
These are considered throughout the document in the general context of the risk assessment, and in particular with respect to case-specific monitoring and general surveillance monitoring. However the document does not specifically address long-term cumulative effects.

2.1.4 European Commission: DG Health and Consumer Protection

First report on the harmonisation of risk assessment procedures part 1: Report of the Scientific Steering Committee's Working Group on Harmonisation of Risk assessment Procedures in the Scientific Committees advising the European Commission in the area of human and environmental health 26-27 October 2000.
Availability: http://europa.eu.int/comm/food/fs/sc/ssc/out84_en.pdf
This very comprehensive report was commissioned to outline approaches to risk assessment used by the EC's different Scientific Advisory Committees and to identify issues of interest across these Committees. It considers the different approaches and tries to identify areas of common interest and areas where progressive harmonisation might be achieved. In the report risk assessment is defined as "a process of evaluation including the identification of the attendant uncertainties, of the likelihood and severity of an adverse effect(s)/events(s) occurring to man or the environment following exposure under defined conditions to a risk source(s)". The report defines risk assessment as comprising hazard identification, hazard characterisation, exposure assessment and risk characterisation. The report is not intended to provide guidelines, but it does identify recommend that a guidance document be developed by the Steering Committee to harmonise some aspects of risk assessment in the EC, including a glossary of terms and a common language to describe different levels of risk. The report also recommends introduction of requirements for monitoring and surveillance for an increasing range of risk sources, in particular issues for which there is a high degree of uncertainty – GM crops may be considered to fall within this. It is unclear whether any progress has been made subsequent to publication of this report, or whether any of the recommendations have been adopted. We have not been able to identify any subsequent reports from this Working Group.

2.2. Guidance documents produced by individual member states

2.2.1 United Kingdom

Availability: http://www.defra.gov.uk/environment/acre/biodiversity/guidance/06.htm
The UK Department for Environment, Food and Rural Affairs (Defra) guidance on the principles of risk assessment and monitoring for the release of GMOs were developed to assist applicants during the introduction of EC Directive 2001/18/EC. Draft guidelines to accompany Directive 2001/18EC were published in November 2002.

Scientific principles and framework
The guidelines state that, in accordance with the precautionary principle, the risk assessment should be transparent, scientifically sound, carried out on a case-by-case basis and re-examined if new information becomes available. The precautionary principle, however, is not defined in the guidelines. The risk assessment is to consider the direct, indirect, immediate and delayed effects of the GMO release; each of these terms is defined. The assessment should also be compared to the risks presented by the use of the unmodified organism in corresponding situations. The guidelines specify a six-step risk assessment procedure. The first four steps consisting of hazard identification, consequence and likelihood assessment and risk calculation. The latter should be made, as far as possible, given the current 'state of the art'. The fifth step requires the analyst(s) to identify risks that need to be managed and how best to manage them. The analyst is then required to re-calculate the overall risk of releasing the GMO taking into account any proposed management strategies.

Hazard identification
The guidelines do not identify or recommend any inductive hazard assessment techniques. They do, however, provide a comprehensive checklist of potential GMO hazards. They also stress that potential adverse effects are not to be discounted on the basis that it is unlikely to occur.

Likelihood and consequence assessment
The guidelines do not identify or discuss any specific techniques to calculate the consequence or likelihood of adverse effects following the release of the GMO. They state that the characteristics of the environment into which the GMO is released, and the manner of the release, will be major factors in the consequences and likelihood of adverse effects.

Uncertainty analysis
The guidelines do not refer to uncertainty in the risk assessment process, or the significance of the risk estimates.

Monitor and review
The applicant is required to submit a monitoring plan as part of the application to market a GMO, designed to confirm the assumptions made in the risk assessments and identify the occurrence of any unanticipated effects. Monitoring must be systematic and continue long enough to identify delayed and indirect effects, and give consideration to mechanisms for identifying and confirming any observed effects.

Long-term and cumulative effects
Monitoring requirements described above are intended to identify these. There is no clear framework for how to approach general surveillance monitoring to identify unanticipated

effects, or cumulative effects, however Defra has commissioned a study to explore how this might be best achieved, in preparation for when the commercial release of GM crops might be expected in the UK.

2.2.2 Italy

Availability: http://bch.minambiente.it/bch.html
The Italian Ministry for the Environment and Territory (Directorate for Environmental Safeguard) has developed a standard methodology for environmental risk assessment for use with the deliberate release of GMOs under EU Directive 2001/18/EC. To date the model has only been applied to experimental releases, but it is the intention that it will also be implemented for assessment of commercial releases.

Scientific principles and framework
Based on application of risk analysis and risk management procedures. Risk analysis is classified as hazard identification followed by risk assessment. Risk assessment is described as the evaluation of the likelihood of the hazard occurring, associated with the presence of the receptor in the receiving environment and determination of the potential exposure to the hazard. The process must be science based and applied on a case-by-case basis. An expert group has developed a method for evaluation of the environmental safety of the deliberate release of GMOs. The method is available for use by regulators, applicants and other stakeholders alike.

The model is based on a logic scheme founded on the assumption that a risk related to release of a GMO into the environment will only occur if the source, diffusion factors migration routes and receptors are present. The model operates via an electronic questionnaire that leads to a qualitative evaluation of risk. Questions refer to the biology of the plant, properties of the trait, agricultural and horticultural practices, the receiving environment etc.

No further information is available at the time of writing the report.

2.3. Summary of guidance documents produced by international organisations

2.3.1 Cartagena Protocol on Biosafety

Availability: http://www.biodiv.org/biosafety/The Parties to the Convention of Biological Diversity adopted the Cartagena Protocol on the 29th January 2000. Annex III of the protocol describes the general principles, methodology and points to consider when conducting a risk assessment for GMOs intended for direct use as food or feed, or for processing (Articles 11 and 15).

Scientific principles and framework
The risk assessment is to be carried out in a scientifically sound manner, in accordance with Annex III and taking into account recognised risk assessment techniques, expertise of, and guidelines developed by, relevant international organisations. The risk assessment is to be carried out on a case-by-case basis and should be compared to the background risk posed by the equivalent non-modified recipients or parental organisms. The protocol specifies the precautionary principle thus: lack of full scientific certainty should not prevent a party to the protocol from taking an “appropriate” decision. The protocol defines a six-step risk assessment procedure. The first step is hazard identification followed by an assessment of the likelihood and consequences of adverse effects. The fourth step is risk calculation followed by

a recommendation as to whether or not the risks are acceptable or manageable. Finally, where there is uncertainty about the level of risk this should be addressed by obtaining more information and/or by management and/or monitoring.

Hazard identification
The protocol does not recommend or detail any specific hazard identification techniques. It simply states that the analyst(s) identify adverse effects on human health and biological diversity associated with novel genotypic and phenotypic characteristics of the organism concerned.

Likelihood and consequence assessment
The protocol does not recommend or identify any specific techniques to identify or quantify the likelihood or consequences of adverse effects. It does, however, state that the likelihood assessment should be based on the level and kind of exposure in the likely potential receiving environment. It recommends that the receiving environment be described in terms of its location, climate, ecological characteristics, biological diversity and centres of origin.

Uncertainty analysis
The protocol does not recommend or identify any techniques to analyse uncertainty, other than obtaining more information or managing/monitoring the problem. The significance of risk is only expressed in terms of how acceptable or manageable they are.

Monitor and review
The protocol does not discuss monitoring techniques other than as a means to deal with uncertainty in the level of risk (see above). It does, however, recommend that the risk assessment take account of the specificity, sensitivity and reliability of methods used to detect and identify the GMO.

Cumulative / long term effects
The protocol does not specify consideration of long term or cumulative effects and does not discuss timescales of releases.

2.3.2 United Nations Environment Programme (UNEP)

International Technical Guidelines for Safety in Biotechnology 1995
These were launched by UNEP in December 1995, four years before the Cartagena Protocol was adopted. The guidelines were developed to provide a common framework for biotechnology safety assessment without prejudice, but as a complement, to the protocol.
Availability: http://www.unep.ch/biosafety

Scientific principles and framework
Familiarity features prominently in the UNEP guidelines. They suggest, for example, that the extent and length of the risk assessment should depend on the analyst(s) familiarity with the organism concerned. Initially risk assessments should be conducted on a case-by-case basis, but as knowledge and experience evolve, the assessment may serve for a functionally equivalent group of organisms. The assessment should be carried out in a scientifically sound manner. The guidelines anticipate that, in most cases, the ecological risks will be low from well-known crop plants (that have been modified by altering or adding only a few genes)

introduced into arable environments. Risks that are identified should be compared to the background risks associated with non-modified organisms. The guidelines go on to state that the risk assessment can range from a routine *ad hoc* judgement by the analyst to adherence to a formalised procedure. It specifies a three-step risk assessment procedure starting with hazard identification. If hazards are identified the assessment proceeds by calculating risk as the combined effect of the consequences and likelihood of the hazard being realised. Finally management strategies, commensurate with the level of risk, should be designed and implemented.

Hazard identification
The guidelines do not identify or recommend any hazard identification techniques.

Likelihood and consequence assessment
The guidelines do not identify or discuss any specific techniques to assess the likelihood or consequences of hazards identified. They do, however, state that forecasting models could be developed in the future that may help the assessment, and that international databases help in the development of models, possibly implying that quantitative approaches might be appropriate. More explicitly they only suggest that full regard be given to experience with the organism elsewhere, relevant literature and consultation with available experts and public authorities.

Uncertainty analysis
The guidelines do not refer to uncertainty within the risk assessment process, or the significance of the risk estimates.

Monitor and review
The guidelines state that monitoring (that may vary from a very simple observation to an extensive research programme) may be used to verify the assumptions of the risk assessment, and should be used to evaluate the efficacy of risk management measures. No further information is given.

Cumulative / long term effects
We could find no reference in the document to either long-term effects or cumulative effects.

United Nations Environment Programme – Global Environment Facility (UNEP-GEF)

Developing countries and countries with economies in transition: UNEP-GEF Biosafety projects

Availability: UNEP/GEF website http://www.unep.ch/biosafety/parcountrieslist.htm

- Provides information on all countries that have previously, or are currently participating in UNEP-GEF Biosafety projects (pilot phase, development, implementation or capacity building).
- Country links provide details of progress within each country, and in some instances regulatory framework documents are provided, e.g. Argentina, Bangladesh. In excess of 100 countries are listed.

2.3.3 Organisation for Economic Co-operation and Development (OECD)

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 30 industrialised countries in North America, Europe and the Pacific, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised Committees and subsidiary groups composed of Member country delegates.

The OECD published its "Safety Considerations for Biotechnology" in 1986 and 1992. The 1986 report was the first attempt to set international safety guidelines for industrial, agricultural and environmental applications of biotechnology. It presented scientific principles that could underlie risk management for the release of GMOs into the environment; the 1992 report follows on from this and *inter alia* defines "Good Development Principles" for the design of safe, small-scale field trials of GM plants and microorganisms. These have now largely been superseded by European guidance document, and developments with the Cartagena Protocol. The reports did not identify or recommend any inductive hazard assessment techniques or discuss uncertainty within the risk assessment process or the significance of the risk estimates. With respect to monitoring, the 1992 report states that scientifically acceptable and environmentally sound field research requires: "formulation of an hypothesis and statement of objectives; development of specific methodologies to introduce, monitor and mitigate the organisms; a precise description of the design of experiments, including planting density and treatment pattern; and a description of specific data to be collected, and of methods for analysis to test for statistical significance".

The **Working Group for Harmonization in Biotechnology** was established in 1995 at a time when the first commercial transgenic crops were being considered for regulatory approval in a number of OECD Member countries. From the beginning, one of its primary goals was to promote international regulatory harmonisation in biotechnology among member countries. Regulatory harmonisation is the attempt to ensure that the information used in risk/safety assessments, as well as the methods used to collect such information, are as similar as possible.

Series on Harmonisation of Regulatory Oversight in Biotechnology No. 32: An Introduction to the Biosafety Consensus Documents of OECD's Working Group for Harmonization in Biotechnology
Availability: http://www.oecd.org
This document explains the background and scope of OECD consensus documents developed by the working group. The Working Group recognised that national authorities and others undertaking risk assessments for GM crops would be going through similar procedures and that much of the information related to the biology of crop plants would be the same. They decided to compile information common to the risk/safety assessment of a number of transgenic products focused on the biology of the host species or crop and traits used in genetic modifications. The aim was to encourage information sharing and prevent duplication of effort among countries by avoiding the need to address the same common issues in each application involving the same organism or trait. These documents therefore compile information relevant to risk/safety assessment of several transgenic products, and focus on either (a) the biology of particular host species or crops, or (b) traits used in genetic modification. They comprise technical information for use during the regulatory assessment

of products of biotechnology and are intended to be mutually accepted among OECD Member countries.

The following consensus documents have been published, and others are in the development phase:

- General Information concerning the Biosafety of Crop Plants Made Virus Resistant through Coat Protein Gene-Mediated Protection (1996)
- Information Used in the Assessment of Environmental Applications Involving *Pseudomonas* (1997)
- The Biology of *Brassica napus* L. (Oilseed Rape) (1997)
- The Biology of *Solanum tuberosum* subsp. *tuberosum* (Potato) (1997)
- The Biology of *Triticum aestivum* (Bread Wheat) (1999)
- General Information Concerning the Genes and their Enzymes that Confer Tolerance to Glyphosate Herbicide (1999)
- General Information Concerning the Genes and their Enzymes that Confer Tolerance to Phosphinothricin Herbicide (1999)
- The Biology of *Picea abies* (L.) *Karst* (Norway Spruce) (1999)
- The Biology of *Picea glauca* (Moench) Voss (White Spruce) (1999)
- The Biology of *Oryza sativa* (Rice) (1999)
- The Biology of *Glycine max* (L.) Merr. (Soybean) (2000)
- The Biology of *Populus* L. (Poplars) (2000)
- The Biology of *Beta vulgaris* L. (Sugar Beet) (2001)
- Information used in the Assessment of Environmental Applications Involving Baculovirus (2002)
- The Biology of *Picea sitchensis* (Bong.) Carr. (Sitka Spruce) (2002)
- The Biology of *Pinus strobus* L. (Eastern White Pine) (2002)
- The Biology of *Prunus sp*. (Stone Fruits) (2002)
- Module II: Herbicide Biochemistry, Herbicide Metabolism and the Residues in Glufosinate-Ammonium (Phosphinothricin)-Tolerant Transgenic Plants (2002)
- The Biology of *Zea maize subsp.mays* (Maize) (2003)
- The Biology of European White Birch (*Betula pendula Roth*) (2003)

Safety Considerations for Biotechnology: Scale-Up of Crop Plants. 1993
Availability: http://www.oecd.org/dataoecd/26/26/1958527.pdf

- Considers specific risk pathways and associated management options for GM crop plants. Preceded Biosafety consensus documents, provides conceptual background.

Environmental Risk Assessment of Transgenic Plants: A Comparison of International Pre-Market Data Requirements. 2004
Availability: Not yet available – classified.

- This document could not be reviewed because it is not yet declassified.

2.3.4 Food and Agricultural Organization of the United Nations (FAO)

The FAO of the United Nations leads international efforts to defeat hunger. FAO supports a science-based evaluation to objectively determine the benefits and risks of individual GMOs on a case-by-case basis prior to release. FAO strives to determine the potential benefits and possible risks associated with the application of modern technologies to increase plant and animal productivity and production, and often acts as an 'honest broker' by providing a forum for discussion. For example, the FAO provides the Secretariat to the Codex Alimentarius Commission (together with the World Health Organisation), which has established an intergovernmental task force on foods derived from biotechnologies.

FAO/World Health Organisation Expert Consultation on the Safety Assessment of Foods Derived from GM Animals, including Fish (FAO Food and Nutrition Paper 79, 2003)
Availability:http://www.fao.org/documents/show_cdr.asp?url_file=/DOCREP/006/Y5316E

- Describes general principles and considerations relevant to risk/safety assessment of GM animals and derived foods, including fish

Working Principles for Risk Analysis for Application in the Framework of the Codex Alimentarius, 2003
*Availability:*http://www.fao.org/documents/show_cdr.asp?url_file=/DOCREP/006/Y4800E/y4800e0o.htm

- Includes general principles and key elements of risk assessment and risk management in the context of food safety and human health

2.4 Summary of specific guidance documents developed by countries outside of the European Union

2.4.1 United States of America. Environmental Protection Agency (US EPA)

In 1998, the US EPA published its finalized 'Guidelines for Ecological Risk Assessment'. The final guidelines expand upon and replace an earlier framework (1992), and included input from stakeholders within and outside the EPA.

a) Guidelines for Ecological Risk Assessment, (EPA/630/R-95/002F, April 1998)
Availability: http://www.epa.gov/ncea

- Detailed discussion of general principles and considerations associated with risk assessment methodology
- Scope is any ecological stressor (chemical, physical or biological)

Scientific principles and framework of risk assessment
These guidelines encompass a wide diversity of environmental stressors including those that are chemical, physical and biological in nature. The guidelines codify principles, terminology and process used in ecological risk assessment by describing the individual components (hazard, exposure, assessment endpoint selection, uncertainty, roles of risk managers and risk assessors etc.) within a logical framework that is useful for decision-making.

Hazard analysis
The guidelines divide ecological risk assessment into three primary phases: problem formulation, analysis, and risk characterization. In problem formulation, risk assessors evaluate goals and select assessment endpoints, prepare a conceptual model and develop an analysis plan. During the analysis phase, assessors evaluate exposure to stressors and the relationship between stressor levels and ecological effects. In the risk characterization phase, assessors estimate risk through integration of exposure and stressor-response profiles and describe risk by discussing lines of evidence and determining ecological adversity.

Likelihood and consequence assessment
The analysis phase includes characterization of exposure and characterization of ecological effects. Both activities evaluate available data for scientific credibility and relevance to assessment endpoints and the conceptual model. Exposure characterization describes sources of stressors, their distribution in the environment, and their contact or co-occurrence with ecological receptors. Ecological effects characterization evaluates stressor- response relationships or evidence that exposure to stressors causes an observed response. The bulk of quantitative uncertainty analysis is performed in the analysis phase, although uncertainty is an important consideration throughout the entire risk assessment. The analysis phase products are summary profiles that describe exposure and the stressor-response relationships.

Uncertainty analysis
There is substantial attention given to the evaluation of uncertainty through all stages of the model, and recommendations are given on how to identify uncertainties in the problem formulation and analysis phases and how to deal with it. There is also clear focus on the importance of statistical evaluation of data at all stages of the assessment process.

Monitor and review
As the model is not specific to GM crops, there is no specific reference to monitoring. However, it is implicit in the approach described that it is flexible with respect to temporal assessments and reviewing of inputs.

Cumulative / long term effects
In the USA there is not usually a requirement to monitor once the crop has been deregulated. The model is so detailed and rigorous, that extrapolation to consider longer-term scenarios should be possible.

2.4.2 Canada. Food Inspection Agency (FIA)

Environmental releases of GM plants in Canada are regulated by the Canadian Food Inspection Agency (CFIA) under powers granted by Part V of the Seed Act and the Canadian Environmental Protection Act. Directive 94-08 of October 2004 (Assessment Criteria for Determining Environmental Safety of Plants With Novel Traits) describes the information used by the CFIA to identify potential adverse environmental impacts associated with the unconfined release of GM plants.

Availability: http://www.inspection.gc.ca/english/plaveg/bio/pbobbve.shtml

- CFIA conducts a case-by-case, environmental safety assessment for all plants with novel traits (PNTs) prior to authorising confined field trials and unconfined releases
- The document describes a five-step, customised approach to environmental safety assessment

Scientific principles and framework
PNTs are defined as plants derived from recombinant DNA technology or conventional breeding techniques that are neither familiar nor substantially equivalent to plants that are in use and generally considered as safe in Canada. The environmental safety assessment consists of five components. The first two require a description of the plants, its modification and novel traits. The third interaction assessment compares the biological characteristics of the modified plants with its unmodified "counterpart", including a post-harvest, residual effect analysis on any three of five indicator species (forage grass, legumes, annual cereal, corn or oilseed). The fourth stage requires an environmental impact assessment for natural and arable ecosystems addressing the degree of change, geographic scope, duration and relative impact on plants, animals, microbes, substance presence/persistence, sustainability, agronomic practice, resource conservation, other concerns and overall environmental quality. Finally, the guidelines require species replacement / competition and seed dormancy studies if there is reason to believe that the behaviour of the plant has been altered in unpredictable ways.

Hazard identification
The guidelines do not identify or recommend any formal hazard identification techniques. The environmental impact and interaction tables provide *de facto* checklists.

Likelihood and consequence assessment
Applicants considering commercialising GM plants are encouraged to include experiments, during confined field trials, designed to meet the regulatory requirements of directive 94-08. Data provided by the applicant to support the interaction, environmental impact assessment and species replacement/competition and seed dormancy studies must be generated using statistically valid experimental designs and protocols. The significance of environmental impacts is addressed in terms of duration, geographical scope and relative impact of the GM plant. The guidelines identify three examples of replacement and seed dormancy analysis in the scientific literature: Crawley et al, 1993; Linder and Schmitt, 1994; and, Rissler and Melon, 1993.

Uncertainty analysis
The guidelines do not refer to uncertainty within the environmental safety assessment.

Monitor and review
The applicant must submit a post release monitoring programme to monitor for unintended or unexpected environmental effects of an authorised product. This is reviewed during the environmental safety assessment of the novel plant in question. The applicant must propose appropriate indicators to evaluate any effects, and these should be based on the characteristics of the PNT. A stewardship plan may be considered sufficient.

Cumulative / long term effects
There is no specific mention of *cumulative long-term effects* in the document.

2.4.3 Australia. Office of the Gene Technology Regulator (OGTR)

In Australia, the Gene Technology Regulator is responsible for protecting human health and safety of the environment by identifying and managing risks posed by, or as a result of, gene technology under the Gene Technology Act 2000 and the Gene Technology Regulations 2001 and corresponding state law. The Risk Analysis Framework provides guidance on how the Office of the Gene Technology Regulator (OGTR) will approach the risk analysis of GMOs under the legislation. The second framework was finalised in January 2005. The guidelines conform to the requirements of the Australian and New Zealand standard on risk management (AS/NZ 4360:2004)

Availability: http://www.ogtr.gov.au/

- Risk analysis = risk assessment + risk management + risk communication.
- Detailed discussion of the steps and elements of risk assessment.
- Discussion of risk management measures and the relationship between assessment and management.
- Discusses and attributes importance to consideration of some issues in risk assessment such as dealing with uncertainties.
- Clearly defines terms that are used in the risk analysis framework.

Scientific principles and framework

The risk assessment is to be scientific and transparent to applicants and the broader community alike. It will be conducted on a case-by-case basis and will consider short and long-term risks. The OGTR will use 'best practice' risk assessment methodologies when conducting the assessment. The risk will be compared to the risks posed by the unmodified organisms, and they will be re-examined in the light of new information. The Regulator will, however, assess the significance of incomplete or absent information, and if uncertainty about the environmental impact remains a licence will not be granted. Similarly, if a risk cannot be managed a licence will not be granted.

Within the framework, the first step in risk assessment is establishing the risk context, i.e. the scope and boundaries of the risk assessment. The next step is to assess the risks in terms of "what might happen", "how might it happen" "will it be serious if it happens", "how likely is it to happen" and finally "what is the risk". This requires a hazard identification step in which sources and causal pathways of harm are identified, and the level of certainty in the hazard identification process. The second risk assessment step addresses the probability of harm and consequences following exposure to the hazard, the level of certainty in the risk estimate and the significance of the risks. The framework also identifies management options and communication of risk. The final step develops and implements the risk management plan including monitoring during and after the release where necessary

Hazard identification

Defined as the process of analysing hazards and the events that give rise to harm. The framework does not detail specific techniques that should be employed to identify hazards, but lists techniques employed by the OGTR. Hazards are identified by consideration of causal pathways that result in harm; a list of potential hazards is provided that will be specifically considered by the OGTR. The possibility of synergistic, additive, antagonistic, cumulative or aggregate effects should be considered. The end point of this step is identification of hazards that warrant detailed estimation of likelihood and consequence.

Likelihood and consequence assessment
The consequence of adverse outcomes or events is considered in terms of severity, effect on baseline conditions, spatial and temporal extent, potential for cumulative adverse impacts and reversibility, and describes a scale by which these are to be assessed. The likelihood of the event occurring is then assessed based on available evidence; again, a scale is described. Risk estimation is presented in a matrix whereby the likelihood of an adverse effect occurring is mapped against the consequences of the event and given a value (negligible to high).

Uncertainty analysis
The aim of the risk assessment is to apply a structured, systematic, predictable approach to the evaluation of risk, and the relative merits of qualitative and quantitative risk assessments are presented. The framework requires the applicant to address the level of certainty in their likelihood and consequence assessments and provides guidance on how to approach it and how to reduce uncertainty in certain areas of the risk assessment.

Monitor and review
The risk analysis framework includes a risk management component that includes options to reduce or mitigate any identified risks. The requirement for monitoring can be incorporated at this stage.

Cumulative / long term effects
The regulations require the OGTR to consider the GMO in terms of the short and long term, and the possibility of cumulative effects. Management options might include the requirement for monitoring in response to identified potential risks.

2.4.4 New Zealand, Environmental Risk Management Authority (ERMA)

Availability: http://www.ermanz.govt.nz/The New Zealand 'Hazardous Substances and New Organisms Act 1996' (HSNO) is to protect the environment, and the health and safety of people and communities, by preventing or managing the adverse effects of hazardous substances and new organisms. Section 25 of the 1996 prohibits the import, development, field-testing or release of new organisms, including *inter alia* GMOs, without prior approval of the Environmental Risk Management Authority (ERMA). Technical guides ER-TG-01-1 9/99 and ER-TG-03-1 7/00 produced by ERMA discuss techniques for identifying risks and preparing information on risks, costs and benefits for applications under section 25 of the HSNO Act.

a) Identifying risks for applications under the Hazardous Substances and New Organisms Act. ERMA, 1999

Availability: http://www.ermanz.govt.nz/resources/publications/pdfs/ER-TG-01-1.pdf

- Detailed guidance on the process of identifying potential risks

b) Preparing information on risks, costs and benefits for applications under the Hazardous Substances and New Organisms Act. ERMA, 2000

Availability: http://www.ermanz.govt.nz/resources/publications/pdfs/ER-TG-03-1.pdf

- Description of the components of risk and options for risk characterization (e.g., qualitative versus quantitative, etc.)
- Consideration of risks in a decision-making context

Scientific principles and framework
Section 7 of the HSNO Act requires ERMA to take into account the need for caution in managing adverse environmental effects when there is scientific and technical uncertainty about those effects. ERMA will use recognised risk identification, assessment, evaluation and management techniques when evaluating applications under Part V of the Act. Information provided by applicants must be "necessary and sufficient" for decision-making. Assessments should be conducted on a case-by-case basis and the results compared to the baseline, i.e. what would happen if the application were refused. The guidelines adhere to the Australian and New Zealand risk management standard (AS/NZS 4360: 1999). The risk assessment consists of five steps: establish the context; hazard identification; calculate risk by combining estimates of likelihood and consequence; and treat risks. Monitoring and reviewing occurs at each step, together with consultation and communication with interested parties.

Hazard identification
The guidelines encourage applicants to demonstrate that they have conscientiously considered the widest possible range of obvious and non-obvious risks. The hazard identification must examine all possibilities of harm regardless of the likelihood of occurrence. The analysis must be thorough and systematic and may include stakeholders and interested parties. The guidelines provide a comprehensive list of hazard identification techniques, including: informal brainstorming, analogy to known cases and failure analysis, the Delphi technique, checklists, fault and event trees and HAZOP analysis.

Likelihood and consequence assessment
Neither of the guidelines discusses specific methods to estimate the likelihood or consequences of hazards. They do, however, contrast qualitative, semi-quantitative and quantitative approaches. Qualitative measures of likelihood, consequence and overall risk based on AS/NZS 4360: 1999 are provided as examples. The guidelines note that quantitative approaches may include various forms of statistical analysis, fault and event tree analysis, and extrapolation. The quality and validity of these approaches depends on the availability of data, and on the accuracy and completeness of the numerical values and methods (e.g. experiments, models) used to derive the data.

Uncertainty analysis
The guidelines distinguish between variability and uncertainty. They suggest that probability or frequency distributions can be used to analyse variability. Two sources of uncertainty are identified: sampling error and lack of knowledge (about the consequences or likelihood of risk). They recommend checking information for bias, statistical competence and peer review, and obtaining further information where appropriate. The guidelines encourage applicants to consider the significance of adverse environmental effects, specifically their distribution over time and space, and whether they are acute, chronic or irreversible.

Monitor and review
Monitoring and reviewing are explicitly highlighted in the risk assessment framework but these components are not discussed further in the guidelines.

Cumulative / long term effects
Consideration of these is implicit in the document, particularly as it is a generic document covering risks to humans of exposure to hazardous substances. However, there are no specific guidelines with respect to monitoring for these.

c) Decision Making: A Technical Guide to Identifying, Assessing and Evaluating Risks, Costs and Benefits, ERMA, March 2004. ER-TG-05-1 03/04
Availability: http://www.ermanz.govt.nz/resources/publications/pdfs/ER-TG-05-01.pdf

This guide has been developed to provide guidance to the ERMA and to Agency staff, and to provide stakeholders with assurance that the ERMA is adopting best practice approaches. The Guide is applicable to all decisions made by the Authority and therefore covers import, manufacture, release or development of hazardous substances and new organisms including GMOs. The guide states that "At present there is no published technical guide to ecological risk assessment, and the best reference is to the United States Environmental Protection Agency '*Guidelines for Ecological Risk Assessment (1998)* (reviewed in 2.3.1 above), which is a refined version of the ERMA framework.

The guide addresses the elements of the decision making process, and includes definition of the overall process, and the individual steps within the process, i.e. identification, assessment, and weighing-up of adverse and beneficial effects (risks, costs and benefits). It is a best practice guide and contains details of the particular tools or techniques that ERMA will apply to the different tasks required by the decision making process. Techniques to ensure risks, costs and benefits are fully and realistically identified, and generic descriptors to represent likelihood, magnitude and risk/benefit are described. Calculation of the level of risk and assessment of uncertainty are covered in detail. Consideration of long-term and cumulative effects is referred to throughout the document; estimates of risk must take account of these components. The document states that risk treatment and review and monitoring are "not applicable to applications for new organisms for full release", implying that if these are required the release is not acceptable.

2.5. Other National and Regional guidance documents

ASEAN (Association of Southeast Asian Nations) – ASEAN Guidelines on Risk Assessment of Agriculture-Related GMOs, 1999
Availability: http://www.aseansec.org/6226.htm

- Intended to ensure a common framework among ASEAN member countries for assessment of risks associated with the transboundary movement of agriculture-related GMOs
- These Guidelines provide a common framework for assessment of risks of agriculture-related GMOs to human health and the environment; and scientific basis for decisions relating to the release of agriculture-related GMOs in ASEAN Member Countries. They address issues related to food safety
- Includes description of information requirements related to risk assessment and risk management, including for numerous specific types of GMOs including plants, fish, other vertebrates, invertebrates, several categories of microorganisms, foods
- No reference to long term and/or cumulative risks, or uncertainty analysis.

Singapore Guidelines on the Release of Agriculture-Related GMOs. Genetic Modification Advisory Committee
Availability: http://www.gmac.gov.sg/guidelines/agriculture.html

- These guidelines are virtually identical to the guidelines developed by the Association of Southeast Asian Nations (above)

- Includes description of information requirements related to risk assessment and risk management, including for numerous specific types of LMOs including plants, fish, other vertebrates, invertebrates, several categories of microorganisms, and foods

BIO-EARN (East African Regional Programme and Research Network for Biotechnology, Biosafety, and Biotechnology Policy Development) – Resource Book for the Implementation of Biosafety in East Africa
Availability: http://www.bio-earn.org/resource%20book/Home.htm

- Risk assessment must be science-based, case-by-case, distinguish between *need-to-know* and *nice-to-know* information, an iterative process, that allows the reviewers to obtain additional information from the applicant as it is needed
- Risk assessment entails identification of characteristics associated with the GMO that may result in potential risks; evaluation of the likelihood and degree of harm; consideration of the consequence should the hazard be realised and identification of risk management strategies to minimise harm
- Detailed list of scientific and technical information that should be provided in the risk assessment. No specific tools or techniques for hazard analysis etc provided; no mention of uncertainty analysis.

Switzerland – Ordinance on the Release of Organisms into the Environment.
Availability http://www.environnement-suisse.ch/imperia/md/content/stobobio/biotech/odeb/14.pdf

- Not specific to GMOs
- Lists considerations for determining the probability and extent of potential damage due to release of organisms into the environment, as well as considerations for determining required safety measures

2.6. Risk assessment approaches used in other areas

International Organization for Standardization (ISO)

General guidance on risk assessment and risk management
Availability: http://www.iso.org
Environmental Management: The ISO 14000 Family of International Standards (2002)

- Various standards related to Environmental Management; the specific standards were not obtained for review.

2.6.1 International Plant Protection Convention

Pest risk analysis for quarantine pests including analysis of environmental risks and living modified organisms (LMO), International Standard for Phytosanitary Measures #11, 2004 (ISPM 11)
Availability: http://www.ippc.int/IPP/En/default.jsp
ISPM 11 is applicable to any LMO that meets the definition of a quarantine pest, namely any LMO that is a potential plant pest (any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products), which is of economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled

- First stage in the pest risk analysis (PRA) is to identify the pest(s) and pathways that are of quarantine concern and should be considered for risk analysis. The context of the need for the PRA must be clearly stated. The actual PRA process consists of pest categorisation, assessment of the probability of introduction and spread, assessment of the potential economic consequences, including environmental impacts.
- Detailed discussion of the elements of methodology is provided, in particular estimating the probability and potential economic consequences, including environmental impacts, of introduction and spread. Assessment of the degree of uncertainty should be documented when concluding the PRA stages, but no specific guidance is given.
- Includes detailed discussion of pest risk management options. The context of these assessments are consideration of future likelihood of entry and establishment of specific pests, so in some ways are considering potential long term effects more directly that some GMO risk assessment approaches.

2.6.2 European and Mediterranean Plant Protection Organisation (EPPO)

Availability: http://www.eppo.org/QUARANTINE/quarantine.htm
EPPO has developed "Guidelines on Pest Risk Analysis - a decision support scheme for quarantine pests". This standard is based on ISPM 11 and was approved and adopted by EPPO in September 2005.

- It provides a simple scheme based on a sequence of questions for deciding whether an organism has the characteristics of a quarantine pest, and if appropriate to identify potential management options.
- Stages of pest risk analysis (PRA) for quarantine pests are: initiation, pest categorization, probability of introduction, assessment of potential economic consequences and pest risk management. Detailed guidance is provided on each of these stages.
- Assessment of the degree of uncertainty should be documented when concluding the PRA stages but no specific guidance is given.
- Includes detailed discussion of pest risk management options and provisions for monitoring and review. The context of these assessments are consideration of future likelihood of entry and establishment of specific pests, so in some ways are considering potential long term effects more directly that some GMO risk assessment approaches.
- The scheme can also be used for PRAs initiated by the identification of a pathway or the review of a policy. Expert judgement may be used in answering the questions.

2.6.3 UK Department for Environment Food and Rural Affairs (Defra)

Standard Methodology to assess the risks from non-native species considered possible problems to the environment (2005).
Availability: http://www.defra.gov.uk/wildlife-countryside/resprog/findings/non-native-risks/index.htmIn the UK this scheme has been adopted for assessment of the risks posed by any non-native organisms to species, habitats or ecosystems in all or part of the UK.

- Provides the first structured framework for evaluating the potential for any non-native organisms, whether intentionally or unintentionally introduced, to enter, establish, spread and cause significant impacts in all or part of the UK.
- Based on a six-module approach – i) assessment of invasive attributes, ii) pathway risk assessment, iii) receptor risk assessment, iv) economic impact assessment, v)

summarising risks and uncertainties leading to the final risk assessment; vi: risk management module.

- Detailed guidance on application and setting the scope and boundaries of the assessment is provided; terms used throughout the scheme are established at the outset; descriptors for values for risk assessments are given. Uncertainty is considered in detail and guidance is provided on its assessment; the model proposes further work in this area.
- Spreadsheets for summarising the level of risk and uncertainty, invasive attributes and economic impact have been created, and new methods for quantifying economic impact and summarising risk and uncertainty have been developed.
- The model has already been used in real assessments for a number of scenarios in the UK.

2.6.4 Codex Alimentarius Commission

The scope of all guidance produced by the Codex Alimentarius Commission is food safety; guidance does not cover environmental risks.
Availability: http://www.codexalimentarius.net/web/index_en.jsp

a) Principles for the Risk Analysis of Foods Derived from Modern Biotechnology, 2003

- Lists principles for risk assessment and risk management related to food safety of genetically modified foods, including some elements of annex III of the Protocol

b) Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant DNA Plants, 2003

- Describes considerations for the assessment and management of risks associated with foods consisting of, or derived from, genetically modified plants

c) Guideline for the Conduct of Food Safety Assessment of Foods Produced Using Recombinant-DNA Microorganisms, 2003

- Describes considerations and approaches for the safety assessment of foods produced using recombinant-DNA micro-organisms

2.6.5 North American Plant Protection Organization

Regional Standard for Phytosanitary Measures #14: Importation and Release (into the environment) of Transgenic Plants, in NAPPO Member Countries (October 2003)
Availability: http://www.nappo.org/Standards/Std-e.html

- Guidance on evaluation of risks to plant or plant health associated with import and release of transgenic plants
- Divided into three modules on (1) importation into contained facilities, (2) confined release into the environment, and (3) unconfined release into the environment
- Lists information requirements regarding the transgenic plant, risk management measures, assessment criteria, potential for reproduction and survival, potential for interactions with sexually compatible relatives, and potential for effects on non-target organisms

Scientists' Working Group on Biosafety

Manual for Assessing Ecological and Human Health Effects of Genetically Engineered Organisms. 1998. Publication of The Edmonds Institute
Availability: http://www.edmonds-institute.org/manual.html

- Detailed flowchart-based approach for hazard identification and for consideration of specific risk pathways
- Risk management options also discussed
- Includes some case-studies

International Organization for Biological Control

Global Working Group on Transgenic Organisms in Integrated pest Management and Biological Control, and the Scientific and Technical Advisory Panel of the Global Environment Facility: Series on Environmental Risk Assessment of Genetically Modified Organisms – Volume 1, A Case Study of Bt Maize in Kenya
Availability: CABI Publishing (orders@cabi.org)

- Detailed case-study development of the methodology for a risk assessment, focusing on a particular trait in a particular receiving environment
- Includes detailed chapters on risk pathways related to (a) impacts on non-target organisms, (b) gene flow and its consequences, (c) insect resistance
- Includes a component that helps to frame the risk assessment in the broader context of decision-making, taking into account risks of alternatives, and incorporating public and stakeholder inputs

2.6.6 Framework specific for cumulative risk assessment

Availability: http://www.epa.gov/osa/spc/2cumrisk.htm

- The US Environmental Protection Agency published the "Framework for cumulative risk assessment" in May 2003 in response to increasing focus placed on combined risks from aggregate exposure to multiple agents or stressors, primarily in the context of exposure to chemicals. The framework document defines cumulative risk assessment as "analysis, characterisation and possible quantification of the combined risks to human health or the environment from multiple agents or stressors". The 2003 report was intended to a first step in the development of clear guidelines for cumulative risk assessment in the USA.
- The framework is conceptually similar to the approach used in ecological risk assessments, but it is distinctive in several areas:
    i. Its focus on the combined effects of more than one agent or stressor makes it different from many assessments, in which, if multiple stressors are evaluated, they are usually evaluated and presented individually;
    ii. Because multiple stressors are affecting the same population, there is increased focus on the specific populations potentially affected rather than on hypothetical receptors;
    iii. Consideration of cumulative risks may generate interest in a wider variety of non-chemical stressors than do traditional risk assessments.
- The framework describes three main phases to a cumulative risk assessment: (1) planning, scoping, and problem formulation, (2) analysis, and (3) risk characterization.
- In the first phase, a team of risk managers, risk assessors, and other stakeholders establishes the goals, breadth, depth, and focus of the assessment. The stressors and the health or environmental effects to be evaluated and the relationships among various stressor exposures and potential effects are identified. The data needed is identified, the

approach to be taken, and the types of results expected during the analysis phase are developed.

- The analysis phase includes developing profiles of exposure, considering interactions (if any) among stressors, and predicting risks to the population or populations assessed. It is in this phase that difficult technical issues such as the toxicity of mixtures, the vulnerability of populations, or the interactions among stressors that may be chemical or non-chemical are addressed and, hopefully resolved. The end of this phase is an analysis of the risks associated with the multiple stressors to which the study population or populations are exposed.
- The risk characterisation phase puts the risk estimates into perspective in terms of their significance, the reliability of the estimates, and the overall confidence in the assessment.
- Hazard analysis should be quantitative where possible and different techniques for achieving this are discussed. However, there is acknowledgement that cumulative risk assessment need not necessarily be quantitative, providing analysis and characterisation are effectively carried out. The difficulty of creating a 'common metric' for dissimilar risks is also discussed and it is acknowledged that there is a need for further work in this area.
- The framework places great emphasis on making sure assumptions made in the assessment are clear, and consideration of the different types of uncertainty and their analysis. The framework does not consider approaches for management or mitigation.
- This framework is aimed primarily at cumulative risks due to chemical exposure, and may be too complex and far-removed in its scope for application to GM crops, however it may offer some useful 'conceptual' guidance.

Appendix 3

Review of regulatory context and needs.
Full responses received from Member States.

Respondent 1

1) What do you understand by "long term" – 10, 50, 100 years or other?

The term "long term" is not interpreted as a fixed period but rather as a time period that exceeds the time period of the consent on a specific product. Therefore it can be used in order to broaden the view in the risk assessment to the time period after the consent. The term "long term" will be defined on a case-to-case basis. For example, the placing on the market of a genetically modified tree will require a different approach of the definition of "long term" than the placing of the market of a perennial plant. The term "long term" generally implies more than one generation cycle of a GMO. "Long term" can also be influenced by intensity of usage of a certain GMO, or a specific trait in various GMOs.

2) What sort of effects do you think might emerge after this period of time? What are your reasons of thinking this?

- Expansive dissemination of the GMO in different habitats (reason: see experience with invasive species)
- Accumulated effects (e.g. gene stacking, multiple resistances) due to combination of traits
- Uncontrolled dissemination of transgenes
- Long-term change of agricultural practice due to intensification of cultivation
- Soil alterations due to long term use of certain GMOs or change of agricultural practice
  Bt-resistant pest species, due to selective pressure of long-term use of Bt-GMO varieties
- HR weeds due to selective pressure of application of HR-GMO varieties and herbicides, or transmission of HR-transgenes
- Long-term change/loss of natural habitats/ecosystems
- Subtle elimination of a wild species by a cultivated GMO (e.g. wild apple replaced by cultivated apples in certain habitats); loss of non-GMO varieties used in agriculture
- Continuous contamination of crops (e.g. experience in USA with Pharm crops and experiences with unwanted contamination events in other countries)

3) What monitoring arrangements do you have in place that would support identification of such effects, should they occur? Do you envisage that it will be possible to prove cause and effect given your current monitoring arrangements?

Concept for National Biodiversity Monitoring being currently developed

Concept for General Surveillance of GMOs; see
Traxler A., Heissenberger A., Frank G., Lethmayer Ch. & H. Gaugitsch (2001). Ecological monitoring of genetically modified organisms. Umweltbundesamt Monographien; Band 147, Vienna 2001, pp 87.

It will be difficult enough to monitor the effects mentioned above with such an intensity that in case an effect occurs it will be detected. If an effect will be detected or not depends on the statistical design of the monitoring approach. Any monitoring system must foresee that the effects that it wants to monitor, i.e. the hypotheses that are formulated, are detected by the statistical design chosen. In order to prove a cause-effect relationship all variables that influence a certain parameter have to be "co-monitored".

However, as this is in most cases not possible, the main variables have to be chosen in order to possibly establish a cause-effect relationship. However, there will be variables that influence a certain parameter, which we don't even know.

4) What do you understand by cumulative effects?

- Effects that arise from the introduction/placing on the market of several different GMOs
- Effects that arise from the introduction of the same "plant protection system" in several GMOs (e.g. same Bt or HR trait in several plants)
- Effects that arise due to intensification of agricultural practice by the use

5) What sort of cumulative effects do you think might emerge after what you have defined as the long term in Q2? What are your reasons for thinking this?

- Soil alterations due to cumulative use of certain herbicides/GMOs
- Development of multiple herbicide resistance in weed species
- Accelerated selection of Bt resistant pest species
- Transgene contaminations in non-GMO crop varieties
- Change/loss of natural habitats and/or biodiversity due to intensification of agricultural production

6) Do you think the monitoring arrangements in place in your member state are adequate to identify these effects should they occur?

At the moment, no.

7) Do you think the current EU risk assessment framework enables applicants to address adequately the potential long-term cumulative effects of GMOs? Do applicants generally pay sufficient attention to this aspect of applications? What do you think would improve this aspect of the authorisation process?

No, applicants do not at all address adequately the potential of long-term cumulative effects of GMOs! Monitoring plans accompanying applications must be considered insufficient in most cases.

In the current step-by-step procedure in the risk assessment of GMOs only short-term effects with few individuals and under few environmental conditions are tested. In most cases no special restrictions on the general use of GMOs are introduced upon consent; although in specific cases such restrictions (time of use, place of use) were considered as measures against adverse long term effects when drafting Dir 2001/18/EC.

The current approach is to analyse the GM plant itself, however effects of GMOs in combination with the agricultural practice should be analysed.

8) Do you have specific criteria for deciding what are acceptable and unacceptable risks? If so, can you let us know what these are and why you have identified these?

For environmental effects – at the moment, no. Definition is dependent on protection goals and on consensus in society.

For Human health effects, specifically the assessment of toxicology, allergology as well as substantial equivalence see:

Spök A., Hofer H., Lehner P., Valenta R., Stirn S. & H. Gaugitsch (2005). Risk Assessment of GMO Products in the European Union. Toxicity assessment, allergenicity assessment and substantial equivalence in practice and proposals for improvement and standardisation. Umweltbundesamt Bericht Band 253, Vienna 2005, pp 131.

Spök A., Gaugitsch H., Laffer S., Pauli G., Saito H., Sampson H., Sibanda E., Thomas W., van Hage M. & R. Valenta (2005). Suggestions for the Assessment of the Allergenic Potential of Genetically Modified Organisms. Int. Arch. Allergy Immunol. 137: 167-180

9) Are the any models used by other regulatory authorities worldwide that you would like to be incorporated into the European approach that might provide for a better assessment of long-term cumulative risks?

No

10) Are you aware of any reports of unexpected effects of the release of a GMO?

See for example:
Tappeser, B., Eckelkamp, C. & B. Weber (2001). Analysis of observed adverse effects from the release of genetically modified organisms. Umweltbundesamt Monographien Band 148, Vienna, 2001, pp 71.

Other published or unpublished reports, e.g.:
Stem split of GM soybean under stress conditions
Loss of unripe cotton balls on GMO-cotton
Change of fat composition in cow milk upon feeding with GM-soybean
Gene stacking of herbicide tolerance traits in Canada

Cumulative long-term effects of genetically modified (GM) crops on human/animal health and the environment: risk assessment methodologies
Reference: No 07-0402/2005/414455/MAR/B4

Respondent 2

1) **What do you understand by 'long term' - 10 years, 50 years, 100 years or other?**
"Long –term" effects could entail a span of 10 years and more.

2) **What sort of effects do you think might emerge after this period of time? What are your reasons for thinking this?**
Effects could occur on public health (allergies and toxicity) and/or the environment (GMO cross with on GMO). The provision in the national law is to grant a permit for five years, maximum.

3) **What monitoring arrangements do you have in place that would support identification of such effects, should they occur? Do you envisage that it will be possible to prove cause and effect given your current monitoring arrangements?**
The monitoring arrangements included in the national law have been set according to Directive 2001/18/EC. In this country no notification has been submitted or examined for a GMO to be placed on the market, therefore there is no hands-on experience on such issue.

4) **What do you understand by cumulative effects?**
Cumulative effects are the indirect and at the same time long-term effects caused by the intended release or placing on the market of GMOs. These could be both environmental and health effects.

5) **What sort of cumulative effects do you think might emerge after what you have defined as the long term in Q2? What are your reasons for thinking this?**
The cumulative effects which might emerge could involve the following:
   - Escape of GMO plants to the wild and affecting the wild flora.
   - Health effects occurring long after the placing on the market of GMO products such as allergies or toxicity.

6) **Do you think the monitoring arrangements in place in your member state are adequate to identify these effects, should they occur?**
As no application has been submitted to our CA for the placing on the market or cultivation of GMO products the answer could only be theoretical: in case of such an event (placing on the market, or cultivation of GMO products) the monitoring plan should be very detailed and control by the authorities very rigorous.

7) **Do you think the current EU risk assessment framework enables applicants to address adequately the potential long-term cumulative effects of GMOs? Do applicants generally pay sufficient attention to this aspect of applications? What do you think would improve this aspect of the authorisation process?**
The general surveillance plan should become more detailed and prescriptive concerning the measures which need to be taken, as well as more demanding on the necessary preventive measures. Additionally further assurances for its proper implementation should be provided by the applicant.

   The need to have a case specific monitoring plan should become more urgent taking into consideration the event (no matter how unlikely) of accidental release into the environment.

The issue of unintended release into the environment should be examined more thoroughly both in the general surveillance plan and the case specific monitoring. There should be improvements of the accuracy and precision of methods of analysis and their adoption and publication as official methods. Problems are particularly faced with the analysis of compound feeds.

8) Do you have specific criteria for deciding what are acceptable and unacceptable risks? If so, can you let us know what these are and why you have identified these?
We did not so far develop such criteria.

9) Are there any models used by other regulatory authorities worldwide that you would like to be incorporated into the European approach that might provide for a better assessment of long-term cumulative risks?
The following issues are considered as significant:

- The feeding studies should become more prolonged instead of the time span presented by the notifiers.
- More thorough evaluation of substantial equivalence.
- Further investigation on the long term consequences of GMO products on flora and fauna.

10) Are you aware of any reports of unexpected effects of the release of a GMO?
The event of the unexpected release into the environment of GMO products destined for feed use occurring in Japan. There are also other reports alerting for the need for further investigation of the effects of GMO products.

Cumulative long-term effects of genetically modified (GM) crops on human/animal health and the environment: risk assessment methodologies
Reference: No 07-0402/2005/414455/MAR/B4

Respondent 3

1) **What do you understand by 'long term' - 10 years, 50 years, 100 years or other?**
We can hardly expect specific information on the detected "long term" effects of any GM crop in a notification to be provided for a period longer than 10 years, because the first GM crops started to be commercially cultivated only 10 years ago. The risk assessment as a part of the notification under part C of Directive 2001/18/EC is usually based on the data available from field trials and, if possible, the experience gained in other countries where the GMO has already been placed on the market.
However, the assessment of possible effects that may appear during the longer period, 20 to 50 years, should be taken into consideration in the risk assessment and in the monitoring plan. The post-market monitoring should cover such a "long term" period.

2) **What sort of effects do you think might emerge after this period of time? What are your reasons for thinking this?**
The possible effects of GM crops could be of two types:
- effects caused by the characteristic of the organism and the modification
- effects emerging from the change of agricultural practices used

Some of the risks are common for agronomical practices using either modified or non-modified plants, e.g. the development of resistance of pests (insects, weeds).
Potential long-term effects may include:
- effect on biodiversity, e.g. changes in the populations of weeds and consequently organisms feeding on weeds (insects, birds – see the UK farm scale trials), changes in the populations of target and non-target organisms.
- gene transfer leading to the spreading of the herbicide resistance trait to the wild relatives of the crop and thus creating persistent weeds
- development of resistant pests populations, like the European corn borer, which can cause first an economic damage, but in a long term also means the increase in pesticide use
- changes in the use of herbicides, e.g. focus on one or two types of herbicides, and consequent changes in the spectrum of weeds or emergence of resistant weeds

Positive long-term effect of herbicide tolerant crops could be the decrease of soil erosion resulting from the post-emergent application of the herbicide.
Positive effect of insect resistant (Bt) crops is the reduction of the use of chemical insecticides.
Effects on soil organisms can be positive or negative, that should be a subject of further studies.

3) **What monitoring arrangements do you have in place that would support identification of such effects, should they occur? Do you envisage that it will be possible to prove cause and effect given your current monitoring arrangements?**
Some multi-year studies with GM crops are carried out in this country, but they are not aimed at systematic long-term monitoring of environmental and health effects.

A monitoring network for non-modified crops has been in place for more than 20 years in this country. Besides agronomical performance and quality also long-term effects of used agronomical practices in different agroecological conditions are monitored in trials funded by the Ministry of Agriculture. The developed methodology for studying long term effects of the crop and agricultural practices on pests, diseases, biogeochemistry and soil microorganisms can be applied to the GM-crops as well. Monitoring of GM crops should be included in this network or a new GM network should be established in EU member states.

4) What do you understand by cumulative effects?

Directive 2001/18/EC, Annex II: PRINCIPLES FOR THE ENVIRONMENTAL RISK ASSESSMENT.

Definitions: *"Cumulative long-term effects" refers to the accumulated* ***effects of consents*** *on human health and the environment, including inter alia flora and fauna, soil fertility, soil degradation of organic material, the feed/ food chain, biological diversity, animal health and resistance problems in relation to antibiotics.*

Cumulative effects are - in our opinion - effects occurring at the same time and at the same place or area. These are, of course, not just negative effects, but also positive or indifferent ones.

5) What sort of cumulative effects do you think might emerge after what you have defined as the long term in Q2? What are your reasons for thinking this?

Cumulative effects may consist of the effects given under 3), in the future cumulative effects will probably be connected also with an increasing number of cultivated GM crops and a spectrum of new traits. These effects are very difficult to anticipate at present.

Cumulative long-term effects of cultivation of GM crops are most likely to emerge from the change in agronomic practices (e.g. different application of herbicides in case of herbicide tolerant crops) than from the genetic modification itself. According to the Annex II of the Directive 2001/18/EC, such effects have to be considered in the environmental risk assessment in case of placing of GMOs on the market.

6) Do you think the monitoring arrangements in place in your member state are adequate to identify these effects, should they occur?

No, the monitoring arrangements are not sufficient (see 4). The monitoring of possible effects of GM crops should be carried out both by the authorities and the notifier. The methodology should be developed in accordance with the obtained data and should react flexibly to the new traits and connected changes in the agronomic practice.

7) Do you think the current EU risk assessment framework enables applicants to address adequately the potential long-term cumulative effects of GMOs? Do applicants generally pay sufficient attention to this aspect of applications? What do you think would improve this aspect of the authorisation process?

The EU legislative (*Directive 2001/18/EC, Annex II and Commission Decision 2002/623/EC)* sets only a general framework for the risk assessment, especially as potential long-term cumulative effects are concerned. Consequently the corresponding parts of the

notifications are usually quite brief. To facilitate the more detailed risk assessment in this respect, the existing framework should be supplemented by specific guidelines, case studies etc., focused on the identified possible long-term effects.
The improvement of this aspect of the authorisation process is an important task not only for the applicants, but also for the Authorities. The applicants should provide the assessment of potential long-term effects of the notified GMO, but they are not in a position to provide an analysis of the cumulative effects caused by the overall GM crops use (rate of the acceptance of biotechnology applications in agriculture, different crops, traits).
The output from this project will surely contribute to the improvement of addressing the long-term risk assessment as well.

8) Do you have specific criteria for deciding what are acceptable and unacceptable risks? If so, can you let us know what these are and why you have identified these?
The environmental risk assessment of a GM crop and its use should be based on comparison to the use of the corresponding non-modified crop (see *Directive 2001/18/EC, Annex II B. General Principles: "In accordance with the precautionary principle, the following general principles should be followed when performing the e.r.a.:*
- identified characteristics of the GMO and its use which have the potential to cause adverse effects should be compared to those presented by the non-modified organism from which it is derived and its use under corresponding situations; …")
The acceptable risks are the risks lower or on the same level as the risks of non-modified crop variety. A risk / benefit analysis should be made as well.

9) Are there any models used by other regulatory authorities worldwide that you would like to be incorporated into the European approach that might provide for a better assessment of long-term cumulative risks?
-

10) Are you aware of any reports of unexpected effects of the release of a GMO?
No, as far as we know, all the published effects of releases of GMOs were the effects taken into consideration in the appropriate risk assessments.

Respondent 4

1) **What do you understand by 'long term' - 10 years, 50 years, 100 years or other?**
Between 20 - 50 years.

2) **What sort of effects do you think might emerge after this period of time? What are your reasons for thinking this?**
During this period of time possible effects would be:
- More cases of contamination of grain with unauthorised grain (similar to the Bt maize in the Starlink case). As companies develop more GMOs with stacked genes and traits, the inherent weaknesses in current PCR-based systems to differentiate between combinations of hybrids will appear. So for example, current PCR methods will NOT differentiate between a separate consignment of NK603 maize and MON 810 maize or a hybrid of the two or even the presence of a third unauthorised hybrid crossed with the two parents.

- For the Southern Mediterranean region we would predict cases of hybridisation and introgression of GM oilseed rape (if approved for cultivation) with concomitant glyphosate resistance appearing in weeds. Various factors would increase the chance of this occurring in this region: it shares pollination windows with various important agricultural weeds, rubble walls prevent the implementation of the usual weed control methods and the plant has a history of hybridisation.

- In a worse case scenario, there could be unexpected health problems in sectors of the EU population exposed to a particular modification. Our current health risk assessments are based on our current knowledge of what constitutes an allergy-generating food and our current databases. Given the varied eating habits of humans it would take time to make the particular links.

3) **What monitoring arrangements do you have in place that would support identification of such effects, should they occur? Do you envisage that it will be possible to prove cause and effect given your current monitoring arrangements?**
Current monitoring techniques are not sufficient to prove cause and effect for points one and three mentioned above.

4) **What do you understand by cumulative effects?**
The effects generated over time and by a combination of factors.

5) **What sort of cumulative effects do you think might emerge after what you have defined as the long term in Q2? What are your reasons for thinking this?**
These are difficult to predict.

6) **Do you think the monitoring arrangements in place in your member state are adequate to identify these effects, should they occur?**
No, current monitoring effects are not sufficient to detect cumulative effects.

7) **Do you think the current EU risk assessment framework enables applicants to address adequately the potential long-term cumulative effects of GMOs? Do**

applicants generally pay sufficient attention to this aspect of applications? What do you think would improve this aspect of the authorisation process?
No, the current EU regulations concentrate on determining whether a negative event will occur but are not strong on determining potential long-term effects. In our experience applicants often concentrate on proving why they do not have to present information and not on providing information.

Some improvements to the current system could be:

- to streamline the requirements for approval for food and feed. So for example feeding tests on chickens and sub-chronic toxicity tests should be standard to support a food and feed request.
- lengthen the time of feeding tests.
- carry out a study to determine how relevant the current animals used in such tests are to the human dietary habits.
- use independent bodies not linked or paid by companies to provide critical data linked to safety or nutritional qualities of GMOs.
- provide further guidelines on what constitutes substantial equivalence. For example if a potato shares the same fatty acids, amino acids, minerals, vitamins and levels of glycoalkaloids as the non-transformed variety BUT there is evidence that the levels of other glycosylated compounds vary would this still constitute substantial equivalence?
- monitor closely results from sub-chronic toxicity tests. We have been provided with tests where there were aberrations seen in the liver, yet the company stated that there were no macroscopic changes.

8) **Do you have specific criteria for deciding what are acceptable and unacceptable risks? If so, can you let us know what these are and why you have identified these?**
We are in the process of developing these.

9) **Are there any models used by other regulatory authorities worldwide that you would like to be incorporated into the European approach that might provide for a better assessment of long-term cumulative risks?**
We are not aware of any of these.

10) **Are you aware of any reports of unexpected effects of the release of a GMO?**
The Starlink case – where a type of maize not intended for human consumption entered the human food chain in taco shells and other snack foods.

Cumulative long-term effects of genetically modified (GM) crops on human/animal health and the environment: risk assessment methodologies
Reference: No 07-0402/2005/414455/MAR/B4

Respondent 5

1) **What do you understand by 'long term'- 10 years, 50 years, 100 years or other?**
Long term would be any effect occurring after 10 years or longer.

2) **What sort of effects do you think might emerge after this period of time? What are your reasons for thinking this?**
Because of the specificity of effects of genetically modified organisms (GMOs) it is not wise to start guessing in a general way about possible long-term effects. Every GMO is different and should be assessed on a case-by-case basis. Only a thorough risk assessment could shed light on the possible long-term effects of a particular GMO. If potential hazardous long-term effects are identified in the risk assessment for this specific GMO, specific monitoring is required as part of the permit.

3) **What monitoring arrangements do you have in place that would support identification of such effects, should they occur? Do you envisage that it will be possible to prove cause and effect given your current monitoring arrangements?**
In this country many different monitoring networks exist that could be applied for the observation of potential long-term effects of GMOs that are unforeseen in the risk assessment. It would be too lengthy to describe them all. Briefly, systems exist for the monitoring of plant diseases, amphibians, plants, birds, marine life, etc. Specific monitoring requirements for GMOs, based on potential hazards identified in the risk assessment, are included in the permit for release into the environment.

A discussion on proving cause and effect in the case of GMOs is at this moment purely theoretical in case of unexpected long-term effects of GMOs. It is therefore not possible to predict whether proving cause and effect in practice will in all cases be possible. It is not likely that this will be impossible. Lots of experience exists for proving cause and effect for other organisms than GMOs.

4) **What do you understand by cumulative effects?**
Cumulative effects are effects that pile up.

5) **What sort of cumulative effects do you think might emerge after what you have defined as the long term in Q2? What are your reasons for thinking this?**
The emergence of cumulative effects, if they occur, depends on the GMO in question. It is not possible to predict these possible cumulative effects if it is not clear what sort of GMOs will be on the market in the future.

6) **Do you think the monitoring arrangements in place in your member state are adequate to identify these effects, should they occur?**
If an adverse effect occurs, current monitoring arrangements in this country, or the monitoring requirements as stated in the permit, will possibly lead to the detection of this effect.

7) **Do you think the current EU risk assessment framework enables applicants to address adequately the potential long-term cumulative effects of GMOs? Do applicants generally pay sufficient attention to this aspect of applications? What do you think would improve this aspect of the authorization process?**

Yes, the current EU risk assessment framework enables applicants to address the potential long-term cumulative effects of GMOs in an adequate manner. Applicants have to assess these effects in the risk assessment. The current application process works well for these aspects.

8) **Do you have specific criteria for deciding what are acceptable and unacceptable risks? If so, can you let us know what these are and why you have identified these?**
We use the criteria given in directive 2001/18/EC to decide whether risks are acceptable or not. Directive 2001/18/EC provides a framework to make such decisions.

9) **Are there any models used by other regulatory authorities worldwide that you would like to be incorporated into the European approach that might provide for a better assessment of long-term cumulative risks?**
No.

10) **Are you aware of any reports of unexpected effects of the release of a GMO?**
No.

Respondent 6

1) **What do you understand by 'long term' - 10 years, 50 years, 100 years or other?**
Depends on the context – species, environment, human effects etc. Consider 10 years to be adequate but e.g. for forest trees it would be inadequate. Very complex question to answer.

2) **What sort of effects do you think might emerge after this period of time? What are your reasons for thinking this?**
It is easier to focus on immediate effects and health effects, which are relatively short term, allergies might be one longer-term effect we could expect to see. Environmental effects are much longer term anyway, e.g. herbicide resistance effects could take much longer than 10 years to be detected in the environment, therefore it is necessary to think longer term anyway. Forestry, fruit trees, forage grasses, horticultural and agricultural crops all have different rotations and life cycle therefore need to consider on case-by-case basis. Having said that, gene flow and its consequences are expected to be the greatest anticipated effects, also effects on seedbanks.

3) **What monitoring arrangements do you have in place that would support identification of such effects, should they occur? Do you envisage that it will be possible to prove cause and effect given your current monitoring arrangements?**
No monitoring programmes are in place at the moment specifically for GMOs; the generic schemes that are in place could pick up effects of growing GM crops, but not really been evaluated yet. The monitoring networks in place when we approach commercial release of GM crops will need to be instructed and/or educated on what to look for. The Biotech Advisory Board has reviewed the monitoring that is currently going on, but no results yet. GM crops are currently a long way off at the moment.

The agricultural community is quite opposed to GM crops at the moment so the prospects for cultivation of GM crops is currently limited. At the National level, authorisations are reviewed after the EC decisions, and a decision is then made as to suitability for the country.

4) **What do you understand by cumulative effects?**
A sum of factors which individually do not have an effect but when combined may have an effect. These scenarios become increasingly complex.

5) **What sort of cumulative effects do you think might emerge after what you have defined as the long term in Q2? What are your reasons for thinking this?**
Again, comes back to consideration on a case-by-case basis.

6) **Do you think the monitoring arrangements in place in your member state are adequate to identify these effects, should they occur?**
There are none in place at present, but CA will look deeper into this when it becomes necessary.

7) **Do you think the current EU risk assessment framework enables applicants to address adequately the potential long-term cumulative effects of GMOs? Do**

applicants generally pay sufficient attention to this aspect of applications? What do you think would improve this aspect of the authorisation process?
We do not think the applicants generally pay sufficient attention to long term risks; notifications present very little in the way of peer-reviewed data, often data to support risk assessments is poorly presented and with poor hypotheses, making its validity questionable. This makes it difficult to critically review the data. We also believe that the notifications present risk from a biased perspective. However, the framework for 2001/18/EC does not provide guidance on data collection, analysis and presentation so it is not necessarily the applicants' fault; there needs to be clear guidance on what should be collected, possibly focussing on key areas of interest, but this would require an EU-wide agreement. CAs could be much more exacting in their requirements for the risk assessments. Possibly there is a need for a review of the roles of the CA and the applicants.

Independent research in these areas would be very valuable - currently we are too reliant on information provided by the notifiers. But there may be difficulties getting the companies to cooperate (the UK's farm scale evaluation trials were thought to be a good example).

8) **Do you have specific criteria for deciding what are acceptable and unacceptable risks? If so, can you let us know what these are and why you have identified these?**
We do not have specific criteria, and again it comes back to case-by-case assessments. No specific indicators have been identified yet. We would be much more critical about assessment for crops that are likely to be grown in this country. Benefits to society and the country must be demonstrated – we would be less stringent about a crop for which clear benefits can be demonstrated.

9) **Are there any models used by other regulatory authorities worldwide that you would like to be incorporated into the European approach that might provide for a better assessment of long-term cumulative risks?**
No.

10) **Are you aware of any reports of unexpected effects of the release of a GMO?**
The Network Biosafety Service has identified a couple of reports [awaiting info about these from the interviewee].

Respondent 7

1) **What do you understand by 'long term' - 10 years, 50 years, 100 years or other?**
This country wants to see no effects, irrespective of time, therefore long term refer to very long way into the future. Any risks that are identified for the short term should be extrapolated and considered in the long term in the ERA. Also depends on the crop you are looking at – the standard life time for a forest tree is very different from an agricultural crop, therefore for forest species the long term is 100 years plus. Also ecological timeframes are different from agricultural timeframes.

2) **What sort of effects do you think might emerge after this period of time? What are your reasons for thinking this?**
All compartments of the environment might be affected, cannot focus on just one area.

3) **What monitoring arrangements do you have in place that would support identification of such effects, should they occur? Do you envisage that it will be possible to prove cause and effect given your current monitoring arrangements?**
See answer to Q7 below.

4) **What do you understand by cumulative effects?**
Cumulative effects are the effects of having more than one GMO, which leads to a change in the potential risks; this also affects the long-term risks.

5) **What sort of cumulative effects do you think might emerge after what you have defined as the long term in Q2? What are your reasons for thinking this?**
All compartments of the environment might be affected, cannot focus on just one area.

6) **Do you think the monitoring arrangements in place in your member state are adequate to identify these effects, should they occur?**
Monitoring programmes are in place for conventional grazing, forestry, intensive agriculture is monitored less.

7) **Do you think the current EU risk assessment framework enables applicants to address adequately the potential long-term cumulative effects of GMOs? Do applicants generally pay sufficient attention to this aspect of applications? What do you think would improve this aspect of the authorisation process?**
The tools for monitoring are available. The lead CAs should use the tools and put pressure on the notifier to use these tools. CAs should make their own assessments and see if they agree with the notifier. Checklists are provided in the Directive. RAs are slightly *ad hoc*; it is difficult to formalise the risk assessment process because of the risk identification step (risk perception differs depending on who is doing the RA).

   Applicants do generally pay sufficient attention to these risks and do a good job, but too little effort is put into monitoring; they need to do more on monitoring.

8) **Do you have specific criteria for deciding what are acceptable and unacceptable risks? If so, can you let us know what these are and why you have identified these?**

We do not have any specific criteria for acceptable/unacceptable risks, there is no EU-wide guidance on this therefore it is up to National legislation. Ethical reasons come into play at this point. Acceptability of risks is a philosophical, internal value for each CA, not an economic consideration and will differ from crop to crop.
CA weighs disadvantages versus the advantages. If the GM crop offers clear advantages for the CA, i.e. for the environment or the general public, the CA would be prepared to accept greater risks. This is not an exact science! Unaware of how things are done in other MSs; quality of assessments varies MS to MS, UK (ACRE) is generally of very high quality, we do not have a similar group, we have a group that is half scientists, half politicians. We believe that assessments should not be blurred by politics. Influence of politics should be at the end of the scientific assessment process, to bring in concept of ethical assessment of level of acceptability of risks.

9) **Are there any models used by other regulatory authorities worldwide that you would like to be incorporated into the European approach that might provide for a better assessment of long-term cumulative risks?**
No.

10) **Are you aware of any reports of unexpected effects of the release of a GMO?**
No, there is no monitoring for this – should look at the USA for reports of these!

Respondent 8

1) **What do you understand by 'long term' - 10 years, 50 years, 100 years or other?**
   In reference to environmental issues, 10 years or more.
   The magnitude, likelihood and timing of a long-term effect can change depending on the stability of the receiving environment that could drastically change in 10 or 20 years.

2) **What sort of effects do you think might emerge after this period of time? What are your reasons for thinking this?**
   - Loss of biodiversity due to gene flow to sexually compatible plants or establishment of the engineered plant as an invasive species in the receiving environment;
   - Changes in insect community structure and function due to reduction of complexity in agricultural landscapes by reducing plant (crop and weed) diversity. It can affect the ecosystem stability by top-down or bottom-up effects;
   - Changes in soil microbial profile, diversity or community structure that could lead to loss of soil biodiversity and thus changes in biogeochemical cycles and reduced soil fertility;
   - Further potential and specific effects could be identified performing a case-specific environmental risk assessment and/or analysing the results of monitoring activities of the GMOs' releases present at the moment worldwide.

3) **What monitoring arrangements do you have in place that would support identification of such effects, should they occur? Do you envisage that it will be possible to prove cause and effect given your current monitoring arrangements?**
   Monitoring activities set up for the identification of GMOs effects (long and short term) are only at regional level and they are related to:
   - Research activities to identify bio-indicators and define experimental standard protocols (most of them on soil ecosystems).
   - Research activities and data collection finalised to the definition of baselines.

   While the monitoring activities at national level are not directly built up for the monitoring of effects related to GMOs and their release into the environment, we consider that they are suitable to be modified and adapted for this object.

4) **What do you understand by cumulative effects?**
   In this framework we distinguish between cumulative and synergistic effects.
   - *Cumulative effects*: Engineering of organisms could lead to a non-target expression of genes such as toxins, allergens and carcinogens. The repeated exposure or intake of such elements can determine adverse effects that are detectable only over long periods.
   - **Synergistic effects*: They can arise when a combination of GMOs is released in neighbouring sites concurring to an adverse effect or when***

different characteristics of a single GMO (linked to the genetic modification and/or to the biological features) act together resulting in a harmful effect.

5) What sort of cumulative effects do you think might emerge after what you have defined as the long term in Q2? What are your reasons for thinking this?

- Undetected toxins, allergens and carcinogens can enter in the food chain with cumulative effects;
- Decrease in insect diversity can be determined by repeated exposure of a target species to a toxin or reducing the agricultural landscape diversity, which is likely to result from developments such as herbicide resistance.

6) Do you think the monitoring arrangements in place in your member state are adequate to identify these effects, should they occur?

Not at the moment because of the lack of databases concerning environmental baselines, lack of indicators, lack of monitoring activities on GMOs at national level.

7) Do you think the current EU risk assessment framework enables applicants to address adequately the potential long-term cumulative effects of GMOs?

No

Do applicants generally pay sufficient attention to this aspect of applications?

No

What do you think would improve this aspect of the authorisation process?

- Environmental Risk Assessment standard methodology;
- Further research activities not only related to gene technology but to ecological interactions, genomic, proteomic and population dynamic;
- Results of monitoring activities (Case Specific and General Surveillance) of the GMOs' releases present at the moment worldwide.

8) Do you have specific criteria for deciding what are acceptable and unacceptable risks? If so, can you let us know what these are and why you have identified these?

No.

9) Are there any models used by other regulatory authorities worldwide that you would like to be incorporated into the European approach that might provide for a better assessment of long-term cumulative risks?

The government has promoted a project for the development of an ERA standard methodology. This methodology has been implemented on the deliberate release of Genetically Modified Higher Plants for experimental purpose. The next step of the project will be the implementation of the methodology to the deliberate release of Genetically Modified Microrganisms for experimental purpose and to the deliberate release of Genetically Modified Higher Plants for commercialisation.

10) Are you aware of any reports of unexpected effects of the release of a GMO?

No

Respondent 9

1) **What do you understand by "long term" – 10, 50, 100 years or other?**
Two types of effect. A change that is not reversible within 15 years. A small change which is not easily detected but is in the same direction over many (10 plus years) building up a significant environmental change that is not reversible in the short term.

2) **What sort of effects do you think might emerge after this period of time? What are your reasons for thinking this?**
In answering this question, we are limiting our consideration to this country and to the range of GM crops which are currently available for cultivation in the EU or have pending applications for marketing consents for cultivation. We do not intend to speculate about the impact of GMOs, which at some point may receive marketing approval but which are not in the regulatory pipeline. We also note that an effect could be good, bad or neutral.

In the foreseeable future, herbicide tolerance is the only trait that will be bred into varieties suitable for cultivation in this country. The insects targeted by *Bt* genes in approved GM crops and those with pending applications do not currently have pest status in this country. GM crops containing these genes are therefore unlikely to be grown here.

Effects that might emerge over a long timescale include those on a landscape scale, such as changes in cultivation patterns and management practices. This in turn could affect certain species directly and affect the balance of farmland biodiversity, depending on how these changes are managed. However similar effects are likely to be found arising from the introduction of any new or different agricultural crop or crop management system and the possibility of such effects is not restricted to GM crops. The emergence of agricultural weeds with herbicide tolerance to broad spectrum herbicides is possible in certain rotations. This is most likely to occur through the emergence of volunteer crop plants and repeated herbicide usage. There could be gene flow from oilseed rape to sexually compatible 'wild relatives'. The effect of this will depend on the availability and use of herbicides that are used as an alternative. The outcome of the UK farm scale evaluations are an example of indirect effects due to new weed management practices made possible by GM HT crops.

3) **What monitoring arrangements do you have in place that would support identification of such effects, should they occur? Do you envisage that it will be possible to prove cause and effect given your current monitoring arrangements?**
This country does not have specific environmental monitoring arrangements for GM crops, because none are yet grown here. But there are other environmental and agronomic monitoring programmes that are carried out in this country for certain indicators. It is possible that large effects, for example in cultivation patterns on a regional scale might be attributable to GM cropping, but for the most part, it is very difficult to envisage being able to link an effect identified

through general surveillance to a particular GMO or to GM crops more generally. Conventional agriculture already has such a gross effect on farmland biodiversity, this is likely to swamp any effects due to GM crops. Hypothetical links would need to be tested experimentally.

4) What do you understand by cumulative effects?

The combined impacts that accrue over time and space from the cultivation of GM crops. This might include the interactive effects of different GMOs as well as to incremental changes resulting from increases in the scale (over time and space) of a particular GMO.

5) What sort of cumulative effects do you think might emerge after what you have defined as the long term in Q2? What are your reasons for thinking this?

Cumulative effects are difficult to predict but could include the evolution of herbicide-tolerant agricultural weeds (through herbicide usage in particular but possibly through gene flow). Gene stacking could result in GM crops with tolerance to a number of broad-spectrum herbicides. Such tolerances could also arise in conventional (non-GM) crops.

6) Do you think the monitoring arrangements in place in your member state are adequate to identify these effects, should they occur?

Yes, if these effects have an adverse effect on the environment as compared to the effects associated with the cultivation, management and use of their non-GM equivalents.

7) Do you think the current EU risk assessment framework enables applicants to address adequately the potential long-term cumulative effects of GMOs? Do applicants generally pay sufficient attention to this aspect of applications? What do you think would improve this aspect of the authorisation process?

The current framework presents applicants with adequate opportunity to address long-term cumulative effects of GMOs. Potential long-term cumulative effects of GMOs for the most part will have to be dealt with by general surveillance because of the difficulty in predicting them. Case-specific monitoring may also be pertinent if the risk can be identified but not dealt with in the environmental risk assessment (e.g. due to scale). A clearer understanding of the principles of case-specific monitoring and general surveillance at Member State level would be advantageous. We also consider that the interaction between 2001/18/EC and 91/414/EC in the regulation/monitoring of pesticide use on herbicide tolerant crops needs clarification.

8) Do you have specific criteria for deciding what are acceptable and unacceptable risks? If so, can you let us know what these are and why you have identified these?

Risks to human health and the environment are assessed in accordance with the criteria laid out in Annex 2 of Directive 2001/18/EC. The risk of an adverse effect on human health or the environment as a result of a particular GM crop release (and the management practices associated with the release) are compared

to those associated with equivalent non-GM crops and the conventional management practices associated with them. A risk is judged to be unacceptable if comparisons under realistic conditions reveal a significant adverse effect on human health or the environment and measures can not be put in place to sufficiently prevent such events occurring. Adverse effects identified in lower-tier testing (for example effects on non-target insects in no-choice bioassays) may be acceptable if subsequent field testing has found that the effect does not occur under realistic conditions. In such cases there would normally be a requirement for case-specific monitoring to check that the assumptions of the environmental risk assessment.

9) **Are there any models used by other regulatory authorities worldwide that you would like to be incorporated into the European approach that might provide for a better assessment of long-term cumulative risks?**
We are not aware of any models of this nature.

10) **Are you aware of any reports of unexpected effects of the release of a GMO?**
We are not aware of any substantiated reports of unexpected effects resulting from the release of GMOs.

Appendix 4

Notes from the Workshop on 31/10/05

CSL, Sand Hutton, York

Attendees:

CSL:

1. Christine Henry
2. Vicky Jackson
3. Andrew Cuthbertson
4. James Blackburn
5. Steven Parker
6. Nigel Boatman
7. Theo Allnutt
8. Sarah Hugo

Newcastle University:

9. Prof Faith Williams

VLA:

10. Chris Teale (by videolink)

RM Consultants Ltd:

11. David Nicholls
12. Agnieszka Stachowicz

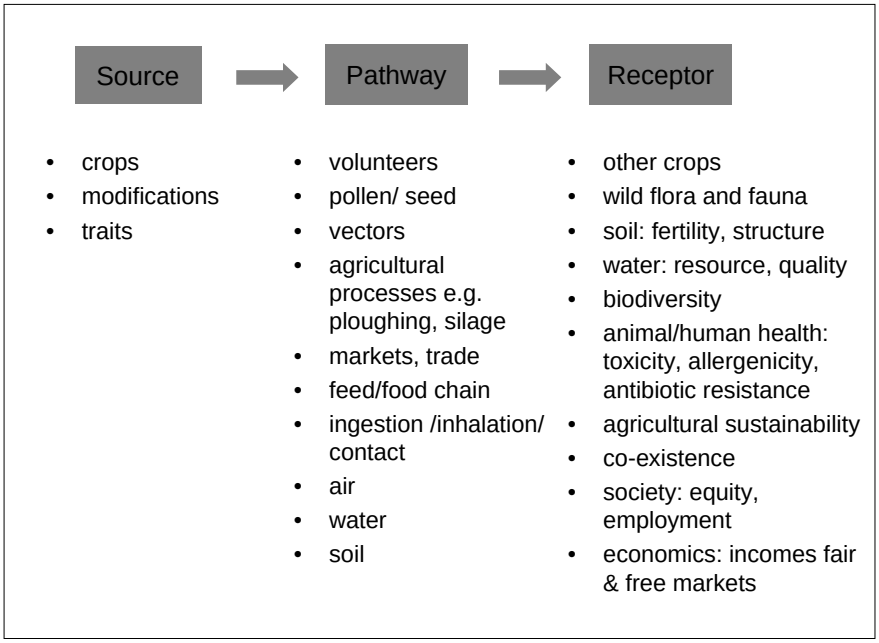
The aim of the workshop was to identify the range of potential long term and cumulative risks to human/animal health and the environment from GM crops, by bringing together experts in various aspects of the topic in facilitated brainstorming.

The following notes record the findings in terms of both potential risks identified, and features, events and processes that may change and hence affect risks.

I

II Breakout Groups 1 – Scenarios

This session was intended to start participants thinking and talking freely about the topic. The participants divided into three groups of three or four and spent 20 minutes imagining (based on evidence or realistic speculation) some scenarios in which long term or cumulative effects could occur. No specific structure was imposed on their discussions, although a Source-Pathway-Receptor model (Figure 2) was suggested as a possible framework for prompting discussion and structuring the results.



III Figure 2 Source-Pathway-Receptor model - prompts for Scenarios

Each group then outlined the scenario and the risks they envisaged, with the following results:

13. Effects on jobs in chemical industry if demand for agrichemicals is reduced/ increased
14. Effects on research employment and priorities;
15. Climate change – exotic pests become native pests – this could change the need for and effectiveness of (both conventional and) GM crops
16. Larger field margins needed to provide a buffer around GM crops – various secondary ecological and agricultural effects
17. Fewer insects & spiders – effects on food chain for birds;
18. Monitoring becomes more difficult if there is widespread GM cultivation - costs of covering large areas *vs* spatial sampling problems
19. There may be greater land cover by particular crops that give the best economic rewards for farmers (cf Oil Seed Rape in 1970s) – effects on biodiversity and agricultural markets
20. Interactions with climate change – may lead to greater or lesser propensity to grow crops that are more likely to be GM - e.g. maize may become more viable in N Europe due to warmer climate
21. Long term effects may be independent of the GM construct itself – more to do with the scale of cultivation of that crop
22. More (different?) proteins about in the environment – more risk of sensitisation to allergies
23. Gene stacking – possible increase in toxicity
24. 'Superweed' scenario: introgression of pest/ herbicide-resistance into a wild plant, taking it beyond current, equilibrium population to become an invasive weed
25. Increased proportion of land used for GM – greater pressure on non-GM growers

26. Antibiotic-resistance genes used in the construction of GM plants could theoretically be passed to bacteria in the environment or to bacteria in farm animals. For example GM maize could be eaten either as silage or in animal feed, and this could impact on the animals' gut bacteria. This might, if it became widespread in the long term or led to the dissemination of new types of resistant bacteria, compromise the effectiveness of the antibiotics whose resistance genes were used in the GM constructs. These antibiotics in general remain very useful for the treatment of many animal diseases, and the use of these older antibiotics for animal treatment, allows the reservation of more advanced antimicrobials for humans.
27. Possibility of passage of antibiotic resistance genes originating from GM plants to bacteria from animals or the environment and then to human bacteria via the food chain or other routes. Would new codes of practice be required in abattoirs to prevent such events happening?
28. More maize may be grown, due to its GM-related advantages. Maize harbours *Fusarium*, which can then be passed to wheat – a problem for wheat growers;

IV Brainstorming – Long Term Effects

Participants were asked to identify what could change in the long term that might affect the source, receptor or pathway. This following prompts were applied:

Features, Events, Processes (FEPs) that may change over time and affect S, R or P

29. recessive genes
30. dormant/ latent traits
31. climate change
32. land use, soils, habitats
33. agricultural practice
34. food consumption
35. behaviour and activities
36. population distribution
37. regulation
38. organisational structures
39. information availability

Deviations: how the FEPs might change, or what might go wrong

40. More/Less
41. Early/Late
42. Not Done
43. Beyond Intent

The results, under each of the FEP prompts are shown below.

IV.i.1 Recessive genes/dormant/latent traits

In general, these may lead to harmful (or beneficial) effects that are not noticed during the consent period. They could result from a modification of an existing recessive gene or from a GM that is itself recessive.

44. Suppression of dormant traits may be removed in long term by (i) natural mutation or (ii) changes in the environment;
45. Polymorphisms (human mainly, not animal)
46. 'Silent spread': almost by definition, recessive genes will not be noticed until expressed, unless there is specific genetic monitoring. It may be possible to calculate the probability of occurrence of expression (if the recessive gene/ trait is known about in advance). Probably more likely to occur in wild relatives than in GM crop itself.

IV.i.2 Climate change

Climate change and its many secondary effects may alter the vulnerability of the environment to the effects of GM crops. It is important to notice that GM is only part of the climate change issue.

47. There are long lead-in times for R&D – we may be focussing on the wrong issues now
48. GM crops could give a benefit in enabling society to adapt to climate change – we may be able to design crops that cope better with the new conditions. But there are dangers in seeking and relying on such quick 'technical fixes' - we have a poor understanding of interactions.
49. Market acceptability of GM may increase if it becomes seen as a solution to a major issue - e.g. how to adapt to climate change – rather than just for the commercial gain of the industry;
50. Danger of becoming dependent on a narrow genetic base. But on the other hand, GM crops could lead to greater diversity: since traits can be passed between species it may enable farmers to grow a wider variety of crops in one area.
51. Cultivation of large areas of the same crop with a narrow genetic base could lead to increased vulnerability to new plant diseases or new strains of old plant diseases that have become particularly adapted to infect plants with this narrow genetic base.

Note: as well as designing-in specific traits, GM can also be used to increase the rate of natural mutations, then screen for and select the useful ones. This may be seen as ethically more acceptable.

IV.i.3 Land use, soils, and habitats

52. Urbanisation – less land available – GM and conventional crops will be forced into closer proximity, hence greater risk of contamination/ transfer. But a potential advantage of GM is in allowing us to use limited land resources more effectively.
53. There are two co-existent but opposite directions in animal husbandry: the organic/ extensive trend and the intensification trend. Intensification is likely to dominate on balance, though the organic/ extensive movement may also increase – the distribution will be bimodal.
54. Greater vulnerability to e.g. epidemics of plant diseases that might be able to affect a GM crop of narrow genetic base, grown extensively – also due to greater

cross-border trade (but environmental concerns may lead to reduction in food miles)

55. Possible reduction in need for crop rotation due to GM properties – e.g. resistance to soil –borne pests and diseases. This could have long term effects on soil structure.
56. Effects of planting another crop on land that has previously been used for a GM crop?

IV.i.4 Organisational factors

57. Control of technology. GM technology will not necessarily be concentrated in hands of a few companies – for example India is starting to see small scale GM (cf also the growth of small scale producers of pharmaceutical generics)
58. Note: We are not asked in this study to assess the risks of what happens outside the EU, or of the imports of such crops into the EU. But we may the same trend in the EU – bringing a potential issue of regulating many small producers – and their ability to do robust risk assessments. There is also an issue of responsibility where EU industries are marketing the technology into the developing world
59. Acquisitions and mergers of companies – how will regulators maintain control?
60. Greater liaison between plant breeders and agri-chem industry. Farmers may tend to buy seed and chemicals as a package.

IV.i.5 Human behaviour and activities

61. Trend has been to greater choice of foods and greater diet awareness. But environmental and energy use concerns may limit this – desire to reduce food miles
62. Use of pesticides – may go up or down. Risks from pesticides include spray drift to neighbours.

IV.i.6 Regulation and institutional monitoring/control

63. Currently the regulator grants licence to the industry that develops and markets the GM crop. What if this changes, such that the licence goes with the farmer, or with the land? (Not likely in foreseeable future so far as we are aware)
64. Could GM crops be admitted as organic – if GM enables them to be grown without artificial fertilisers, pesticides? This would have various impacts on markets, uptake of GM
65. Interacting environmental legislation – e.g. Water Framework Directive, Waste Directive(s).
66. What about farm seed that is kept beyond the consent period?

V Brainstorming - Cumulative Effects:

The consideration of cumulative risks was similar in structure to that for long term risks. Participants were asked to identify potential risks by considering the additive, synergistic or antagonistic effects of

67. multiple genes

68. multiple traits
69. multiple releases
70. large scale cultivation

The results are shown below.

V.i.1 Multiple genes/ traits in the GM crop(s)

71. Multiple genes are no more likely to 'escape' together than the product of their individual escape probabilities - unless they are on the same chromosome. That should be a designed-in factor that it is known about in advance, and hence that can in principle be monitored for.
72. Multiple traits, however, may act synergistically to give the plant a greater than additive advantage in the wild.

V.i.2 Multiple releases of different/same GMO

73. More opportunities to sensitise people to allergenic traits (second stimulus more likely to be encountered);
74. Because modifications tend to be (on) the same genetic sequence – more chance of 'genetic meltdown'.

V.i.3 Large scale cultivation

75. How to monitor? – Spatial sampling problems – and who will interpret data – possible need for controlled, generic monitoring by regulator, not just leaving it to each industry; Possible need for comprehensive genetic library of current position, to provide baseline data against which changes can be detected.
76. Greater vulnerability to disease if there is a trend to monoculture or fewer varieties and possible diminution of the wider pool of genetic diversity available in currently grown crops.
77. Will GM lead to monoculture or diversity? Will legislation be needed to prevent monoculture. How will market demand affect this?
78. More choice of viable crops to grow, due to GM, may lead to greater volatility in what farmers choose to grow from year to year.

VI

VII Breakout Groups 2 - Mitigation & Monitoring

This session aimed to identify possibilities and issues in monitoring and mitigation Participants worked in groups of three/ four, and spent approximately 15 minutes identifying (based on evidence or realistic speculation) mitigation and monitoring measures for four example scenarios, chosen from the preceding sessions.

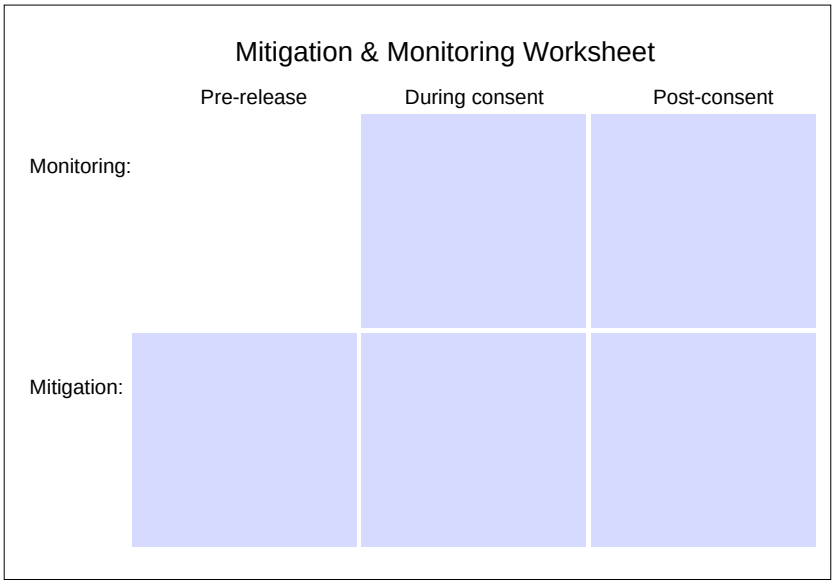
Monitoring was defined as detection of changes or risks, mitigation as–proactive measures to reduce likelihood, or reactive measures to minimise consequences

Participants were asked to consider both technical aspects of mitigation (how) and the organisational/societal aspects (who)

The worksheet shown in Figure 3 below was used as a prompt/aid to discussion.

Figure 3 : Worksheet for Mitigation and Monitoring Session

1. 'Superweed' scenario: Introgression into wild plant, taking it beyond current pest-controlled population to become an invasive weed.



79. One would hope and expect to do most mitigation pre-release – e.g. assessment of hybridisation potential and weediness of crop, barriers, and male sterility. But pre-release trials – may not be economic or safe to do on a scale that gives you usable numbers that can be scaled up to commercial release.
80. There is a need for careful baselining if we are to monitor effects - for many things there are no good baselines.
81. May need to do specific monitoring e.g. of gut bacteria in animals.
82. Post-consent – often too late by time it is noticed.
83. Need to develop good indicators in the pre-release period (e.g. adjacent hedgerows) in order to focus monitoring effectively.

Silent spread of recessive genes/dormant traits (harmful trait in GM crop itself, or vulnerability in interacting species)

84. The general problem will be that you do not know what you are looking for!

85. Genomics may be able to help predict when to expect an expression of the recessive gene, and there may also be some historic data from analogous cases. It really needs generic surveillance monitoring. This could be of the environment or of the final products.
86. Current regulatory system assumes that it will ensure that there are no unsafe effects on human health before allowing a release, so monitoring for such effects might be considered anathematic.

Antibiotic-resistance genes in plants– passed to environmental bacteria – hence to animal bacteria – compromising wish to keep simple antibiotics for animals, complex ones reserved for humans

87. Note antibiotic –resistance is a marker used to help select plants that have picked up the intended but undetectable gene. The use of antibiotic-resistance as a marker is already being withdrawn, but EC had specifically asked about it.
88. How do vets pick up growth of antibiotic-resistance in animals? Are there effective mechanisms for sharing and analysing such information? Antimicrobial resistance in bacteria from food-producing animals is monitored on an ongoing basis by Defra and results are published annually. There is also periodic monitoring of resistance in intestinal bacteria recovered from cattle, sheep and pigs after slaughter for human consumption.
89. Reactive mitigations, could include limiting the crop use to non-food purposes, and issuing appropriate animal husbandry guidance where possible and supported by relevant research findings.

More maize grown, due to its GM-induced advantages. It harbours *Fusarium*, which can then be passed to wheat – problem for wheat growers

90. Need to look for good indicators of trends and changes, taking account of seasonality.
91. Will be very expensive to monitor during consent period if not well focussed.
92. May also need to consider limiting locations for release

VIII

IX General Remarks

93. While the general regulatory principle is that industries should take responsibility for risk-assessing their own activities, and providing assurance of their acceptability, long term and cumulative effects raise bigger social/political/ethical & economic questions. Is it right/reasonable to ask industry to assess effects beyond the scope of any individual release?
94. Are we designing assessment methods for industries, or for regulators? Who should be doing assessment?
95. What powers does the regulator have to require impose monitoring beyond the consent period? Need to check original Directive(s) and national legislation.
96. If there are adverse effects with consequential losses what mechanisms are in place to offset these? Does the “polluter pays” principle apply?

IX.i.1.1.1.1.1

Appendix 5

Toolbox catalogue

This Appendix lists the main tools and techniques that may be helpful at various stages in the assessment of long term and cumulative risks from GM crops.

For each step in the assessment framework (Section 3.3) that requires supporting tools, the table lists the potential tools, outlining their specific uses and advantages within the overall aim of that step, and limitations on their capabilities or constraints on their use.

The tools are listed under each step in generally increasing order of sophistication and time required.

STEP (numbers as in Section 3)	AIM	TOOLS	SPECIFIC USES	LIMITATIONS / CONSTRAINTS	REFERENCES
3 Hazard Identification	Identify intended or unintended events associated with the release that could lead to adverse effects.	**Checklists**	Initial, high-level identification of general types of potentially harmful characteristics and adverse effects	Checklist approaches are 'closed' and non-exhaustive. The method does not prompt consideration of what else might occur.	For a typical list of characteristics and effects see Annex II of Directive 2001/18.
		Analytical/ Inductive desk study methods such as: **Holistic Holographic Analysis (HHA)** **Failure Modes and Effects Analysis (FMEA)**	Structured breakdown of the system into physical elements, processes etc, by reference to which an individual analyst can be prompted to identify hazards. The process is more open-ended than a checklist, because the analyst is required to think inductively about what could go wrong in each element, rather than simply assessing whether a particular characteristic or harm may arise.	Time consuming, requiring a detailed, methodical approach	Hayes 2003, 2004 FMEA: many standard texts and articles available

STEP (numbers as in Section 3)	AIM	TOOLS	SPECIFIC USES	LIMITATIONS / CONSTRAINTS	REFERENCES
		Structured Brainstorming	Open-ended approach, using interactions between group members with different perspectives to encourage creative thinking	Brainstorming needs to be carefully structured, and led by an experienced facilitator, to ensure that it remains on track and covers all the aspects. The balance between breadth and detail needs careful management, as time available is often a major limitation. Preparation should involve both the design of an overall structure that will be comprehensive, and maintain attention and interest, and the development of suitable prompts – cf Themes, FEPs as described in main text Participants should have experience in all the potentially relevant disciplines, such as genetics, ecology, agriculture, veterinary science and human health, and be open to creative 'what-if' thinking. Finding a time suitable for all required participants can be a practical difficulty.	There are many standard textbooks and articles on brainstorming, but a good description of the general technique, although written for application to a different domain, can be found in the guidance to the Eurocontrol Safety Assessment Methodology: Functional Hazard Analysis. This is available from the Eurocontrol website: www.eurocontrol.int

STEP (numbers as in Section 3)	AIM	TOOLS	SPECIFIC USES	LIMITATIONS / CONSTRAINTS	REFERENCES
4 Hazard Analysis	Identify and understand the casual and contributory factors for each Hazard, and its potential effects, and analyse the relationships between and within causes, Hazards and effects.	Graphical techniques to represent the network of causes and effects **Fault trees** work back from a 'top event' to show how causal factors (base events) combine to lead to a top event **Event trees** work forwards from a defined event to show its potential outcomes. **Bow tie diagrams** link a fault tree to an event tree **Cause-consequence diagrams** and **Influence diagrams** represent linkages between events with more flexibility	As well as providing a basis for evaluating risks in Step 5, the construction of such diagrams is valuable in its own right, in forcing clarity of thinking about how potential harm can be realised and helping the assessor to identify the main pathways or contributors to risk. By showing what events, errors and circumstances have to conspire and what barriers have to fail for harm to occur, it provides a systematic basis for considering where risk can be mitigated in Step 8.	These diagrams are most easily applied to 'binary' events – events that either do occur or do not. Fault trees are a specific, rather limited, instance of using the rules of probability and Boolean logic It is not straightforward to represent events that are matters of degree rather than binary. Time-dependence and feedbacks are also difficult to represent. More sophisticated variants of the basic tools may need to be obtained/ developed and a higher level of analytical experience and is required to do this.	Many standard textbooks and articles available – also several commercial software packages.

STEP (numbers as in Section 3)	AIM	TOOLS	SPECIFIC USES	LIMITATIONS / CONSTRAINTS	REFERENCES
5 Assess Likelihoods, Consequences and Risks	Assess how likely it is that each hazard will occur. Identify the various possible outcomes of each hazard. For each outcome, assess its severity (how bad it would be) and the probability of that outcome actually occurring (given that the hazard itself has occurred) Assess the level of risk – this will be a function of the likelihood of the hazard and the severities and probabilities of its consequences	**Subjective elicitation** Many techniques are available for ensuring that subjective data are elicited from individual experts or groups in ways that minimise, or at least reveal, uncertainty and bias. These include for example, Extreme Values consensus, Delphi Technique and Paired Comparisons. **Experimental evidence** e.g. from Farm Scale Trials **Computational l prediction/ simulation** **Natural analogues** – evidence from history or palaeontology about the impacts of introduction or evolution of variant species in the past. **Complex systems** analysis	Where adequate quantitative data are available, fault trees, event trees and other types of cause-consequence diagram as developed in Step 4 can be used to quantify likelihoods, consequences and risks.	Require general experience in mathematical modelling and in each specific technique.	Ayyub 2001 (elicitation) (complex systems) Stolk, 2003 and 2005.

STEP (numbers as in Section 3)	AIM	TOOLS	SPECIFIC USES	LIMITATIONS / CONSTRAINTS	REFERENCES
7 Consider Uncertainties	What are the main uncertainties in the assessment models, data or assumptions? How might they change the conclusions? In view of these uncertainties, is there sufficient confidence to make a decision? If not, what could be done to reduce the uncertainties?	**Worst case scoping calculations**	Adequate if, for example, the release can be shown to be acceptable even with extreme worst-case assumptions.	Identifying the worst-case combination of input parameters may not be obvious, for complex environmental systems	
		Sensitivity tests	Provide a richer picture of the potential range of outcomes than simple best-estimate/ worst-case calculations Identifying which parameters/ processes are critical	Time-consuming when there are many inputs to be varied. Danger of missing a critical combination of parameter values that leads to high risk, because of the difficulty of understanding complex system behaviour	
		Probabilistic or stochastic modelling (e.g. Monte Carlo analysis) Input data are specified using statistical distributions, rather than single point estimates, and the corresponding distribution of outputs (risk levels) is calculated.	Provides a much richer, and truer, picture of the variability and critical parameters than sensitivity testing, and can be less labour-intensive in the long run, because the selection of cases to be considered is automated.	Requires experience of mathematical modelling and statistics. Gathering input data distributions is more time-consuming than single-point estimates.	Commercial software packages include @RISK and CrystalBall

STEP (numbers as in Section 3)	AIM	TOOLS	SPECIFIC USES	LIMITATIONS / CONSTRAINTS	REFERENCES
8 Identification of Mitigation & Monitoring Methods	What could/ should be done to reduce the risk? (return to Step 3 and re-assess as appropriate – the mitigation/ monitoring methods may change the hazards)	Tools for identifying potential mitigations mirror those for hazard identification – including simple **checklists** , through desk based**, systematic consideration of each element of the system** (diagrams produced in Step 4 can provide a structure for this) and **structured brainstorming.**	Systematic and comprehensive identification and evaluation of potential measures to reduce the likelihood of the hazards, and/ or reduce the probability and / or severity of their consequences.	As for Hazard Identification	

STEP (numbers as in Section 3)	AIM	TOOLS	SPECIFIC USES	LIMITATIONS / CONSTRAINTS	REFERENCES
		For each identified method, its effectiveness (including consideration of any negative effects) and practicability should be assessed. This can be carried out: • by simple **subjective ranking,** using a matrix of effectiveness and practicability; • by **reference to the hazard analysis**– it may be possible to see which mitigations control the most critical links in the chains of cause and effect; • or by **re-running the assessment.** Note that it is necessary to return to the hazard identification step (Step 3) since mitigations can introduce new hazards.			

1

Appendix 6

Case studies

6.1. Case Study 1

C/NL/98/11 Roundup Ready oilseed rape, event GT73 (Monsanto – updated version July 2003).

Trait: Tolerance to the herbicide glyphosate ('Roundup Ready').
Scope: The notification covers the importation and storage of GT73 oilseed rape and its use as feed as well as in the processing for feed, and its industrial uses as or in products.
Current status: European Commission decision 31 August 2005[9]; consent not yet issued.
Note: Originally submitted in 1998 under Directive 90/220/EC, which did not require elucidation of monitoring plans, these have been added in subsequent submissions to the lead competent authority.

Scenario 1: Spillage and introgression of GT73 to feral OSR leading to decline in numbers of organic farms and consequent loss of biodiversity

Background information for scenario 1:

Oilseed rape frequently forms feral populations outside cultivated areas in man-made and semi-natural habitats, and such populations are particularly widespread in field margins, on disturbed ground and along roadsides throughout oilseed rape growing countries. Such feral populations often result from seed shed in the field at harvest, spread by contractors and lost during transport (e.g. to crushing plants). In agricultural situations this seed can germinate and emerge in subsequent crops as 'volunteer' weeds, populations of which can persist in fields for over 10 years after the oilseed rape crop had been harvested. The typical seedbank population density of feral oilseed rape is 100 seeds m^{-2}, which although small compared to the total weed seedbank in an arable soil (commonly 1000 to 10,000 m^{-2}) is similar to the established stand density of oilseed rape crops. According to agronomic experiments, even if only 1/100th of the feral OSR seedbank germinated in a 'break' year (oilseed rape is commonly grown as a break crop every two to four years), it would have a large impact as an impurity in the oilseed rape crop. Simulations have shown that when no attempt is made to control feral OSR populations in field situations it can take around 16 years for impurity in yield to fall below 1% (see report 'The potential for oilseed rape feral (volunteer) weeds to cause impurities in later oilseed rape crops' (2003)[10]. The application to market GT73 oilseed rape is restricted to import and processing. This first scenario addresses the possibility of spillage of GT73 leading to the establishment of feral or 'escaped' populations and consequent geneflow to non-GM oilseed rape crops. The scenario is based on the premise that at least some of this spilled seed is not cleaned up and goes on to germinate. (In actual fact European Commission Decision 2005/465/EC requires the consent holder to put in place appropriate measures in case of accidental grain spillage of GT73. However for

[9] Document number 2005/635/EC. OJ L228 pp 11-13 03/09/2005.
[10] http://www.defra.gov.uk/environment/gm/research/pdf/epg_rg0114.pdf

the purposes of this risk assessment it is assumed that no prior risk analysis has been carried out).

Framing The Question:

In order to make a well-informed decision regarding the risk of a product (or a process) it is important to adequately frame the question that is being asked. This question must be defined in risk terms, in a way that best informs the decision to be made. In the case of commercialisation of GT73, the following question is advanced:

"What are the risks that long-term and cumulative effects of GT73 commercialisation will result in serious harm to the environment (and to human and animal health)?"

Once the risks have been elucidated (and quantified) it leads on to the following secondary question:

"Given the risks associated with GT73 commercialisation, are there satisfactory measures that can be put in place to prevent, detect and mitigate the harm?"

Clearly mitigation is not possible in the case of irreversible harm, and this potential outcome must be taken into account in the analysis.

Once the above questions have been answered it must be decided whether the risks are tolerable, and whether or not commercialisation of GT73 can go ahead (with or without conditions to prevent, detect and mitigate any perceived harm). It should be noted that the above questions are generic and can be applied to any GM crop seeking authorization. Depending on the type of GMO and the particular hazards associated with it, additional specific questions could be asked to better disclose and pinpoint the risks.

Framing The Risks:

Criteria are used to determine whether a risk is tolerable. Criteria may, therefore, be defined in terms of hazard type; for example, commercialisation of a GMO may be deemed as unacceptable if geneflow leads to contamination of crops (e.g. seed crops) with unauthorized trait. Criteria may also be in the form of severity. For example, it may be considered acceptable to have a low level of contamination. Of course what is considered an acceptable low level by one individual or body may be considered unacceptable by another. In addition criteria presented by a notifier are likely to be different from those put forward by the regulator and the types of criteria used are likely to dependent upon the types of hazard that have been identified for the different crops. In the case of GT73 spillage leading to contamination of crops, the risks we are concerned about are those affecting organic crops (although equally conventional or other GM crops could be considered as well). In this case measures of risks may include economic risks (will GT73 contamination result in a reduction in profit; if so, for whom?), employment risks (will contaminated organic crops be unmarketable, leading to unemployment of farm workers?), risk to wildlife (will widespread contamination result in organic farms converting back to conventional farming practices, with

consequent loss of wildlife habitats) or environmental (will campaigns to eradicate feral populations of GT73 lead to destruction of wildlife habitats).

Defining Criteria:

In the case of GT73 spillage leading to the contamination of conventional crops, one of the primary defining criteria may be the level of crops rejected for having a GT73 presence (the application for commercial release of GT73 does not include cultivation). Regulators may decide, in consultation with the industry, that the possible rejection of 0.1% of seed crops, for example, is an acceptable risk to take. In terms of food/feed the primary criteria may be that less than 0.3% of conventional food/feed crops require labelling. Similar (perhaps more stringent) thresholds may be applied to organic crops. The exact values would need to be carefully considered from an economic, environmental (human health, if applicable) and perhaps consumer choice/ethics point of view.

Hazard Identification:

This scenario is concerned with the risk implications of spillage, and introgression into feral OSR crops by the GT73 herbicide-tolerance gene, such that it has the potential to cross-pollinate with OSR crops (both conventional and GM). Table 1 shows the possible effects of GT73 contamination on a number of human health and environmental themes. It should be noted that this contamination of crops in this scenario is not seen as having direct human health effects, but that other separate scenarios could be envisaged GT73 (e.g. the silent spread of recessive genes) that lead to potential direct effects.

Table 1 - **Potential consequences if the hazard is realised.**

	Potential effect (give brief rationale or example if not obvious)	
Theme	Positive effect? (benefit)	Negative effect (harm)
Human health	None envisaged.	Increased pesticide use due to conversion of organic farmland to conventional may lead to negative health effects on the public (pesticide residues) and farm workers (pesticide poisoning).
Animal health	None envisaged.	More intensive farming (less organic farms) may lead to reduced animal welfare in some instances.
Quality of life	None envisaged.	May result in more expensive non-GM food/feed (and less choice if organic products are in short supply). This may be more problematical for families on low incomes.

	Potential effect (give brief rationale or example if not obvious)	
Theme	Positive effect? (benefit)	Negative effect (harm)
Environment for wildlife	None envisaged.	May result in the use of more environmentally harmful herbicides to control Glyphosate-resistant feral OSR plants/volunteers.
Air	None envisaged.	None envisaged.
Water	None envisaged.	Detrimental effect if more/stronger herbicides are used to eradicate GT73 volunteers/feral plants.
Land	None envisaged.	Restricts potential uses of the land (e.g. for seed crops) if cannot be controlled. If crops (e.g. seed crops) are disposed to landfill it reduces the volume available for other waste and adds to the environmental burden.
Greener business	None envisaged.	Possible use of more harmful herbicides; more landfill
Sustainable resource use	None envisaged.	Energy and resources will have to be expended to control GT73 volunteers/ feral weeds – though a small effect in terms or the big sustainability picture; more organic produce may have to be sourced abroad.
Limit and adapt to climate change	None envisaged.	Secondary effect of energy use (above) and possible CO_2 emission from landfill.
Flood risk	Very small effects due to changes in field margin vegetation (affects slope stability, runoff, hydraulic resistance of flooded channel etc. relative to current flora). Could be good or bad, depending on location and hydrology.	
Effectiveness of regulation	None envisaged.	If the problem of crop contamination arises, it could be a relatively high profile issue, diverting funds from elsewhere.

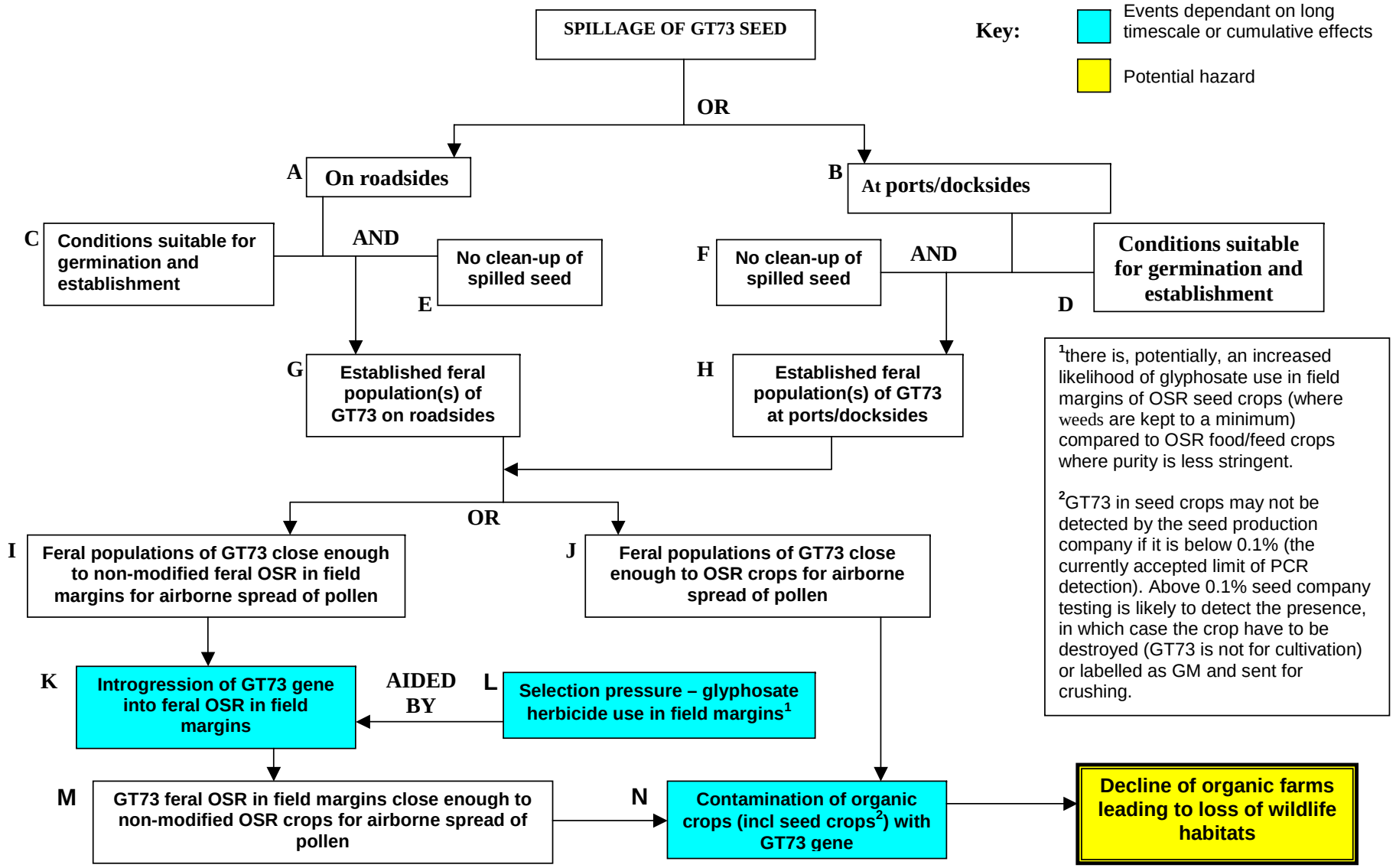
Assess Likelihood of the Hazard

Context is very important in helping to characterise and provide a meaningful assessment of likelihood, and it may be necessary to include some type of 'threshold' (or grading) as far as magnitude of events is concerned. For

example, the question may be asked "what is the likelihood of geneflow occurring between a GM crop and a wild relative?" (although geneflow is not necessarily a hazard in itself, it can lead to a hazard). If scientific studies show that 1 plant in a whole population will be fertilized by GM pollen, does this mean that the likelihood is 'high'. Presumably if half the population was fertilized the likelihood must also be deemed 'high'. Clearly it is necessary, therefore, to express likelihood in terms of the effect. Directive 2001/18 requires that an appropriate safety and emergency response should be included in the technical dossier submitted to the national competent authority, for example, to facilitate the control of GMOs or their retrieval in the event of severe risk. It may be appropriate, therefore, to apply the risk assessment procedure to the proposed emergency response in order to ensure it will not have any detrimental long-term effects itself. For example, if it was found that the crop had a deleterious effect on on-target insects the emergency plan may involve applying herbicide to the crop. However this in itself may have a devastating effect on weed populations and hence on the insects that feed on them. A more environmentally benign emergency treatment may then be sought.

It should be borne in mind that the information used to assess the likelihood of the hazard being realised is necessarily based on a best estimation of the events and conditions existing during the period of the consent. It is not possible to verify all the data used for this scenario because not all of the required information is public knowledge (some information will only be known to the notifier e.g. transport information), and hence not all the associated inferences will be strictly accurate. However the cause/consequence diagram presented below (figure 1) does give a useful overview of the processes and interactions that must be considered in order to produce an overall assessment of likelihood. When an actual risk assessment is being carried out the notifier can include this specific information in their own assessment of likelihood (or the Competent Authority can request this type of information) and it can be put forward as evidence to support any conclusions.

Figure 1: Cause/consequence diagram showing steps neededfor spillage and introgression of GT73 to feral OSR leading to contamination of OSR crops



Likelihood scores:

Spillage of GT73 on roadsides – *likelihood assessment*: MEDIUM

Reasoning: Spillage of OSR on roadsides is a regular occurrence, particularly at harvest time when the crop is being transported from field to farm, and from farm to crushing plant. Oilseed rape seed especially is prone to spillage, due to its small size and spherical shape. The fact that spillage occurs is not only evident visually from the number of OSR plants that can be seen growing along many roadsides in OSR producing countries, it is also confirmed by research conducted in the showing that roadside feral oilseed rape populations are constantly replenished by seed spilling from vehicles on their way to oilseed crushing plants (Crawley, Brown 1995). Such studies have led some researchers to conclude that significant losses of GM OSR seed are likely to occur along seed transport routes. It must be stated, however, that because the consent application for GT73 is limited to import and processing, spillage on roads is less likely than for OSR crops that are cultivated in the EU. In the case of GT73, then, the possibility of harvested seed being shed during transportation from fields is nil (assuming, of course, that there are effective procedures in place to prevent unauthorised cultivation). One means by which GT73 seed may be spilled onto roads is through seed transport from ports to crushing plants. However, due to the relatively high costs associated with road transportation, most GT73 is likely to be processed at crushing plants based near to the port of entry; because such crushing plants are generally based in the port area there should be little need to use public roads. It follows, therefore, that very little GT73 is likely to be moved on public roads, if it is transported in this way some is likely to spill. Consequently there is deemed to be a medium risk of spillage. As stated earlier, however, this assessment is based on a best-estimate basis. The most detailed information concerning the supply chain is likely to be held by the notifier and may include, for example, the precise locations of the crushing plants that will be used, the length of the supply chain from port to crushing plant (including any need for transhipment and the use of public roads), and the likelihood of diverting GT73 seed to less robust transport networks (e.g. if there happened to be mechanical breakdown at the primary crushing plant). All this information can be included in the assessment of likelihood to provide a more accurate measure of final risk.

Spillage of GT73 at ports/docksides – *likelihood assessment*: MEDIUM

Reasoning: Spillage of grain at ports and on docksides is an inevitable result of moving large quantities of a small-sized, free-flowing particles from ships and thence to the final destination (possibly via interim holding areas) and it is a virtual certainty that if a port handles any reasonable quantity of oilseed rape seed some will be spilled. In a study of looking at the origin of feral oilseed rape plants at docks in Scotland, for example, it was concluded that the origin of the seed was from lorries taking seed to large warehouses, and probably from seed blown from operations at the warehouse itself (see Defra report ‘The potential for oilseed rape feral (volunteer) weeds to cause impurities in later oilseed rape crops’ (2003), http://www.defra.gov.uk/environment/gm/research/pdf/epg_rg0114.pdf). Whilst the seed in this case was of conventional varieties, it would seem that spillage is equally likely for GT73 seed. Having established that at least some seed is likely to be spilled at docks, further consideration must be given to the possible amounts of seed spillage, and how to take this into account in the assessment of likelihood.

For example, the likelihood that a small quantity of seed spillage will take place is 'high', whereas the likelihood of that a large amount of seed spillage occurs may be considered 'low'. In this assessment we have considered the two extremes and on balance decided that a 'medium' risk is appropriate.

Conditions suitable for germination/establishments (roads) – *likelihood assessment*: HIGH

Reasoning: The high numbers of feral OSR plants along roadsides has been presented as evidence of the spillage of seeds on roads in step A). The fact that these plants have grown up and are often seen to flower and set seed is evidence of germination and to some extent establishment. The fact that feral populations are constantly replenished may indicate that in the longer term these populations are not self-sustaining, but this is somewhat irrelevant if the criteria we are looking at is the propensity to flower and spread pollen, hence the likelihood assessment is 'high'.

Conditions suitable for germination/establishments (docks) – *likelihood assessment*: HIGH

Reasoning: The presence of feral oilseed rape populations is well documented around ports and docksides (and along riverbanks). Rich (Rich 1991), for example, includes docks in a list of habitats of feral oilseed rape for Great Britain and Ireland ('A common… crucifer of roadsides, waste and cultivated ground, docks, cities and towns, tips, arable fields, riverbanks, etc.'). Oilseed rape is an opportunist species whose seed can remain dormant for many years and then rapidly germinate, grow and flower when conditions become favourable. In this way it can rapidly colonise new habitats and maintain stable, self-sustaining populations.

No clean-up of spilled seed (roads) – *likelihood assessment*: HIGH.

Reasoning: It is very difficult to ensure that bulk containers are adequately sealed to prevent the escape of material as small as OSR seed, whilst still maintaining an adequate ease of handling. It is reasonable to assume, therefore, that if GT73 is transported by road (see above for likelihood), the most significant source of spillage will be from lorries travelling to crushing plants. Such spillage may result from an ill-fitting or inadequately fastened tailgate, from careless loading or simply from seed picked up in the treads of tyres when in the loading area. In such instances the loss of seed is likely to be in small but steady amounts, thus leading to a low density of seeds along the length of the transport route. In such a case it will be very difficult to clean up the spilled seed, even if the loss is discovered once the vehicle reaches its destination.

No clean-up of spilled seed (docks) – *likelihood assessment*: MEDIUM.

Reasoning: Due to the quantity of material transported through ports/docks each day it is inevitable that some spillage will occur. In addition, by their nature, docksides are very difficult places to keep clean. This results from a combination of large area, the fact that bulk quantities of material are being moved around, and the constant tracking of machinery through them. It is likely, then, that during their standard operations GT73 seed will be spilled and a quantity of this will not be adequately cleaned up.

Established feral population(s) of GT73 on roadsides – *likelihood assessment*: HIGH

Reasoning: Establishment of feral populations of GT73 on roadsides is a function of seed spillage, lack of clear-up and the existence of suitable conditions for germination and growth, thus we have already acknowledged that GT73 has the potential to establish. Because Glyphosate herbicide is licensed for control of weeds on road verges its use is likely to result in selection pressure that will favour the establishment of feral populations of GT73. Local authorities are likely to have information on the extent of use of this herbicide on roadsides.

Established feral population(s) of GT73 at ports/docksides – *likelihood assessment*: HIGH

Reasoning: In a similar way to roadsides, establishment of feral populations of GT73 at ports and on docksides is a function of seed spillage, lack of clear-up and the existence of suitable conditions for germination and growth. If these factors are favourable then GT73 has the potential to establish. Glyphosate herbicide is licensed for use on roadsides its use is likely to result in selection pressure that will favour the establishment of feral populations of GT73. Glyphosate herbicide is licensed for control of weeds on industrial sites its use is likely to result in selection pressure that will favour the establishment of feral populations of GT73. Port authorities are likely to have information on the usage of this herbicide at ports, etc.

Feral populations of GT73 close enough to non-GM feral OSR… – *likelihood assessment*: HIGH.

Reasoning: The agricultural landscape is traversed by numerous roads of differing size and usage, many in close proximity to fields. It follows then, that any feral populations of GT73 on roadsides will have a high likelihood of being in close proximity to feral oilseed rape in field margins. In addition, oilseed rape can be described as a high-risk crop in terms of crop-to-crop (and crop to wild relatives) gene flow. Based on the experience of the seed industry regarding separation distance versus seed purity, the Supply Chain Initiative on Modified Agricultural Crops (SCIMAC), a voluntary body of the UK seed industry set up to police the large scale trial and commercial planting of GM crops, has established separation distances for planting GM crops near non-GM crops. In the case of OSR, the following isolation distances apply: Certified seed crops (same species) - 200m; Registered organic crops (same species) - 200m; Non-GM crops (same species) - 50m. At the landscape scale low levels of geneflow have been documented at long distances, and thus genetic isolation of feral OSR is unlikely to be maintained.

Feral populations of GT73 close enough to non-GM crops… – *likelihood assessment*: MEDIUM.

Reasoning: Investigations into geneflow from OSR has shown that pollination declines rapidly over short distances, but then remains at low levels over long distances from source. A 2003 investigation into OSR geneflow found that male-sterile plants positioned up to 26km from the nearest known pollen source were still fertilised, albeit at low levels. The study concluded that placing an upper limit on the distance over which this gene flow occurs is not possible (see ‘Quantifying

landscape-scale gene flow in oilseed rape', DEFRA Project RG0216, published 2003). This suggests that some level of geneflow is inevitable between feral populations and oilseed rape crops. It is thought that insects, particularly honeybees (*Apis mellifera*), bumblebees (*Bombus* sp.) and (possibly to an even greater extent) pollen beetles, play a major role in the transfer of pollen over these long distances. Given the evidence for long range insect-mediated geneflow it seems reasonable to conclude that geneflow from feral populations of GT73 OSR on roadsides to will non-GM crops will occur to a greater or lesser degree depending on distance, hence a likelihood assessment of medium.

Introgression of GT73 cassette into feral OSR in field margins - *likelihood assessment*: MEDIUM.

Reasoning: The backcrossing of GT73 hybrids in field margins, leading to the stable integration of the new gene into the wild population, is likely to be affected by the rate of pollen flow (which is in turn affected by distance between donor plants, pollen viability, outcrossing rate, etc) and the level of the gene in the feral population. It will also be influenced by selection pressure (see L)). Studies show that feral OSR populations decline rapidly during their first two years but they then persist as a residual in many fields at a typical density of 100 m^{-2} (e.g. http://www.royalsoced.org.uk/enquiries/gm_debate/gm_squire.pdf).

Selection pressure – Glyphosate herbicide use in field margins - *likelihood assessment*: LOW.

Reasoning: Where glyphosate herbicide is used to treat field margins it is likely to result in greater selection pressure and lead to the more rapid introgression of the GT73 *epsps* and *gox* gene cassette into the population. Currently there is a lot of emphasis on the wildlife management of field margins, which reduces the likelihood of herbicide use on them. However herbicide treatment may be more likely where seed crops are concerned, due to the strict purity standards that apply as far as weed seed are concerned. This may lead to greater selection pressure in the margins of fields used for seed production.

Feral OSR in field margins close enough to non-modified OSR crops – Glyphosate herbicide use in field margins - *likelihood assessment*: MEDIUM.

Reasoning: Where glyphosate herbicide is used to treat field margins, however, this is likely to result in greater selection pressure and lead to the more rapid introgression of the GT73 cassette into the population. At the present time there is a lot of emphasis on the wildlife management of field margins, which reduces the likelihood of herbicide use, however such treatment may be more likely where seed crops are concerned, where strict purity standards apply and weeds must be controlled.

Contamination of organic crops (incl seed crops) with GT73 cassette - *likelihood assessment*: MEDIUM.

Reasoning: Contamination of organic crops with the GT73 cassette may take place from feral oilseed rape populations in field margins or (more directly) feral populations on roadsides. These two sources can be quantified separately in terms of likelihood or could be combined to calculate an overall assessment of likelihood. Roadside populations, for example, are likely to be at a greater

distances from crops than field margin populations, but the route for geneflow is more direct. Consequently the two sources of geneflow will have different likelihood values allocated to them.

Decline of organic farms leading to loss of wildlife habitats - *likelihood assessment*: LOW.

Reasoning: It is relatively easy to imagine, perhaps even in the short term, the rejection of conventional seed crops due to the adventitious presence of GT73 (the application for commercialisation of GT73 does not include cultivation). It is also relatively easy to imagine food and feed crops becoming contaminated due to geneflow from feral GT73 populations, although presumably levels would generally be below labelling thresholds. It follows then, that contamination of organic crops is also likely to occur, especially if farms are located near docks or transport routes. If this situation does occur, then the consequences for organic farmers are likely to be more serious than for conventional farmers because thresholds for GM contaminants are likely to be lower (perhaps even zero).

Identify Potential Consequences of the Hazard and Assess Their Severities and Probabilities

Hazards associated with contamination of crops with GMOs have been widely considered and are well documented. In the case of conventional oilseed rape grown for food or feed a GM presence may lead to rejection of the crop (with consequent financial consequences) at the crushing plant or may result in a lower market price if the crop requires labelling as GM. In the case of seed crops there is also the possibility of rejection, and in addition the scope for labelling the seed as GM may be limited because GT73 would not be authorized for sowing in the EU.

In the case of organic crops, similar factors apply, although the financial penalties in the case of rejection or labelling may be more severe due to the generally higher production costs and the higher unit price. In addition the possibility of rejection may be greater due to potentially lower acceptable threshold values for organic crops (assuming any threshold for GM contamination is acceptable to the organic sector). Organic crops with an adventitious GM presence below 0.9% may well have to be marketed as conventional crops, because they will not reach the correct organic specifications. Who will the carry the burden of this is not clear, but it could be the farmers themselves if no compensation scheme is in place. If this is the case then, over time, organic farmers may come under increasing financial pressure, and this may ultimately lead to a reduction in the number of organic farms as organic farming ultimately becomes unviable. These farms may then revert back to conventional farming methods, leading to a reduction in wildlife habits and a reduction in the biodiversity associated with organic farming methods. Co-existence arrangements may help to ameliorate this situation, although they are likely to concentrate on avoiding crop to crop geneflow rather than geneflow from feral populations.

As well as growers facing problems when marking the crop there may be also consequences for agronomic practice. Feral OSR populations containing the GT73 cassette may cause problems in other crops where they may manifest themselves

as difficult-to-control weeds. This may require different, potentially more expensive and/or more harmful herbicide usage or mechanical control methods.

If GM varieties of an oilseed rape crop were to be given commercial approval for cultivation in the EU, and were grown widely, then the possibility exists that 'gene stacking' (the accumulation of transgenes encoding different traits) would occur due to cross-pollination between feral GT73 and the cultivated GM crop(s). This might involve transgenes conferring resistance to several herbicides, raising the possibility of multiple herbicide resistance (Orson, Crawley and Brown) and (Beckie, Hall and Warwick). Gene stacking has already been documented in Canada where both glyphosate resistance and glufosinate resistant oilseed rape (canola) are grown. In addition the possibility of generating feral GM plants that are invasive of semi-natural habitats as a result of transgene stacking is conceivable in the long-term, especially if a range of GM crop varieties with resistance to different pests, diseases or other environmental stresses are grown on a commercial scale in the EU[11].

Assess Risk

The likelihood of hazard being realised is combined to form a measure of the risk using the basic equation:

Risk = SUM (Likelihood x Hazard)

Both likelihood (or probability) and hazard are measured on a scale of 0 (no possibility or no hazard) to 5 (certainty or extreme hazard). The output therefore ranges from 0 (zero risk) to 25 (extreme risk). Table 2 illustrates how the overall risk is assessed.

Table 2 – Assessing the overall risk.

Hazard:	**Decline of organic farms leading to loss of wildlife habitats**		
Effect	Likelihood	Hazard	Risk Level = (LxH)
Human health	2	2	4
Animal health	1	2	2
Quality of life	2	2	4
Environment for wildlife	3	3	9
Air	0	0	0
Water	2	3	6
Land	2	2	4
Greener business	1	2	2
Sustainable resource use	1	2	2
Limit and adapt to climate change	1	1	1
Flood risk	1	1	1

[11] see, for example, http://www.gmsciencedebate.org.uk/report/pdf/gmsci-report1-pt5.pdf

Effectiveness of regulation	3	3	9

Compare Risk Against Criteria

With the current example it has been suggested that possible criteria to decide how a risk is tolerable may be defined in terms of percentage contamination of conventional crops. An integral component when comparing risk against criteria is the severity of the hazard.

Consider Uncertainties

In the scenario outlined there are many uncertainties that must be considered, ranging from unknowns regarding the transportation and spillage of GT73 to incomplete information on OSR geneflow (formulae showing fertilization rate with distance but be extremely helpful), likely distance between feral GT73 populations and conventional OSR (feral and crops) and possible introgression rates.

Identification of Prevention, Monitoring and Mitigation Methods

Following the assessment of risk consideration must to be given to methods of monitoring and mitigating that risk. Table 3 shows potential monitoring and mitigation measures for the various events identified in the scenario.

Table 3 – Prevention Monitoring and Mitigation Methods

	Event	Possible prevention measures	Possible monitoring methods	Possible mitigation measures
A	Spillage of GT73 on roadsides	Prevent or reduce the transport of GT73 by road; Use adequately sealed lorries when road transport is necessary.	Random testing of roadside OSR plants along transport routes (e.g. by spray treatment with Glyphosate) will provide evidence as to whether there has been spillage of HT OSR (although this requires prior seed germination). Analytical testing (i.e. PCR would be required for positive identification of GT73 seed/plants).	Standard Operating Procedure (SOP) for reporting and clearing up spillage; Monitor areas where seed has been spilled (and control any OSR plants that germinate – see C).
B	Spillage of GT73 at ports/	Reduce the possibility of	Random testing (see above	SOP for reporting and clearing up

	Event	Possible prevention measures	Possible monitoring methods	Possible mitigation measures
	docksides	spillage (e.g. by fitting appropriate guards to conveyors); Wheel washing of vehicles exiting 'high risk' dock areas.	methods) of OSR plants/seeds in the vicinity of ports (including roads leading from the port).	spillage; Monitor areas where seed has been spilled; Control of OSR plants growing in the port area (e.g. by spray treatment with herbicide other than Glyphosate).
C	Conditions suitable for germination/ establishments (roads)	Environmental conditions along roadsides are fixed and it is unlikely that proactive measures could be carried out to make conditions less conducive to OSR germination/ establishment.	Use of Glyphosate herbicides to test whether growing feral OSR plants are tolerant to the herbicide.	Use of non-Glyphosate herbicides to control Glyphosate tolerant feral OSR identified-by monitoring in A).
D	Conditions suitable for germination/ establishments (ports/ docksides)	At the local scale it may be possible to alter some of the environmental conditions at ports to make them less conducive to OSR germination/ establishment. The most practical way of doing this may be to ensure general cleanliness to prevent the build-up of organic matter suitable for plant growth.	Monitoring for general port cleanliness and the growth of OSR.	Use of non-Glyphosate herbicides to control Glyphosate tolerant feral OSR identified by monitoring in A).
E	No clean up of spilled seed (roadsides)	Requirement by the consent holder to inform operators and users of appropriate management measures in event of spillage; SOP for the clean	Possible statutory monitoring of roadsides use for transportation of GT73.	Routine control of oilseed rape populations growing on roadsides.

	Event	Possible prevention measures	Possible monitoring methods	Possible mitigation measures
		up of spilled seed.		
F	No clean up of spilled seed (ports/docks)	Requirement by the consent holder to inform operators and users of appropriate management measures in event of spillage; SOP for the clean up of spilled seed.	Possible statutory monitoring of ports/docksides where GT73 is imported.	Routine control of oilseed rape populations growing in ports/docksides.
G	Established feral population(s) of GT73 on roadsides	Clean up of spilled seed - see above	Sampling and testing (by glyphosate application or PCR analysis) of feral populations on roadsides.	Notifiers and regulators may need to consider where responsibility lies for control of feral populations on roadsides, and whether additional legislative/ organisational structures need to be put in place to facilitate this.
H	Established feral population(s) of GT73 at ports/docksides	Clean up of spilled seed - see above	Sampling and testing (by glyphosate application or PCR analysis) of feral populations on roadsides.	Control of feral populations of GT73 (using herbicides other than glyphosate) before flowering and/or seed set.
I	Feral populations of GT73 close enough to non-modified feral OSR in field margins for airborne spread of pollen	Control of feral populations before flowering and/or seed set; Removal of OSR plants in close proximity that may be recipients of pollen.	Survey of roadside/field margin OSR populations to provide possible modelling and mitigation information.	Control of feral populations of GT73 on roadsides and in field margins before flowering and/or seed set.
J	Feral populations of GT73 close enough to OSR crops for airborne spread	Control of feral populations of GT73 before flowering.	Survey of feral OSR populations in field margins to provide possible modelling and mitigation	Control of feral populations of GT73 in field margins before flowering.

	Event	Possible prevention measures	Possible monitoring methods	Possible mitigation measures
	of pollen		information.	
K	Introgression of GT73 cassette into feral OSR in field margins	Control of feral populations of GT73 in field margins before flowering – see above.	Sampling and testing of feral OSR populations in field margins.	Control of feral OSR with herbicides other than glyphosate
L	Selection pressure – glyphosate herbicide use in field margins	Restrict the use of glyphosate in areas known to harbour feral GT73 populations	Sampling and testing of feral OSR populations in field margins.	If GT73 in field margins is found to be a problem, information notices could be issued to farmers suggesting limiting the use of Glyphosate.
M	GT73 feral OSR in field margins close to OSR crops for airborne spread of pollen	Control of feral populations of GT73 in field margins before flowering.	Pollen trapping and analysis in fields considered vulnerable to GT73 pollen influx.	Control of feral OSR before flowering with herbicides other than glyphosate
N	Contamination of OSR crops with GT73 cassette	Border rows of male sterile OSR to act as pollen taps in vulnerable fields (e.g. fields next to docks/transport routes)	PCR testing of crops.	Compensation scheme for growers whose crops are financially affected by GT73 contamination.

Decision making:

The above scenario is just one of the possible consequences of commercialisation for import and processing of GT73 oilseed rape. A full risk assessment would go through the above process for a range of potential hazards and would determine a level of risk for each one. Scenario 2 is provided as an abridged example of a potential additional hazard that can be envisaged for GT73, with figure 2 showing the events leading to this hazard in the form of a cause/consequence diagram. Once the full range of hazards has been assessed the resulting risk values could then be integrated into an overall level of risk for the GMO. Based on this overall risk a decision would be made as to whether commercialisation could go ahead, and if so whether any additional prevention, monitoring or mitigation measures would need to be put in place.

Scenario 2: Introgression of GT73 herbicide tolerance gene into wild relatives leading to reduced biodiversity on banksides.

Background information:

There are 17 species from the family Brassicaceae that are native to the UK and are able to hybridize with cultivated oilseed rape (*Brassica napus*) (Scheffler and Dale, 1994). Of these, *Brassica rapa* is the species most likely to receive transgenes directly from *B. napus* on a regular basis. The "wild" form of *B. rapa* (wild turnip, bargeman's cabbage) is a plant of riversides and canal banks (Rich, 1991). This scenario looks at the potential of introgression of GT73 *epsps* and *gox* gene cassette into *B. rapa* on banksides, leading to a decrease in biodiversity.

Assess Likelihood of the Hazard

As examples, likelihood scores for two of the main events leading to reduced biodiversity on banksides by introgression of the GT73 herbicide tolerance gene into wild relatives.

Likelihood scores:

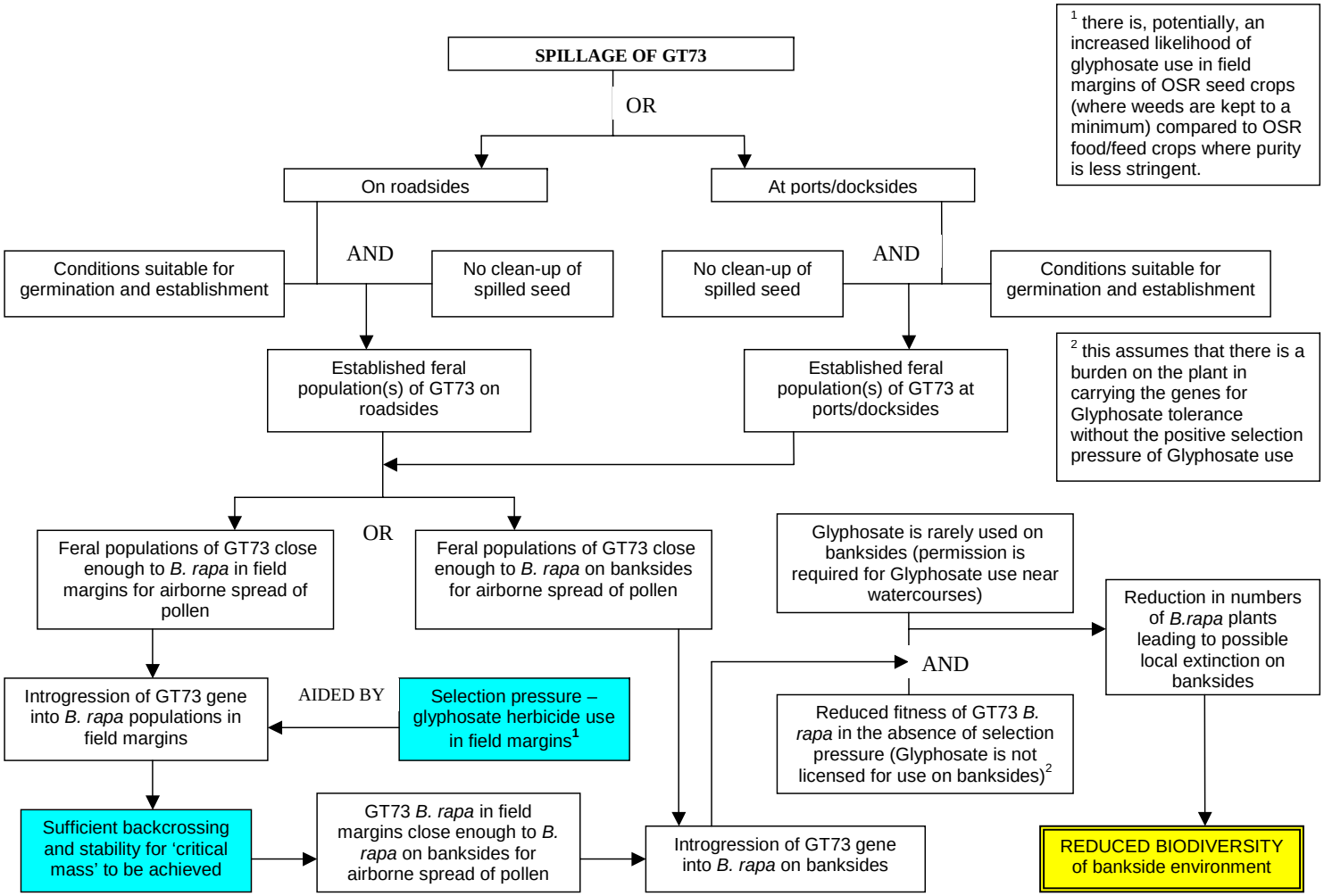
Introgression of GT73 cassette into *B. rapa* on banksides – *likelihood assessment*: MEDIUM

Reasoning: It has been estimated that across the UK around 32,000 hybrids form annually in waterside *B. rapa* exposed to oilseed rape pollen (Wilkinson *et al*.). However, the majority of this geneflow is likely to originate from cultivated OSR, rather than from feral populations, therefore in order to accurately estimate the probability of geneflow we would need to know the area of feral OSR and compare it to the area of cultivated OSR. A Royal Society report, 'Outcrossing among crops and feral descendents – geneflow', states that feral OSR populations persist in many fields at a typical density of 100 m^{-2}, therefore this figure could be used in any estimate of geneflow and introgression.

Reduced fitness of GT73 *B. rapa* in the absence of selection pressure - *likelihood assessment*: HIGH

Reasoning: In the absence of selection pressure (in this case glyphosate use), transgenic tolerance to herbicides is predicted to reduce persistence (David Claessen, Christopher A.Gilligan, Frank van den Bosch 2005). Although glyphosate is licensed for aquatic weed control it is harmful (dangerous) to fish and other aquatic life, and in the UK the Environment Agency or Local River Purification Authority must be consulted before use in or near water. It seems reasonable to suppose, therefore, that glyphosate will not be used on *B. rapa* populations near water, and this may result in decreased fitness due to the extra burden of carrying the gene for glyphosate tolerance.

Figure 2: Cause/consequence diagram showing steps needed for GT73 spillage and introgression wild relatives leading to reduced diversity



Identify Possible Consequences of the Hazard and Assess their Severities and Probabilities

Although the hazards of crop-to-crop geneflow are fairly clear-cut and well-documented (i.e. adulteration of harvested seed and novel traits in volunteer weeds) there is less consensus about the hazards of gene flow to wild relatives. Some people have ethical concerns, considering gene flow to wild plants to be 'genetic pollution' (Daniels and Sheail, 1999). Other hazards are the potential for changes in the persistence, abundance or distribution (i.e. 'weediness') of wild relatives, which might alter the composition of plant communities, and effects on non-target organisms. It is likely that herbicide tolerance will not increase the weediness of plants in non-agricultural habitats where herbicides are not applied.

Following feedback from the case studies involving scenarios 2 and 3, the risk assessment framework was further revised to make the procedure more intuitive and easy to use. Scenario 3 was then used to test this updated framework.

6.2. Case Study 2

C/FR/96/05/10 - Bt11 maize (field or sweet maize) (Syngenta Seeds SAS)

Trait: Resistance to corn borers *Ostrinia nubilalis* and *Sesamia nonagrioides* using the Cry1Ab gene.
Scope: For use as any other maize, including cultivation in the EU. Bt11 also possesses herbicide tolerance but the scope of the notification has been limited to use without the application of glufosinate ammonium herbicides.
Current status: pending authorisation.

Scenario 3: Development of resistance by ECB to Bt11 leading to the decline of predators and parasitoids by exposure to the Cry protein.

Background

Infestation of maize plants by the stem-boring larvae of European Corn Borer (*Ostrinia nubilalis*) can cause significant loss in yield in maize crops. As a means of controlling ECB, Bt11 maize has been engineered to express an insecticidal Cry protein from the bacterium *Bacillus thuringiensis*. Despite early predictions that target pests increasingly exposed to Bt maize would quickly build up resistance to the Cry endotoxin, to date there is no scientific evidence of European Corn Borer (ECB) developing such resistance in the field. This is despite the widespread commercial use of this pest control method and the fact that some laboratory studies show a rapid build up of resistance. Nevertheless, it is widely accepted by the scientific community that the occurrence of insect resistance is a distinct possibility and this has led to the development of Insect Resistant Management strategies which include using high dose rates and non-Bt refuge strategies. For Bt11, refuge areas of 20% for planting areas over 5ha have been proposed. A key biological/behavioural element for the effectiveness of refuges is reliance on the

fact that the adult form of ECB is highly mobile, resulting in a high level of interaction between crop and refuge populations. The following scenario aims to quantify the likelihood of Bt11 resistance in ECB and the possible consequences for predators and parasitoids. The advent of resistance is based on the premise that either 1) a more sedentary strain of ECB develops, resulting in less interaction between the two populations, or 2) there will be large areas of the EU where refuges will not be required. The consequences for predators and parasitoids are based on the hypothetical concept of resistant ECB passing the toxic Cry protein to higher trophic levels.

Framing The Question:

For this scenario we will assume it is a regulatory authority that is posing the question, perhaps due to specific concerns raised by new experimental research. These hypothetical research results are a combination of modelling data showing hitherto unidentified mechanisms operating in support of ECB resistance, and laboratory experiments that highlight toxic food-chain effects in resistant laboratory-bred ECB fed to predators).

In the case of commercialisation of Bt11 the regulatory authority poses the following question:

"What are the risks that the development of Bt resistance in European Corn Borer will lead to a reduction in species diversity through toxic food chain effects?"

In terms of geographic scale the assessment is concerned with all EU Member States having a ECB problem (and consequently all EU Member States likely to grow Bt11). In terms of risk type the assessment is concerned with risk to the environment, specifically to higher trophic levels that predate ECB. The (hypothetical) research concerns toxic food chain effects, but the regulatory authority is also concerned that chronic effects must not be overlooked. With this in mind timescale becomes increasingly important, as chronic effects often take a long time to materialise. The development of resistance itself is likely to take a long time to materialise; hence the assessment is concerned with long-term and cumulative effects that cannot necessarily be confirmed in laboratory or field experiments. This makes the scenario an ideal candidate for risk assessment.

Framing the risks:

For the purposes of this scenario we are assuming that the criteria adopted by the regulatory authority is that any toxic effects on ECB predators and/or parasitoids must be no greater than those currently caused by conventional maize cultivation. In actuality this criteria may be difficult to determine, because different management regimes have different effects on different insect groups. Another way of setting tolerability criteria may be to lay down defined population (or density) limits for different insect groups or species; for example, toxic effects may be expected to be most pronounced for a parasitic species of wasp that lays its eggs within ECB larvae, therefore an population density of 1 wasp per m^{-2} may be considered acceptable (perhaps judged to be equivalent to the density found in conventional maize). Any lower and the hazard may be considered to be unacceptable. Alternatively, certain carabid beetles may be considered to be essential to the food

web in maize, in which case a decline of over 5% would be deemed unacceptable. Defining precise tolerability criteria is probably one of the most difficult tasks of the risk assessment, but it is also one of the most essential because it is against this that the risk is measured and the decision made as to whether that risk is tolerable.

Defining Criteria:

The current scenario is concerned with the toxic effects of the Cry protein on predators and parasitoids following the development of resistance in ECB. Table 4 shows the possible effects of the decline of these non-target insects on key themes.

Table 4 - **Potential consequences if the hazard is realised.**

	Potential effect (give brief rationale or example if not obvious)	
Theme	Positive effect? (benefit)	Negative effect (harm)
Human health	None envisaged.	The hazard is unlikely to have direct negative effects on human health. A conceivable indirect effect may be exposure to Cry toxins through food chain effects (e.g. via game birds that have eaten insects with high levels of the toxin), however the Cry protein in non known to have toxic effects on humans.
Animal health	None envisaged.	No direct negative effects are envisaged. Indirect effects are conceivable, but unlikely (see above).
Quality of life	None envisaged.	Reduction in diversity may be seen as a negative effect on quality of life by many; Loss of maize crops may result in more expensive non-GM food/feed (and less choice if organic products are in short supply). This may be problematical for families on low incomes.
Agricultural sustainability	None envisaged.	Development of resistance in ECB is likely to reduce agricultural sustainability. Likewise loss of predatory insect will reduce sustainability by increasing reliance further on chemical pesticides.
Environment for wildlife	None envisaged.	Disruption to the food web and a lack of arthropod prey is likely to result in a decline in insectivorous species, including predatory arthropods, small mammals and birds.
Air quality	None envisaged.	Possible burning of maize crops to halt spread of resistant ECB may result in air pollution.
Water quality	None envisaged.	Increased pesticide use is likely to result in lower water quality and the decline in some aquatic invertebrates.

	Potential effect (give brief rationale or example if not obvious)	
Theme	Positive effect? (benefit)	Negative effect (harm)
Land use and soils	Loss of maize crops to resistant ECB will result in longer-term movement away from growing maize. This may have positive or negative effects.	
Greener business	None envisaged.	More pesticides used to control resistant ECB.
Sustainable resource use	None envisaged.	More resources used in control and eradication programmes. More pesticides and fossil fuels used.
Limit and adapt to climate change	None envisaged.	None envisaged (except as secondary effect of resource use above).
Regulatory effectiveness	None envisaged.	Monitoring, control and eradication programmes for resistant ECB are likely to divert funds and human resources from other areas.

Hazard Analysis:

The cause/consequence diagram presented below represents the convergence of a number of disparate events, which, together with widespread and continuous cultivation of Bt11, result in a potentially serious hazard to the environment.

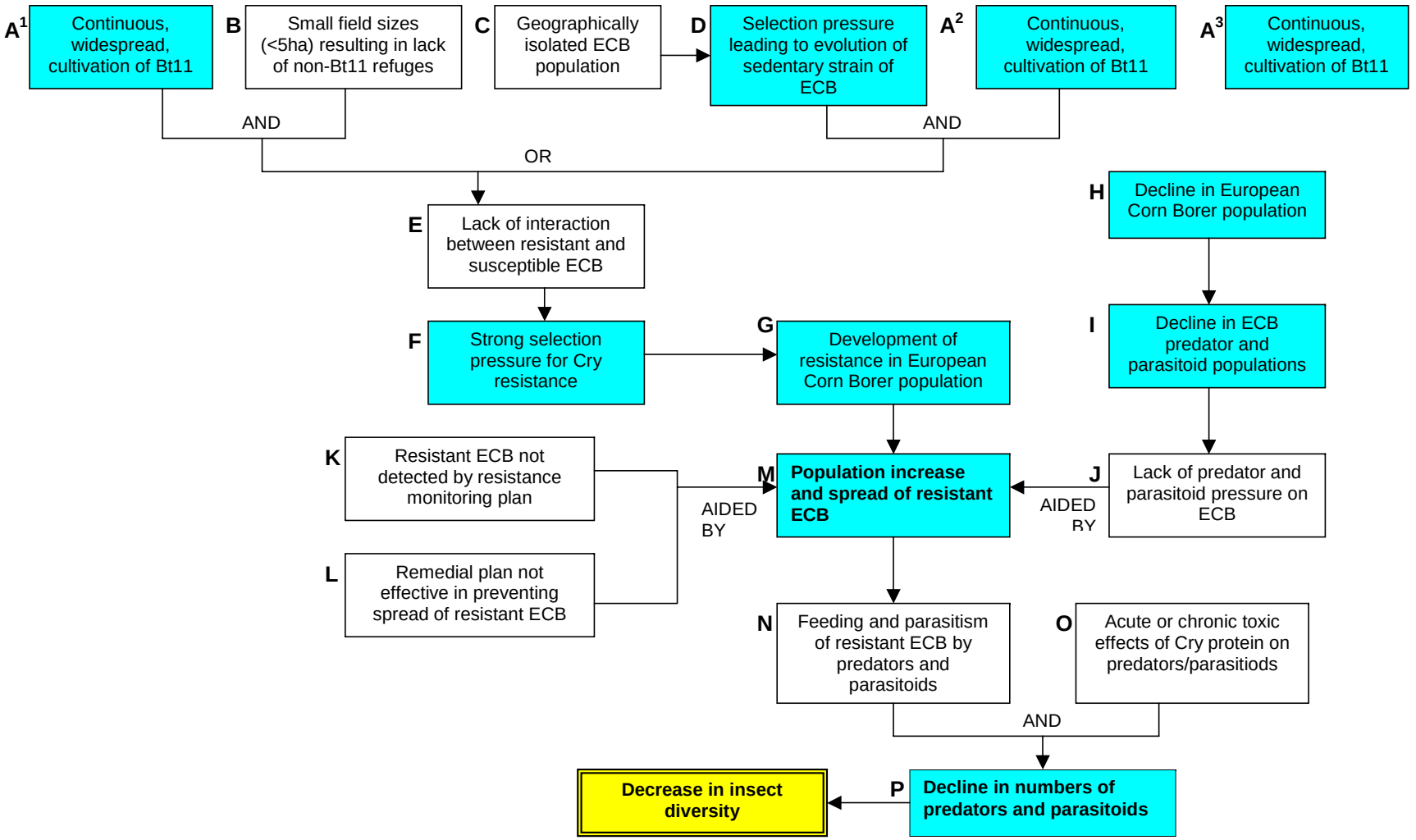


Figure 3: Cause/consequence diagram showing steps needed for the development of resistance by ECB to Bt11 leading to the decline of predators and parasitoids by exposure to Cry toxins.

Assess Likelihood of the Hazard

For each of the events leading to the proposed hazard likelihood scores are identified as follows:

A. Continuous and widespread cultivation of Bt11 maize – *likelihood assessment*: HIGH

Reasoning: There is a high likelihood that in certain areas of the EU where corn borer is endemic there will be continuous and widespread cultivation of Bt11 maize. Evidence for this can be seen in parts of N. America, where conditions favour the growing of maize. As a consequence growers tend to keep growing it as there are higher returns compared to growing less well-suited crops.

B. Small field sizes (<5ha) resulting in large areas of Bt11 without refuges – *likelihood assessment*: MEDIUM

Reasoning: Throughout the EU there are many types of arable farming systems in operation, from very intensive farms with large field sizes to small smaller-scale farms with consequently small field sizes. Many of these smaller farms are located in the newer Member States, some of which have very large geographic areas, including areas where ECB is endemic. Because of the good pest control afforded by Bt11, coupled with the expected savings in pesticide use and associated equipment, it seems likely that growers in these areas will be keen to adopt this technology. Many of these fields are likely to be less than 5ha in size, and this could result in large areas of Bt11 with no refuges. According to the Insect Resistance Management plan field of less than 5ha are likely to benefit from the presence of non-Bt maize on bordering farms, however this may not always be the case.

C. Geographically isolated ECB population – *likelihood assessment*: MEDIUM

Reasoning: It is likely that in some parts of the EU there are small maize growing areas where ECBs are geographically isolated from other ECB populations. This isolation may be due to climatic and/or geographic factors, (genetically isolated plant and insect populations are documented in isolated Swiss valleys surrounded by tall mountains, for example) or may be due to cultural reasons. In these cases where there is a restricted gene pool it can lead to high phenotypic plasticity and rapid evolutionary change.

D. Selection pressure leading to evolution of sedentary strain of ECB – *likelihood assessment*: LOW

Reasoning: Above it has already been determined that there is the possibility of isolated populations of ECB, for example, in geographically isolated valleys. Such populations may be compared to island populations, in that comparatively small evolutionary stimuli can lead to large evolutionary changes. One such change that has been documented with island populations of insects is reduced ability to disperse (e.g. reduction of wings in insects and birds and dandelions without a "parachute"). This occurs because there is a selective pressure against the ability to disperse off the island: if the organism leaves the island they are unlikely to return to mate and contribute to the gene pool of the next generation, so gradually only less-mobile individuals remain to pass on their genes. It is conceivable that ECBs living in an isolated area (e.g. surrounded by inhospitable mountains) may, over a long time period, be subject to this same selection pressure, thus leading to more sedentary forms. Flightlessness also has an energetic advantage because it

requires less energy expenditure (although it becomes unfavourable when predation is a factor).

E. Lack of interaction between ECB in Bt11 crop and ECB in refuge populations – *likelihood assessment*: MEDIUM

Reasoning: Both A) and B) combined, and C), D) and E) combined, result in a lack of interaction between ECB populations in the Bt11 areas and those in refuge areas. The likelihood of this scenario is a product of combining the individual likelihood scores. In reality, because there are two possible routes to achieving the same outcome, we should have two separate likelihood scores, but for the purposes of this case study, which is simply to illustrate the process, we have determined a single overall likelihood value.

F. Strong selection pressure for Cry resistance – *likelihood assessment*: HIGH

Reasoning: Survival of a few Bt11-dwelling ECB larvae and a lack of interaction between them and refuge-dwelling ECB will result in strong selection pressure as far as Cry resistance is concerned. Currently it is widely accepted that resistance to Bt crops is a rare event and is genetically recessive. The concept behind refuges is that rare resistant homozyotes and the few surviving heterozygotes will mate randomly with the high numbers of susceptible homozygotes, thereby delaying the evolution of resistance. This strategy relies on there being an adequate ratio of refuge to crop, and the fact that the adult ECB moth is highly mobile between the two areas. Clearly, if either (or both) of these conditions are not met it provides the opportunity for the resistant homozygotes and the surviving heterozygotes to breed together resulting in introgression of the Bt resistance gene into the population.

G. Development of resistance in European Corn Borer – *likelihood assessment*: MEDIUM

Reasoning: In order to operate evolution requires not only selection pressure, it requires the presence of an appropriate gene in the population. As has already been stated, expert opinion considers that Cry resistance is a rare event in ECB populations. Nevertheless, resistant populations have been bred in the laboratory after several generations, and it would appear credible therefore to predict that Cry resistance will appear in the field given the appropriate selection pressure and enough time. Given the lack of interaction between Cry-resistant and Cry-susceptible ECB individuals, individuals homozygous for resistance are likely to appear in the population with increasing frequency over time.

H. Decline in European Corn Borer populations – *likelihood assessment*: HIGH

Reasoning: The continuous, widespread, cultivation of Bt11 maize is likely to cause a steep decline in ECB numbers due to the fact that it is a highly effective control method compared to conventional pesticides. This is, of course, the intention behind the planting of Bt11.

I. Decline in ECB predator and parasitoid populations – *likelihood assessment*: MEDIUM

Reasoning: The decline ECB numbers is likely to be mirrored by a decline in predator and parasitoid numbers due to a reduction of their food supply. These declines are likely to be offset by predation on corn borers living in refuge areas, although how effective this will be in preventing a decline in numbers is unknown.

J. Lack of predator and parasitoid pressure on ECB – *likelihood assessment*: HIGH

Reasoning: Predator and parasitoid pressure is often one of the limiting factors that keep insect populations at a reasonably low level. Take away this pressure and the population can in some instances explode. In the case of ECB this is unlikely due to the fact that the toxin in Bt11 maize has a more severe limiting effect on population size. However, if this limiting factor is taken away (due to Bt resistance, for example) there would be very little to prevent a population explosion.

K. Resistant ECB not detected by resistance monitoring plan – *likelihood assessment*: LOW

Reasoning: The Resistance Management Plan put forward by the notifier includes a monitoring plan aimed at detecting any development of insect resistance. However, the scale of the plan and the frequency of monitoring is not clear, and without these details it is difficult to make an assessment of the likelihood of resistance going undetected. Given the predicted large-scale cultivation of Bt11 it is not inconceivable that resistance will go undetected at least in the early stages of development. Because of the uncertainties regarding the monitoring plan the likelihood is scored as 'low'. If resistance does develop in an area and it is not detected it may quickly spread to other areas due to natural processes or by inadvertent distribution by humans.

L. Remedial plan not effective in preventing spread of resistant ECB – *likelihood assessment*: LOW

Reasoning: An integral part of the remedial action plan is the implementation of alternative control measures to target pest populations in affected areas. The details of these control measures are not clear, and it is likely that elimination of resistant ECB will be difficult. The primary reason that Bt maize has been developed is because corn borers are particularly difficult to kill with conventional pesticides, since chemical sprays cannot reach boring pest larvae, living as they do in the stems of the plant. An alternative method may be the destruction of the crop, but spraying large areas with herbicide is unlikely to be popular with growers or the public. In addition, this would have to be carried out before the larvae begin to pupate and turn into adult moths. Spraying to kill the adults themselves is unlikely to be practical. Crop burning may be an alternative control method, but this is likely to be unpopular and may require derogation at the EU.

M. Population increase and spread of resistant ECB – *likelihood assessment*: MEDIUM

Reasoning: If Bt resistance develops in ECB and the monitoring and control plans are not effective in the area where the new forms have arisen, then it is possible that there will be a rapid population increase due to a ready food source (Bt maize) and a lack of predators and parasitoids. A large population of resistant ECB will increase the likelihood and rate of spread, thereby leading to the colonisation of new areas. Dispersal is likely to be due to a combination of natural processes and inadvertent human assistance (the latter cause is more likely in the spread of a sedentary strain). Undoubtedly the frequency of resistant homozygous individuals will decline as resistant individuals colonise new areas and interact with susceptible populations, but selection pressures in Bt11 areas will continue to favour the spread of the resistant forms.

N. Feeding and parasitism of resistant ECB by predators and parasitoids – *likelihood assessment*: HIGH

Reasoning: An increase in the numbers of resistant ECB will result in an increase of feeding and parasitism by natural enemies. Natural enemies of ECB include *Orius* spp. (Anthocoridae: pirate bugs), carabids (e.g. *Poecilus cupreus, Metallina lampros, Pseudophonus rufipes*, etc), spiders, parasitic wasps, etc. Feeding on (or in the case of parasitoids, laying eggs within) resistant ECB larvae or adult moths may result in exposure of natural enemies to high levels of Cry toxin.

O. Acute or chronic toxic effects of Cry protein on predators/parasitoids – *likelihood assessment*: MEDIUM

Reasoning: Many independent scientific studies have shown Bt toxins to be specific for particular insect groups such as moths, and this specificity is often cited as an important safety factor because non-target beneficial insects (honey bees, ladybirds, parasitic wasps, etc.) will not be harmed. However, this assumption may not be strictly valid for all insects, particularly when trophic level effects are taken into account. Tri-trophic laboratory studies have shown some evidence that predatory lacewing larvae exhibit increased mortality when fed on caterpillars that had in turn been feeding on Bt maize leaves (Hilbeck, Moar, Pustzai-Carey, Filippini, Bigler 1998). Other insects may exhibit the same response. It is possible, therefore, that widespread Bt11 cultivation could lead to a decline in predators, parasites and pathogens, firstly as corn borer populations decline, and secondarily through toxic food-chain effects caused by feeding on surviving corn borers. Over time such effects may result in a significant decrease in species diversity. Refuge areas may moderate these indirect effects, but at present little data on this subject exists.

Identify possible consequences of the hazard and assess their severities and probabilities

In the scenario presented above there are many areas where there is a theoretical likelihood of an event occurring, but there is very little hard scientific evidence to quantify this likelihood. Certainly there is data for flightlessness in island populations (one of the key criteria proposed for development of Cry resistant ECB), but extrapolating this to remote corn borer populations without solid evidence may be stretching the bounds of scientific credibility. Nevertheless, even though this is just a theoretical hazard, it is important to explore such dangers in order to ascertain that the risk is indeed as low as was thought.

Assess Risk

The likelihood of hazard being realised is combined to form a measure of the risk using the basic equation:

Risk = SUM (Likelihood x Hazard)

Both likelihood (or probability) and hazard are measured on a scale of 0 (no possibility or no hazard) to 5 (certainty or extreme hazard). The output therefore ranges from 0 (zero risk) to 25 (extreme risk). Table 5 displays the overall risk in terms of combined probability and severity.

Table 5 – Assessing the overall risk

Hazard:	**Decline of predators and parasitoids by exposure to the Cry protein**		
Likelihood of Hazard occurring, H			
Effect	Probability, P	Severity, S	Risk Level = f(H,P,S)
Human health	2	2	4
Animal health	1	1	1
Quality of life	2	3	6
Agricultural sustainability	4	4	16
Environment for wildlife	4	5	20
Air quality	1	3	3
Water quality	2	3	6
Land use and soils	2	2	4
Greener business	2	2	4
Sustainable resource use	2	3	6
Limit and adapt to climate change	1	1	1
Regulatory effectiveness	3	5	15

Compare Risk Against Criteria

With the current example it has been suggested that possible criteria to decide how a risk is tolerable may be defined in terms of comparisons with conventional crops.

Identification and Evaluation of Mitigation and Monitoring Methods

Once the assessment of risk has been carried out it is appropriate to consider the type and extent of monitoring and mitigation measures that could be put in place to negate or reduce that risk. Table 6 shows potential monitoring and mitigation measures for the various events leading from resistant ECB to a decline in predators and parasitoids.

Table 6 showing possible monitoring and mitigation methods (key control points are shown in bold)

	Event/ control point	Possible prevention measures	Possible monitoring methods	Possible mitigation measures
A)	Continuous and widespread cultivation of Bt11	The continuous and widespread cultivation of maize is not in itself a problem, but it could lead to problems. To reduce potential problems associated with continuous Bt11 cultivation the consent holder could instigate a grower education programme aimed at introducing rotation into crop management. Grower surveys by the consent holder could be used to monitor for effectiveness of the grower education programme.		
B)	Small field sizes (<5ha) resulting in large areas of Bt11 without refuges	Reduce the threshold that exempts fields of 5ha or less having a non-Bt maize refuge.	Conduct surveys to determine the likelihood of large areas being exempt from having refuges, and use this to inform the IRM policy; Ensure that all fields that are not exempt comply with the requisite area of refuge (currently 20%).	Adjust local refuge requirements in areas where they are inadequate refuges.
C)	**Geographically isolated ECB population**	There are very few measures that could be put in place to avoid the geographic isolation of remote corn borer populations. The most obvious means to limit isolation is to reduce the cultivation of maize (Bt and conventional) in remote areas, but this approach is likely to far outweigh the risk of such populations becoming problematic.		
D)	**Selection pressure leading to evolution of**	There is very little that can be done to prevent or mitigate evolution of sedentary ECB. 'Normal' ECB could be brought into a region to mate with the sedentary forms and dilute the, but this has its own risks and is likely to be unpopular.		

	Event/ control point	Possible prevention measures	Possible monitoring methods	Possible mitigation measures
	sedentary strain of ECB	Sampling and testing may detect the evolution of less mobile forms of ECB in isolated populations (testing could be in the form of laboratory studies or even simple morphological analysis measuring wing area). If sedentary forms are found an eradication programme could be instigated.		
E)	**Lack of interaction between Bt11 crop and refuge populations**	Since a lack of interaction between Bt11 and refuge ECB populations is a product of several disparate events coming together, effective prevention and mitigation measures can only be applied to the root causes. It may, however, be possible to monitor the degree of interaction between corn borer populations by setting up moth traps in the Bt11 areas and refuge areas and conducting mark and recapture experiments.		
F)	**Strong selection pressure for Cry resistance**	Selection pressure is a product of survival of some Bt11-dwelling ECB larvae and lack of interaction with refuge-dwelling ECB. The notifier has already addressed survival rate by ensuring that Bt11 produces a high dose rate of Cry toxin, and it is difficult to envisage what more could be done. Lack of interaction between populations has been dealt with above.		
G)	Development of resistance in European Corn Borer	Bt resistance is a product of several events coming together and the only way of preventing it is to address each of the root causes.	Resistance monitoring for changes in the baseline susceptibility of ECB already forms part of notifier's the IRM plan.	The notifier has outlined a remedial action plan in the event that resistant ECB is detected. This plan includes: informing customers and agents of resistance; increased monitoring; implementing alternative control measures; and possible cessation of Bt11 sales.
H)	**Decline in European Corn Borer populations**	N/A - this is an expected result of growing Bt11 maize.	Monitoring may be appropriate to determine if there is a link between ECB decline and the decline of other species.	N/A - this is an expected result of growing Bt11 maize.
I)	Decline in ECB predator and parasitoid populations	Non-Bt refuges will help to lessen the decline of ECB predators and	Insect trapping techniques can be used to monitor ECB	Increased refuge areas will help to arrest the decline in predator/parasitoid

	Event/ control point	Possible prevention measures	Possible monitoring methods	Possible mitigation measures
		parasitoids. Ensuring other wildlife friendly areas may also provide alternative prey sources.	predator/parasitoid numbers.	numbers.
J)	**Lack of predator and parasitoid pressure on ECB**	The lack of predator and parasitoid pressure on ECB populations is a product of several events and effective prevention and mitigation measures can only be applied to the root causes.		
K)	**Resistant ECB not detected by resistance monitoring plan**	Increasing the scale and frequency of the monitoring programme will help to ensure resistance is detected but will cost more. The risk (of not detecting the occurrence) must be balanced against cost of wider monitoring.		
K)	Remedial plan not effective in preventing spread of resistant ECB	The remedial action plan should specify a range of control measures to enable growers and consent holder operatives to use alternative control methods if the initial ones do not work.	Sampling and resistance testing may be carried out to determine the effectiveness of the remedial action plan.	The remedial action plan is not specific with regard to alternative control measures, but it seems likely that pesticide sprays will be a major control method. If this is ineffective alternative pest management techniques will have to be used.
M)	Population increase and spread of resistant ECB	More robust monitoring and control methods may help to prevent the increase and spread of resistant ECB.	Additional sampling and resistance testing would document the increase and spread of resistance.	Use of conventional pesticides will help control resistant ECB populations to some degree, although chemical control is difficult because chemical treatments are effective only during the short period between eggs hatching and larvae boring into stems. Targeted campaigns (using

	Event/ control point	Possible prevention measures	Possible monitoring methods	Possible mitigation measures
				monitoring data) should aid the elimination of resistant strains.
N)	**Feeding and parasitism of resistant ECB by predators and parasitoids**	Larger refuges or wildlife margins may help to provide a more diverse range of prey items for the natural enemies of ECB.	Sampling of field arthropods could be used to determine the number of alternative (non-ECB) prey items.	If sampling results show a scarcity of non-ECB prey items an increase in refuge area or wildlife margins may help to compensate in future years.
O)	**Acute or chronic toxic effects of Cry protein on predators/ parasitoids**	Diluting the number of resistant ECBs by providing a greater proportion of non-Bt11 habitat may help to ameliorate any toxic effects of Cry proteins.	Sampling and laboratory testing (tri-trophic tests) will help to determine the level of toxicity of resistant ECB to predators and parasitoids.	See above

Decision making:

During a full risk assessment it will be necessary for the assessor to work through the above framework for each of the risks identified in the hazard analysis section. By determining the level of risk for each one it is then possible to combine these risk values, for example using a Boolean combination, to determine the overall risk.

6.3. Case Study 3

C/SE/96/3501 - GM Potato clone EH92-527-1 (BASF)

Organism: **Potato variety 'Prevalent' genetically modified for altered starch composition;**
Trait: **Enhanced amylopectin content (>98% starch); reduced amylose; NptII (resistance to kanamycin);**
Scope: **For use as any other industrial starch potato (including cultivation in the EU);**
Application**: Industrial starch processing; feed use (pulp by-product used as cattle feed) (not evaluated for human consumption);**
Current status**: pending authorisation.**

Background information for case study 3:

In assessing the environmental and feed safety of potato clone EH92-527-1 there is a general consensus amongst official advisory bodies that notification C/SE/96/3501, including the risk assessment and monitoring plans, is a robust application and poses little risk to the environment and human and animal health. In some ways the potato plant, due to its general characteristics, would appear to be an ideal candidate for genetic modification in terms of environmental risk. Potatoes have no sexually compatible wild relatives in Europe, they produce virtually no pollen (any that is produced has a limited dispersal of around 3m), and the seeds are contained in poisonous fruits making dissemination by animals highly unlikely. Experiments conducted by the notifier have shown that viruses, fungi and bacteria attack the EH92-527-1 clone with same frequency as the mother clone, and both the starch and glycoalkoloid content are stable. In addition to these favourable characteristics, EH92-527-1 appears to have an increased vitamin C content and a decreased glycoalkaloid content (i.e. solanine), compared to the mother clone. Both these characteristics may offer health benefits to animals that are fed the pulp by-product. In terms of case-specific monitoring, the notifier has presented plans for testing to determine if any detrimental changes in tuber composition occur (including stability of genes, starch composition and glycoalkaloid content), and to detect any change in the presence of bleomycin resistance associated with open reading frames. General surveillance will monitor growth characteristics, changes in phenology, susceptibility to pests and disease and changes in animals in and around fields. Both plans have been assessed as being generally appropriate and sufficient by the relevant regulatory bodies.

Given the above assessment it would appear that potato clone EH92-527-1 poses very little risk to human health and the environment, certainly in the short term. However, as with any new release to the environment there is always the possibility that not all potential risks have been fully characterised or quantified, especially those that are not immediately obvious. In order to reveal any long-term and cumulative risks and assess

the probability and hazard, we will apply the methods outlined in the risk assessment framework to notification C/SE/96/3501.

Framing The Question:

In the case of commercialisation of potato clone EH92-527-1 the following general question is posed:

"What are the risks associated with the commercialisation of GM potato clone EH92-527-1; are these risks acceptable?"

In order to focus more precisely on the nature of the applied risk assessment the above question is defined in terms of the following criteria:

The decision context: in this instance we will assume that question has been posed by the notifier who wishes to quantify the risk of any potential deleterious effects and determine possible prevention and mitigation measures.

The geographic scale of the decision to be made: in terms of geographic scale, the assessment is concerned with all EU Member States where EH92-527-1 may be grown in the long term. Erring on the side of caution, and considering the potential effect of a possible abolition of the starch quota system, any Member State could in future grow starch potatoes, thus the scale has been increased to cover most of Europe (certain small Mediterranean countries are unlikely to grow EH92-527-1).

The type of risk being considered: in terms of the nature of the risk, the assessment is concerned with deleterious effects on the environment, human and animal health.

Measures of risk: for this example risk is assessed as the percentage decrease in insect species diversity over a given time period, as compared to analogous fields that have grown conventional potato varieties and similar subsequent crops.

The timescales under investigation: potato volunteers can overwinter in the soil from one year to the next, especially if winters are mild, thus giving rise to daughter tubers; true seed can remain viable for at least 7 years (Askew, 1993) before germinating. Both these traits may result in the persistence of volunteers for many years if there is inadequate control; nevertheless it is likely to take a long time for a significant volunteer problem to arise, hence the assessment is concerned with long-term and cumulative effects that will not necessarily be evident in the first few years of commercialisation.

Defining Tolerability Criteria:

As has been stated in the risk assessment framework guidance notes, defining tolerability criteria for a particular risk is not always straightforward, especially given the complexity and interrelated nature of the environment. Various benchmarks and standards exist for GM crops (and crops in general), including

environmental stewardship schemes, codes of practice and independent farm assurance schemes. In addition there are various statutory rules concerning the cultivation of crops and farming practice in general (e.g. in England: the Wildlife and Countryside Act 1981; the Conservation Regulations 1994 and The Hedgerows Regulations 1997). However there is still a need to develop clear criteria for GM crops so that risks can be judged against them.

Ultimately risk assessment comes down to quantifying the likelihood of a particular hazard and the severity of that hazard. If sufficient data are available then likelihood can generally be calculated on a probability basis, from impossibility to certainty. Putting a value on hazard severity, however, is not always a straightforward proposition, especially when it involves environmental concerns, because ethical considerations are an important part of the equation. Growing any type of crop will inevitably have environmental consequences, and whether those consequences are seen as acceptable or not depends on the viewpoint of the individual or the body concerned. Somehow, then, a defined limit needs to be placed on the consequence, so that any effect above this specified limit is considered unacceptable and elicits a particular action (e.g. implementation of mitigation measures or even withdrawal of the consent). Clearly, for limits to be set on severity, a clear understanding of what that the hazard is, or may be, is required. This therefore leads us to the next step in the framework, that of hazard identification.

Hazard Identification:

As mentioned above, existing assessments of consent C/SE/96/3501 conducted by expert committees have concluded that, on the whole, potato clone EH92-527-1 is unlikely to have an adverse effect on human health, animal health or the environment. In some instances, however, these committees have highlighted aspects of the consent application that could, at least theoretically, have the potential to cause problems in these areas. Taking these factors as a starting point, and focussing on potential long-term and cumulative effects, a focussed brainstorming session involving experts in potato biology was used to supplement information obtained at the primary brainstorming session. Analysis of the consent application dossier and consideration of the characteristics of the potato led to the identification of a number of areas where there is potential for long-term and cumulative adverse effects from the cultivation of potato clone EH92-527-1. Some of these effects are likely to be specific to EH92-527-1 and some are of a more generic nature. The potential hazards were been identified are as follows:

Allergenic effects: One of the major health concerns expressed for GM food is its potential to increase allergies in the human population either directly or through the food chain (i.e. via animals that have consumed the GM product). The possibility, therefore, of fatal anaphylaxis in sensitised individuals after unwitting exposure to allergenic proteins is a real danger (Gasson, M.J., 2003). Bioinformatic analysis shows that potato clone EH92-527-1 contains an open reading frame that could theoretically produce a protein (designated ‘ORF4’) with homology to two proteins that are known to be toxic or allergenic (EFSA Journal (2006), 323, 1-20), namely

the bleomycin (*ble*) resistance protein together and the ornithine cyclodeaminase protein of *Agrobacterium* (which is also a mite allergen). In their advice of 14 January 2005 the Advisory Committee on Releases to the Environment (ACRE) recommended that case-specific monitoring should examine the male fertility of potato clone EH92-527-1 to determine if there is any increase in pollen production. Although EH92-527-1 male fertility has been documented as low in trials, ACRE argue that if significant, unexpected pollen production is detected then the presence of ORF4 protein should be examined. The danger of high levels of pollen containing the ORF4 protein is that it could trigger allergic reactions in some people, such as farm workers and members of the public. Such effects may not be evident immediately and may require people to have a period of sensitisation.

Increased numbers of volunteers: During potato harvesting some small tubers are invariably left in the ground after harvest (groundkeepers) and these may give rise to volunteer plants in the next crop. Volunteers may also arise from true seeds, and studies have shown a return of up to 30 million seeds per hectare following a crop of potatoes, with up to 90% viability (SAC 1999). Although many groundkeepers are killed by climatic conditions (e.g. frost and drought) their control can be problematical in some areas and relies on a combination of careful harvesting, cultural and husbandry techniques, and herbicide treatment. In seed potato crops the presence of volunteer plants arising from 'rogue' tubers can lead to the rejection or downgrading of seed potato crops (the dossier is not clear as to whether fields that have been used for the production of EH92-527-1 will subsequently be used for production of conventional seed potatoes, and if so the length of time separating the two events); another crop that can be affected by potato volunteers is vining peas, which are automatically rejected if contaminated with potato berries. Volunteer potatoes can be carriers of various potato diseases, act as a major reservoir of viruses and can act as a 'green bridge' for pests and diseases that cannot persist in the absence of the host. In addition they can be very competitive with other crops, and control of disease and pest problems on volunteers in other crops may be virtually impossible as label-approved recommendations do not exist. In evaluating potato clone EH92-527-1 the notifier has assessed the potential for the genetic modification to result in a change of agronomic performance, and considered the likelihood of this occurrence to be low. The ERA did concede, however, that the period and scale of field trials (245 sites over 9 years) may not be considered long enough to reveal subtle and cumulative effects, and that volunteer management under different agricultural conditions could become more complex. It is possible then, that EH92-527-1 may exhibit a subtle difference in some aspect compared to the recipient clone. Such a difference may only become apparent after several years of cultivation or following cultivation under different climatic conditions. Theoretical examples of altered characteristics that could lead to an increased number of volunteers are:

- Increased numbers of smaller tubers, resulting in more tubers left in the ground after harvest;
- Increased frost resistance of tubers leading to enhanced over-wintering capacity (for example the higher levels of mono- and disaccharides documented for EH92-527-1 may result in an antifreeze effect; or the anhydrous properties of amylopectin could result in less water in the tissues).

Whilst the above attributes have not been documented in EH92-527-1, it is possible that they may only manifest themselves under certain environmental conditions and/or over a long time period.

Increased weediness: Weediness describes a plant's potential to successfully colonize an ecosystem, especially when it may also lead to the displacement of other species. Generally, weediness depends on the selective advantage of many genes functioning in combination, which are unrelated to the genes usually introduced for agronomic reasons. However, traits which enhance tolerance to environmental stresses such as drought, cold or dormancy have the potential to increase the survival and distribution of the plant in managed and unmanaged ecosystems. Additionally, traits which provide for resistance to biotic stresses that play a significant role in the ecology of the plant (e.g., insect or pathogen resistance) could permit the plant to become persistent and/or invasive within and outside of the agricultural ecosystem. Baker (1965) described a number of key characteristics of weeds, including: discontinuous germination and long-lived seeds (and/or tubers in the case of potatoes); rapid growth to reproductive stage; high seed (and/or tuber) output under favourable conditions; high tolerance or plasticity of climatic and edaphic variation; and good competitiveness achieved through, for example, allelochemicals or choking growth. In northern Europe potatoes are only found in cultivated areas, whereas in southern Europe they can also be found in the wild (although they are rarely seen as a 'nuisance' plant in natural ecosystems). At present starch potatoes are grown in Nordic countries, Eastern Europe, Germany, Holland, Belgium and France, but there is a possibility that the quota system could be revised or even abolished in future, leading to the cultivation of EH92-527-1 elsewhere in Europe, including southern Member States. For EH92-527-1 to become invasive the modification would need to result in some (possibly significant) change in its nature compared to other potatoes, resulting in EH92-527-1 displaying 'weedy' characteristics. Whilst clone EH92-527-1 has not been reported as displaying any such characteristics, the notifier, in addressing potential changes in competitive behaviour, states that "it cannot be excluded that a long-term effect will only be measurable after a certain period, e.g. after different culture cycles".

Pleiotrophic effects: Pleiotrophy, when one gene is responsible for the development of several features and characteristics, can lead to unforeseen changes during the development of both transgenic and non-transgenic organisms. Pleiotrophic and positional effects (the influence of the gene's position on its activity) are often concentrated on cell metabolism and can lead to phenotypic changes including altered agronomic characteristics and variations of gene expression levels. If such effects occur during the development of the transgenic organism they are usually addressed successfully, however if they are undetected, perhaps because they are expressed only under certain conditions, such effects can pose a risk if they increase the potential for the spread of transgenes (e.g. increased pollen production), or result in the production of toxic or allergic compounds (Lips, 1998) In a study of transgenic fructane-building potatoes, for example, different flowering behaviour has been observed compared to the donor plants (Becker *et al*, 1998). Such effects may only come to light after long-term cultivation under a range of different growing conditions.

Development of antibiotic resistance: Potato clone EH92-527-1 is one of a number of transgenic crops that employs the NptII gene as a selectable marker, by conferring

resistance to the antibiotic kanamycin. There is concern among some scientists that the use of such marker genes could potentially lead to increased resistance to antibiotics in humans and animals as a result of gene transfer from genetically modified plants to bacteria. These concerns have been well documented and the EFSA has issued scientific advice on the use of antibiotic resistance marker genes in genetically modified plants, with the aim of phasing out the practice. Whilst there is a theoretical potential for such adverse effects associated with EH92-527-1, due to cattle being fed the by-products, the risk is thought to be remote.

Effects on human health: Intolerance to amylopectin is known to occur in some people due to a congenital lack of the appropriate enzymes, and people suffering this condition (especially children) sometimes have to limit their intake. The accidental consumption of potato with 100% amylopectin may have unwanted and unexpected effects on such people, although the notifier maintains that such consumption will have no practical consequence since starch from normal potatoes also causes the problem. There may however be a dose-response effect that is yet to be documented. The Swedish Board of Agriculture, in their statement of 15 April 2004, raised a concern that, because amylopectin is digested more rapidly than amylose, this could result in undesirable effects in diabetics, who could experience a swifter increase in blood glucose levels than would normally be expected from consuming potatoes. The Board acknowledged that it is very unlikely that potato clone EH92-527-1 will enter the human food chain, but stated that this cannot be completely ruled out. As time goes on this risk is likely to increase.

Effects on non-target organisms: Observation of the effect of growing EH92-527-1 on a range of organisms that interact with potatoes (including viruses, fungi, bacteria, insects, snails and worms) has been carried out by the notifier, and no adverse effects have been noted. However it is not unreasonable to expect that the altered starch composition of EH92-527-1 may have an effect on certain soil-dwelling micro-organisms, leading to alterations in the populations of microflora. This in turn could lead to altered decomposition rates and possible perturbations in chemical and nutrient cycling. In addition there is evidence that the level of glycoalkaloids (toxic and anti-nutritional compounds that occur mainly in the above-ground parts of the plant) in potato clone EH92-527-1 is lower than that in the mother variety Prevalent. Whilst a lower concentration of compounds such as solanine may be beneficial for animal health, it may also make EH92-527-1 more liable to attack by herbivorous insects and invertebrates, particularly larvae and snails (Johnston, 1989). Although the notifier could find no evidence for more serious attacks, a subtle increase could, over a long timescale, see a significant increase in the amount of pesticides used on the crop, with concomitant effects on non-target insects and wildlife in general. It is also theoretically possible that glycoalkaloids may be a limiting factor for some insects, such as Colorado Potato Beetle. If glycoalkaloid concentrations in leaves are lower this could lead to rapid growth of this insect pest, a possible extension in its climatic range, and over time result in wider outbreaks in conventional potato crops.

To broaden the scope of the above hazards in relation to the environment and further explore the possible consequences the theme-based approach has been employed. The results are shown in table 7.

Table 7 - **Potential consequences if the hazard/or hazards are realised.**

	Potential effect (give brief rationale or example if not obvious)	
Theme	Positive effect (benefit)	Negative effect (harm)
Human health	None envisaged (potato clone EH92-527-1 is not for human consumption, although the lower glycoalkaloid and higher vitamin C content may actually enhance the nutritional content if it does accidentally enter the human food chain).	Horizontal gene transfer of the kanamycin resistance gene in EH92-527-1 to bacteria in the guts of humans, resulting in antibiotic resistant bacteria and compromised human therapy; Some people exhibit intolerance to amylopectin; Glycaemic effects on diabetes sufferers who might accidentally consume EH92-527-1; Acute or chronic toxicity on people who might accidentally consume EH92-527-1; Allergenic effects may manifest themselves in some people.
Animal health	Lower glycoalkaloids and higher vitamin C content may enhance the nutrition of cattle fed the by-product.	Horizontal gene transfer of the kanamycin resistance gene in EH92-527-1 to bacteria in the guts of animals, resulting in antibiotic resistant bacteria and compromised animal therapy.
Quality of life	Potato starch is perceived to have a higher quality than maize starch (thus commanding a higher price), possibly leading to higher economic returns for growers (and better quality products for consumers).	Chronic allergenic effects of pollen may afflict some people.
Agricultural sustainability	May increase the resilience of the agro-economy due to a wider range of cropping choices.	May cause perturbations in soil-dwelling micro-organisms, potentially leading to disruption in biochemical processes; May decrease the resilience of the agro-economy due to potential volunteer problems in subsequent crops; Increase in pests (phytophagous insects) due to lower glycoalkaloid levels in plant tissues.
Environment for wildlife	There may be benefits for wildlife compared to growing other crops that have higher pesticide inputs or are less wildlife-friendly than potato	Reduction in invertebrates and their predators if more herbicides are used to control volunteers; Reduction in invertebrates and their predators if more pesticides are used to control higher pest numbers.
Air quality	None envisaged.	None envisaged.
Water quality	None envisaged (although 'greener' processing may benefit water quality – see Greener business).	Detrimental effect if more herbicides/insecticides are used to eradicate EH92-527-1 volunteers.
Land use and soils	Increased opportunity for growers to diversify.	Restricts potential uses of the land (e.g. for potato seed crops) if volunteers are more frequent and persistent; If seed/ware potato crops are contaminated and are disposed to landfill it reduces the volume available for other waste and adds to the environmental burden.

	Potential effect (give brief rationale or example if not obvious)	
Theme	Positive effect (benefit)	Negative effect (harm)
Greener business	A reduction in imported (e.g. maize) starch may reduce the number of 'product-miles'; Fewer chemicals may be required during the processing of EH92-527-1 starch.	Possible greater use of harmful herbicides
Sustainable resource use	None envisaged.	Energy and resources will have to be expended to control EH92-527-1 volunteers
Limit and adapt to climate change	None envisaged.	Secondary effect of energy use (see above).
Regulatory effectiveness	None envisaged.	If the problem of conventional seed or ware potato contamination arises, it could be a relatively high profile issue, diverting funds from elsewhere.

To further test and refine the proposed risk assessment methodology in relation to long-term and cumulative effects it is necessary to select a scenario from the list of hypothetical hazards. In this case it has been decided to examine more closely the risk of increased numbers of volunteers leading to reduced wildlife diversity due to an increase in herbicide application.

Scenario 4: Increased numbers of volunteers

In this hypothetical scenario we have chosen to assess the risk to the environment posed by a potential increase in the number of potato volunteers due to possible phenotypic (tuber size) and physiological (frost resistance) changes resulting from the modification. We assume that the increase in the numbers of volunteer plants, although slight, has been calculated to be significant over the lifetime of the consent, and this has presented the notifier with the option of either extending the rotation period for EH92-527-1 or increasing the frequency of herbicide use. For economic reasons we anticipate that the notifier has chosen to recommend to contracted growers an increase in herbicide use. An expert committee have assessed the proposal and concluded that an increase in the use of broad-leaved herbicides will in turn impact on native weed populations and have possible knock-on effects for other wildlife. The notifier wishes to quantify this effect.

As this particular scenario is focussing on the potential consequences of an increase in the number of potato volunteers it is appropriate to revisit the initial question and make it more specific to the identified hazard. In this case the following specific question is posed concerning the commercialisation of potato clone EH92-527-1:

"What are the risks that the increased potato volunteers will result in a decrease in species diversity due to increased herbicide use?"

Having restricted the focus of the question it is appropriate to look again at how the question is framed. In the case of the risk to wildlife diversity the following criteria are considered important:

The decision context: again we will assume that question has been posed by the notifier wishing to quantify the risk of any potential deleterious effects on the environment and determine possible prevention and mitigation measures.

The geographic scale of the decision to be made: In the first instance this may be restricted to Nordic counties where commercial cropping will first be introduced.

The type of risk being considered: in terms of the nature of the risk, the assessment is concerned with deleterious effects on wildlife diversity. This could be in terms of numbers of organisms, species diversity or species richness.

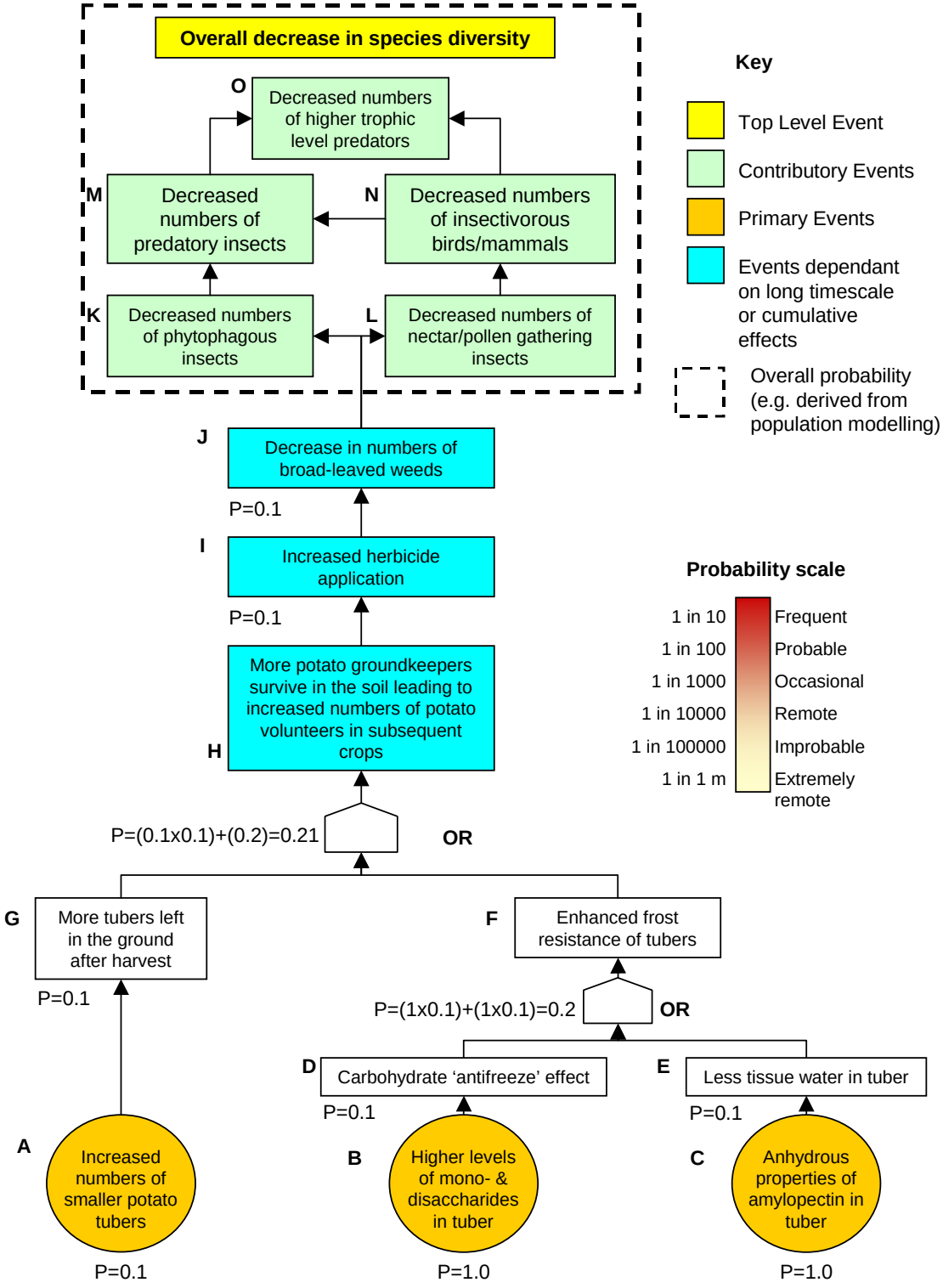
Measures of risk: for this example we will assume that risk is assessed as the percentage decrease in wildlife numbers over a given time period, as compared to analogous fields that have grown conventional potato varieties and similar subsequent crops.

The timescales under investigation: potato volunteers can overwinter in the soil from one year to the next, especially if winters are mild, thus giving rise to daughter tubers; true seed can remain viable for at least 7 years (Askew, 1993) before germinating. Both these traits may result in the persistence of volunteers for many years if there is inadequate control; nevertheless it is likely to take a long time for a significant volunteer problem to arise, hence the assessment is concerned with long-term and cumulative effects that will not necessarily be evident in the first few years of commercialisation.

Hazard Analysis

In order to understand the factors that lead to the perceived hazard, and to understand the relationships between the contributing factors, graphical analysis has been employed. Figure 4 is a rationalised fault tree showing the different interrelated events that have to combine for the hazard to be realised. Probability values have been assigned to each event.

Figure 4: Cause-consequence diagram showing how an increase in potato volunteers could elicit a decrease in wildlife diversity due to increased herbicide use over time.



The cause/consequence diagram in figure 4, above, provides a systematic description of the combinations of possible occurrences in the agro-ecosystem which can result in a decline in wildlife diversity (the Top Event). The tree has been constructed by deducing each of the preconditions for the top event and then successively for the next level of event, until the basic causes are identified. The probabilities are calculated using 'AND' and 'OR' logic gates. At an OR gate the probabilities are added to give the probability of the next event; at an AND gate the probabilities are multiplied.

For each of the events leading to the anticipated hazard, probability values have been ascribed to give the likelihood of a particular outcome. Assigning probabilities in itself, however, is not a straightforward process, because most of the events that must occur for the hazard to be realised do not simply have a 'yes' or 'no' outcome: in nature things are generally a lot more complicated than this. In fact, for most events considered there are different gradations of effect. If we look at the first event ('Increased numbers of smaller potato tubers'), we could ask the question 'how many smaller tubers must there be for the effect to merit concern?'. Would a 5% increase or a 50% increase in the number of smaller tubers be significant? Presumably, if the tubers are only slightly smaller they will not pass through the harvesting machinery, and the problem will be negated. This begs the question 'how much smaller do the tubers need to be before they are considered problematical?'. In the case of this scenario it has been decided that 20% is a realistic threshold value for each effect, thus each probability value has been assigned on the basis that the event will cause a 20% change (this can be an increase or a decrease) in the factor under consideration. The rationale behind each probability values is as follows:

A. Increased numbers of smaller potato tubers – *Probability*: 0.1

Reasoning: The notifier has reported that the yield of EH92-527-1 is significantly lower (statistically speaking) than Prevalent. Lower yield can manifest itself either as fewer tubers or the same number of tubers of a smaller size (or a combination of the two). The notifier has not stated which of these factors is responsible, therefore for this scenario we will assume an increase in the number of smaller tubers is the reason for the drop in yield. In this instance it is not possible to put an absolute probability value on this event due to lack of data, however it seems reasonable to consider the likelihood of a greater number of smaller tubers as 'probable' (P=0.1).

B. Higher levels of mono- and disaccharides in tuber – *Probability*: 1.0

Reasoning: Compositional analysis of EH92-527-1 by the notifier has confirmed that the levels of fructose and saccharose are higher in the modified clone compared to the donor plant. Due to the fact that this increase in sugar levels is statistically significant, the probability is given as 1.

C. Anhydrous properties of amylopectin in tuber – *Probability*: 1.0

Reasoning: Compositional analysis of EH92-527-1 has confirmed that it has a higher amylopectin content than the parent plant, consisting of almost 100% of amylopectin. Due to the configuration of the amylopectin molecule this type of starch is known to have increased anhydrous properties compared to amylase starch.

D. Carbohydrate 'antifreeze' effect – *Probability*: 0.1

Reasoning: Increased sugar content could theoretically result in greater freezing resistance, giving tubers an increased ability to survive over the winter.

E. Less tissue water in tuber – *Probability*: 0.1

Reasoning: The anhydrous properties of amylopectin are likely to result in less water in the tissues of EH92-527-1 potato tubers.

F. Enhanced frost resistance of tubers – *Probability*: 0.2

Reasoning: A lower tissue-water content, or an antifreeze effect caused by a higher sugar content, could lead to enhanced frost resistance in EH92-527-1 tubers. Frost tolerance studies have been performed by the notifier and did not show any difference compared with Prevalent. However the field trials only appear to have been conducted in a single year, and laboratory trials are unlikely to duplicate all of the conditions existing in commercial cultivation over time.

G. More tubers left in the ground after harvest – *Probability*: 0.1

Reasoning: Given an increased number of small tubers it is likely that there will be a greater number of tubers left in the ground following harvesting, as not all will be removed by potato harvesting machinery. A frequent (1 in 10) chance of this happening would seem realistic, although field observations could be used to increase the accuracy of this estimate.

H. More potato groundkeepers survive in the soil – *Probability*: 0.21

Reasoning: With the potential for greater number of tubers being left in the ground, or the possibility of enhanced frost resistance, there is an increased likelihood of larger numbers of groundkeepers surviving from one year to the next. The probabilities of the two separate branches are added together at the OR gate to calculate the overall likelihood of the event taking place. With a larger number of groundkeepers surviving in the soil from 1 year to the next it is highly likely that there will be a larger number of potato volunteers in the subsequent crop

I. Increased herbicide application – *Probability*: 0.1

Reasoning: Given the agronomic problems associated with the control of potato volunteers it is highly likely that growers will increase their herbicide applications to deal with the higher numbers in subsequent crops, hence the likelihood of increased herbicide application is assumed to be a frequent.

J. Decrease in numbers of broad-leaved weeds – *Probability*: 0.1

Reasoning: Increased herbicide application to kill potato volunteers in subsequent crops is highly likely lead to a decrease in the numbers of common broad-leaved weeds (e.g. *Brassica rapa, Chenopodium album, Fumaria officinalis, Papaver rhoeas* etc.) in the field, hence this has been given a probability of 0.1.

Due to the inherent complexity of biological systems, assessing the probability values relating to decreased numbers of insects, birds and mammals (events K to O, outlined below) living in a defined area or ecosystem is a highly complex issue.

In such cases a population modelling approach is likely to provide the most accurate probability estimates, and this requires high quality data relating to the environment of concern. As this is beyond the remit of this project we are unable to attach probabilities to the following events.

K. Decreased numbers of phytophagous insects

Reasoning: A great many insects (and other invertebrates) in arable fields rely on broad-leaved weeds as a primary food source. These include sap-sucking insects, such aphids and froghoppers and shield bugs, as well as foliage-eating insects and invertebrates including leaf cutter bees, caterpillars, slugs and snails, etc. Fewer broad-leaved weeds are very likely to have a detrimental effect on the numbers of these insects and invertebrates in the field of concern.

L. Decreased numbers of nectar/pollen gathering insects

Reasoning: In the same way that phytophagous insects rely on broad-leaved weeds, many nectar and pollen-gathering insects are dependent on flowering broad-leaved weeds in the arable environment to provide them with a food source. Examples of such insects are bees, butterflies and pollen beetles. A decrease in the number of such weeds is likely to have a direct effect on these insects, although it must be noted that bumble bees and butterflies can fly long distances and a decline in weeds may have little immediate impact on them. However, if weeds die over large areas and over several years, the effects on nectar resources would become more important. Such was an important conclusion of the UK Farm Scale Evaluation (FSE) trials.

M. Decreased numbers of predatory insects

Reasoning: Predatory insects, such as carabids spiders, lacewings, ladybirds, wasps and centipedes all feed on other smaller insects. With a reduction in their prey items their numbers are also likely to decrease.

N. Decreased insectivorous/granivorous birds/mammals

Reasoning: A number of birds and small mammals (and amphibians such as frogs) feed on insects and invertebrates, and often this is their primary food source or is an important food source at a particular life stage. Many birds require a regular supply of insects in order to feed their chicks the high protein diet they require for rapid development. Grey partridges are especially susceptible to variations in insect food supply, and studies have shown that partridge density is inversely related to the number of herbicide applications, and positively related to the number of broad-leaf weed groups (Campbell *et al.*, 1997). Broad-leaved weeds also provide a useful source of seeds for granivorous birds and mammals.

O. Decreased numbers of higher trophic level predators

Reasoning: A decrease in the number of insectivorous and granivorous birds and mammals may lead to a decrease in the numbers of higher trophic level predators, such as owls, raptors, foxes, weasels, etc. that prey on small mammals and birds. This conclusion is supported by the FSE results, which show that a decline in arable weed populations is likely to result in adverse effects on organisms at higher trophic levels, compared with conventionally managed crops (although the

FSE trials did not involve GM potatoes, the effects of weed management practices can be extrapolated to other crops).

Top Level Event: Decreased species diversity. As has already been described, the probabilities of each event have been assigned on the basis that there has been a 20% change in the characteristic about which we are concerned. In the case of wildlife diversity, this means that the probability value for the top event is applicable to an overall 20% decrease in species in and around the field where potato clone EH92-527-1 has been grown. If, on the other hand, a threshold value of lower than 20% was chosen (e.g. 10% reduction in species diversity), there would be a greater likelihood of the hazard being realised and the final probability value would be higher; similarly if the threshold were higher than 20%, the probability value would be lower.

Assessing Likelihoods, Consequences and Risks

In the scenario outlined above are described a series of interrelated causal events leading to a decrease in species diversity. To be able to attach meaningful probabilities to the individual events it has been necessary to apply a threshold value to the different stages. The overall probability value, relating to the top level event, is then calculated by combining the probability of each lower event using a standard metric (e.g. OR and AND gates). This, however, is just one part of the risk equation, since risk is generally expressed as a combination of 1. the likelihood of an adverse effect, and 2. the severity (or hazard) of that particular effect. In order to complete the assessment of risk we need, therefore, to ascertain exactly how severe the decline in wildlife diversity is, and for this application we can use a similar scale to the probability scale, from 0 (insignificant) to 1 (extreme hazard). This scale, however, need not be linear, as there may be instances where a small effect is inconsequential but a larger effect crosses a critical threshold and causes severe consequences such as environmental instability. Figure 5 represents a simplified hazard severity curve for an ecosystem with a critical threshold (or ‘tipping point’) at the 10% level. If this hypothetical 10% level is exceeded, then the relationships between the existing species break down and the severity in terms of effect on the ecosystem as a whole increases markedly. Using figure 5 for the volunteer potato scenario being considered, a 20% decline in species diversity can be judged to be moderately severe and is assigned a hazard rating of 0.5 (see dashed arrows).

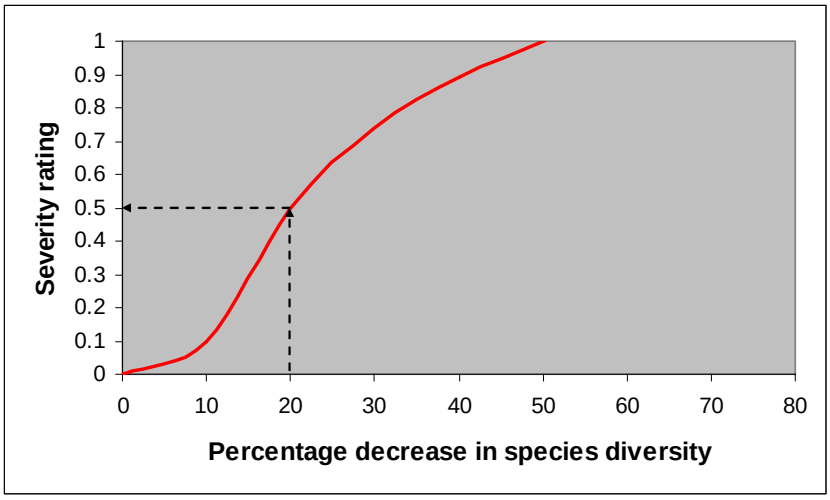


Fig. 5 – Hypothetical hazard severity curve for ecosystem species diversity

Using this type of approach it is possible to predict (or at least estimate) the likelihood of a particular hazard being realised and the severity of that hazard. The next stage, therefore, is to calculate the risk posed by these factors. In the case of this scenario, both hazard likelihood (probability) and hazard severity are measured on a scale of 0 to 1. The output therefore also ranges from 0 (zero risk) to 1 (extreme risk). The actual equation is:

Risk* = SUM (Probability of Hazard x Expected Severity of Consequence)

*of a 20% decreased wildlife diversity.

The decision as to whether a particular risk is tolerable must then be assessed in light of the predefined tolerability criteria defined in Step 2.

Assess Uncertainties

With any type of risk assessment there is likely to be a degree of uncertainty in the values assigned to each event. In the case of transgenic crops this uncertainty is increased because most GM crops have only been grown experimentally on a relatively small scale over a relatively short period of time. Coupled with this, when such crops are released into the wider environment they enter a highly complex system with a great many interdependent relationships. Determining exactly how the crop will affect the environment, and how the crop itself will be affected by the environment, is a difficult task. In the case of Scenario 4 it must be acknowledges that there are many gaps in the information required for a comprehensive risk assessment, especially concerning the interrelationships between different trophic levels, and several steps must rely on subjective estimates. Much more data is needed to fill these gaps.

Compare Risk Against Criteria

The release should be categorised as either acceptable or unacceptable when compared to the criteria set under Step 2. At present appropriate criteria needs to be developed for GM crops.

Identification and Evaluation of Mitigation and Monitoring Methods

Having carried out an assessment of risk for the identified hazard it is appropriate now to consider the type and extent of monitoring and mitigation measures that could be put in place to negate or reduce that risk. Table 8 shows potential monitoring and mitigation measures for the various events leading from increased numbers of volunteer potatoes to decreased species diversity.

Table 8 – Potential prevention monitoring and mitigation methods

	Event	**Possible prevention measures**	**Possible monitoring methods**	**Possible mitigation measures**
A	Increased numbers of smaller potato tubers	Because EH92-527-1 is a clone there is little that can be done to prevent the production of smaller tubers.	Monitoring of potato fields after harvest should detect greater numbers of small tubers.	Greater care during harvest will reduce the number of potatoes left in the ground.
B	Higher levels of mono- & disaccharides in tuber	N/A	Sampling and testing may alert the notifier of the problem and allow appropriate changes to management practices.	The levels of mono- & disaccharides may be influenced by environmental conditions – changing these conditions may reduce these levels
C	Anhydrous properties of amylopectin in tuber	The anhydrous properties of EH92-527-1 is likely to be fixed, therefore there are no prevention measures; Sampling and testing may alert the notifier of the problem and allow appropriate changes to management practices.		
D	Carbohydrate 'antifreeze' effect	The antifreeze properties of EH92-527-1 is likely to be fixed, therefore there are no prevention measures; Further laboratory and field testing may help to determine the extent of any problem.		
E	Less tissue water in tuber	The quantity of tissue water of EH92-527-1 is likely to be fixed, therefore there are no prevention measures; Further laboratory and field testing may help to determine the extent of any problem.		
F	Enhanced frost resistance of tubers	Reduce the number of groundkeepers remaining in the soil (improved harvesting and cultural methods); Field testing and monitoring needs to be carried out under a range of different environmental conditions.		

G	More tubers left in the ground after harvest	Greater care harvesting	Post-harvest sampling to determine groundkeeper numbers	Improved cultural methods to remove 'rouge' tubers
H	More potato groundkeepers survive in the soil leading to increased numbers of potato volunteers in subsequent crops	Improved harvesting and cultural methods to ensure as few groundkeepers remain in the soil	Farmland potato volunteer surveys	Spot-spraying of volunteers to avoid total weed eradication
I	Increased herbicide application	Use methods of controlling volunteers other than herbicides	Farmer pesticide-use surveys	Increase wildlife-friendly habitats
J	Decrease in numbers of broad-leaved weeds	Restrictions on herbicide applications	Arable weed surveys	Increase wildlife-friendly habitats
	Decreased species diversity	Increase wildlife-friendly habitats	Farmland surveys of invertebrates, birds, mammals.	Diversity action plans may help wildlife to recover

An increase in potato volunteers leading to an increase in herbicide use is one of theoretical consequences of the cultivation of potato clone EH92-527-1. To carry out a full risk assessment it is necessary to investigate each of the potential hazards that have been highlighted and determine the level of risk for each one. Once the full range of hazards has been assessed the resulting risk values could then be integrated into an overall risk for EH92-527-1. Based on this overall risk the decision as to whether commercialisation could go ahead would be made. Any such decision should take into account the possible prevention, monitoring or mitigation measures presented above

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6.4. Case Study 4

This case study is of a generic nature and is concerned with potential long-term and cumulative effects of maize cultivation. For the sake of argument it will be applied to MON 810, although it could readily be applied to any other GM maize line.

C/F/95/12-02 - Insect Resistant Maize MON 810 (Monsanto)

Organism: maize line MON 810;
Trait: insect resistance employing the CryIA(b) insect-control protein;
Scope: for use as any other maize, including cultivation;
Current status: consent issued August 1998.

Background information

This particular case study is concerned with risks that are based primarily on theoretical hazards rather than observational data. The subject of the assessment is MON 810 maize, although the majority of the hazards discussed could apply equally well to other genetically modified organisms.

1. Framing The Question:

In the case of the risk assessment of genetically modified maize the following general question can be posed:

"What are the risks associated with the commercialisation of GM maize; are these risks acceptable?"

In order to focus more precisely on the nature of the applied risk assessment the above question is defined in terms of the following criteria:

a. The decision context: in this instance we will assume that question has been posed by the regulator who wishes to assess the risk of any potential deleterious effects from long-term cultivation and determine possible monitoring and mitigation measures.

b. The geographic scale of the decision to be made: in terms of geographic scale, the assessment is concerned with all EU Member States where insect-protected maize is likely to be grown.

c. The type of risk being considered: in terms of the nature of the risk, the assessment is concerned with deleterious effects on the environment, human and animal health.

d. Measures of risk: for this example risk is assessed relative to cultivation of conventional maize, with the stipulation that any effects on human health, animal health and the environment will be no greater than the effects posed by analogous conventional varieties grown in the same environment.

e. The timescales under investigation: the assessment is concerned with potential risks that extend throughout the duration of the consent period, and possibly beyond.

2. Defining Tolerability Criteria:

The following tolerability criteria may be considered applicable:

- Benchmarks and standards: the degree of risk should be no greater than that posed by the unmodified crop, unless it can be demonstrated that there are distinct benefits that offset the risk (see below).

- Formal requirements: the principle of As Low As Reasonably Possible (ALARP) may be deemed appropriate, although consideration would need to be given to the concept of 'reasonable'. Stakeholder meetings may be the most appropriate way of defining what this concept.
- Benefits versus risks: to receive a favourable risk assessment, where a risk is identified there must be an obvious and comparable benefit (or benefits) which offset that risk. Such benefits may be economic (e.g. the insect resistance properties allow the production of maize in areas where it was previously economically unviable); environmental (e.g. reduction in pesticide use) or health-related (reduction in worker-exposure to pesticides).
- Level of confidence: confidence levels will inevitably be difficult to determine in the case of long-term effects. However the risk assessment process is likely to help determine what needs monitoring, as well as the spatial scale and the geographical extent of monitoring. Once more data becomes available the level of confidence is likely to increase.
- Ethical considerations: moral judgements can be very disparate and may vary with time. To attempt to reach a consensus it may be necessary to canvass the opinions of a wide range of interested parties.

3. Hazard Identification:

The toolbox catalogue in appendix 5 provides a number of tools and techniques that can be used for hazard identification, from simple checklists to more specialized methods such as Holistic Holographic Analysis. In this instance we have chosen to look at the outputs from the brainstorming session of 31/10/05. In particular we are interested in the 'blue sky' scenarios that were identified, which although being somewhat outside the main consensus of scientific opinion, could conceivably occur due to the long term and widespread cultivation of GM crops. Due to their speculative nature such scenarios are likely to be useful in testing the applicability of the framework to more hypothesis-based risks, rather than simply relying on evidence-based risk assessment that may be limiting in terms of long-term cumulative effects. The main exploratory scenarios put forward are outlined below:

- '**Genetic meltdown**' which describes a genomic crisis occurring due to an extraordinarily high rate of mutation (a phenomenon known to occur in viruses and perhaps in other organisms). The concept behind this theory is that although mutation contributes to variability and variability drives the success of a population in the face of a changing environment, most mutations are deleterious. Therefore, when a high mutation rate gives rise to a great many deleterious mutations it will eventually cause the population go extinct.
- '**Silent spread' of recessive genes/dormant traits** refers to genes or traits that are not overtly expressed in the crop (thus their effects are not detected during the assessment process), but remain concealed in the genome until triggered by genetic recombination or some other external event (such as altered environmental conditions).
- **Compromised human and animal therapy** due to the use of antibiotic-resistance marker genes in plants. In this scenario antibiotic resistance genes in transgenic plants have the potential to transfer to bacteria (e.g. in cows'

stomach) leading to increased resistance to antibiotics in human and animal pathogens (note, the nptII gene was not integrated in the case of MON 810). Whilst this is a long-established theory there remain wide differences of opinion as to the likelihood of it occurring, and the potential consequences if it did happen.

- **Increased *Fusarium* infection of wheat** may occur due to the increased cultivation of maize, grown more widely due to its GM-induced advantages. Maize is known to harbour *Fusarium* which can then be passed on to nearby wheat crops, thus creating disease problems for wheat growers. Evidence suggests that this scenario is unlikely to happen in the case of insect resistant maize (secondary infections are reduced due to lower rates of pest attack) but it may occur with other traits such as herbicide tolerance.

In order to broaden the scope of any potential positive and negative effects in relation to human health, animal health and the environment the theme-based approach was applied to the above scenarios. The results are shown in table 9, below.

Table 9 - **Potential consequences if the hazard/or hazards are realised.**

	Potential effect	
Theme	Positive effect (benefit)	Negative effect (harm)
Human health	Potential production of beneficial compounds due to rearrangement of genes during transformation process.	Potential production of deleterious compounds (e.g. pollen allergens) due to rearrangement of genes during transformation process; Potential loss of simple antibiotics
Animal health	Reduced fungal infection resulting in less mycotoxins	Potential production of deleterious (e.g. toxic) compounds due to rearrangement of genes during transformation process; Potential loss of simple antibiotics
Quality of life	Economic benefits of low-input maize production	Potential allergenic effects of pollen may afflict some people.
Agricultural sustainability	Increase productivity for maize growers in areas with endemic corn borer populations	May decrease agronomic resilience due to monoculture of maize; Fusarium infection may lead to yield reduction in wheat
Environment for wildlife	Reduction in use of pesticides will benefit non-pest species	Maize monoculture may lead to less species diversity
Air quality	None envisaged.	None envisaged.
Water quality	Less pesticide runoff	Potential Cry protein leachates in soil
Land use and soils	Allows wider range of cropping	Cry proteins may harm soil organisms and cause detrimental effects on fauna/structure
Greener business	Reduction in use of pesticides	Possible detrimental effects on non-target organisms

	Potential effect	
Theme	Positive effect (benefit)	Negative effect (harm)
Sustainable resource use	Less energy and resources will need to be expended to control insect pests	'Genetic meltdown' may lead to loss of maize varieties/germplasm; Cry toxicity may lead to decline in non-target/beneficial organisms.
Limit and adapt to climate change	Secondary effect of energy use (see above).	None envisaged.
Regulatory effectiveness	None envisaged.	If the problems outlined in the above scenarios occur it could divert funds from elsewhere.

To demonstrate the applicability of the risk assessment methodology in terms of theoretical risks, a specific scenario must be selected from the list of hypothetical hazards. In this case it has been decided to examine more closely the risk of the 'silent spread' of recessive genes/dormant traits leading to unintended effects in maize volunteers.

Scenario 5: 'Silent spread' of recessive genes/dormant traits

In this 'blue sky' scenario we have chosen to assess the risk to the environment posed by a deleterious recessive gene unintentionally produced in the maize genome during insertion of the transgene. As we now have a more specific risk to assess it is appropriate to revisit the initial question and revise it appropriately. In this case the following specific question is posed concerning the commercialisation of maize line MON 810:

"What are the risks that a deleterious recessive gene will result harmful effect on the environment?"

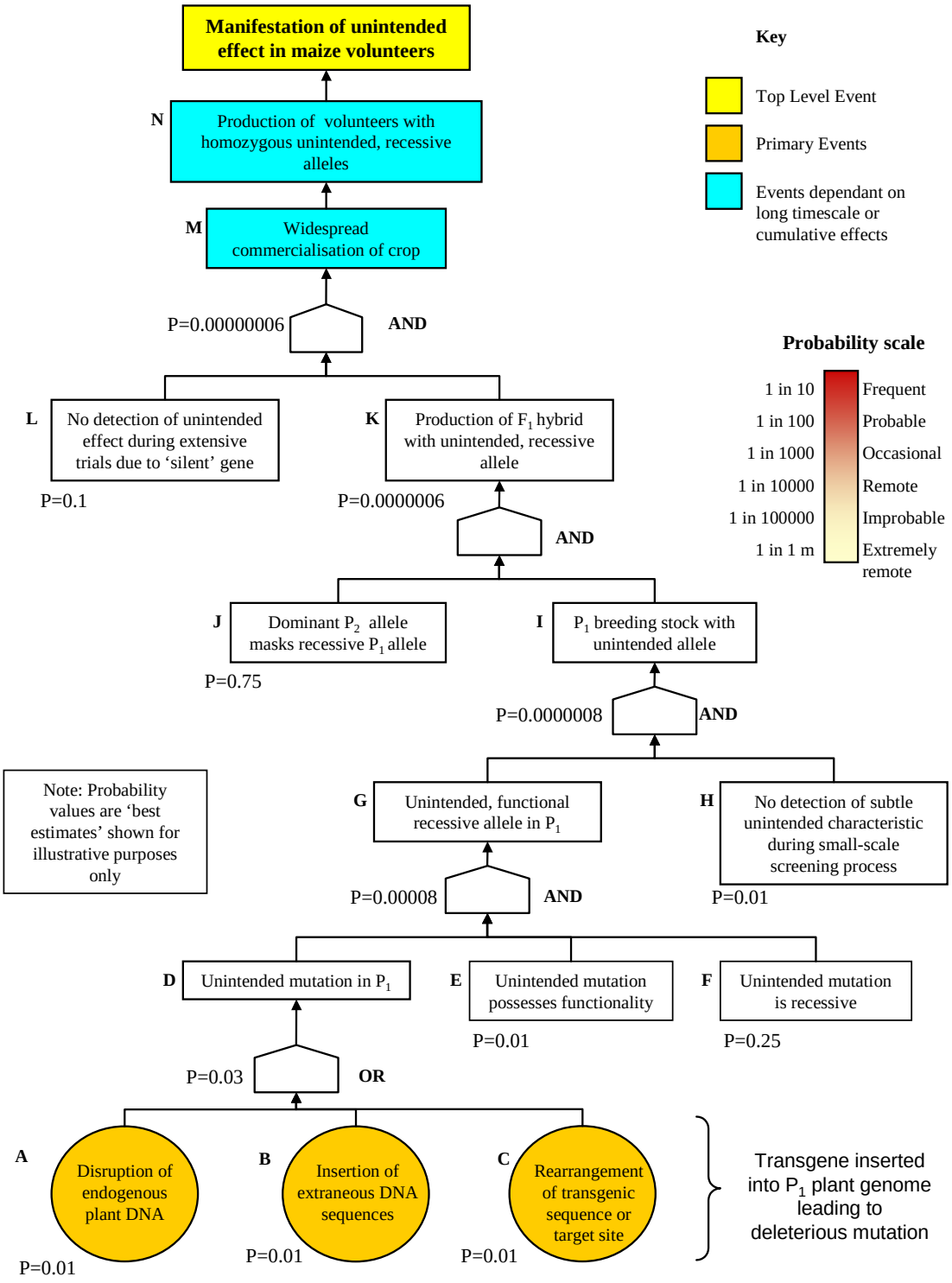
It should be noted that the question could equally apply to harmful effects on human and animal health, but for the purpose of this assessment we are restricting its scope to the environment. Having rephrased question it may be appropriate to look again at how it is framed.

4. Hazard Analysis

To gain a better understanding of the factors that may lead to the perceived hazard, and to understand the relationships between the contributing factors, graphical analysis has been employed. Figure 6 is a cause/consequence diagram showing the different interrelated events that are required to combine for the hazard to be realised. 'Best estimate' probability values have been assigned to

each event to demonstrate how the likelihood values might be derived, however, because the chosen scenario is a theoretical concept there are very little scientific data to support these values.

Figure 6: Cause-consequence diagram showing how the genetic modification process may lead to unexpected detrimental effects over time.



The cause/consequence diagram in figure 6 explores the likelihood that transgene insertion into the plant genome will lead to a functional deleterious mutation. It is based on the premise that, in addition to 'perfect' transgene integrations, several rearrangements of the construct and of the target site can occur. Termed 'insertional mutagenesis' this disruption of gene sequences can have deleterious effects on gene function and lead either to gene silencing or the expression of unintended novel genes. *Agrobacterium*-mediated integration in particular can result in complex integration patterns, including directed and inverted repeats (Krizkova and Hrouda, 1998), and the presence of such rearrangements is often associated with instability in transgene expression (e.g. Kumar and Fladung, 2000). At present, even given a known nucleotide sequence, it is inherently impossible to predict the fate and the site of the integration of a particular transgene construct into the plant genome. The events listed B), C) and D) describe some of the factors which can result in sequence disruption.

A) Disruption of endogenous plant DNA – *Probability*: 0.01
Reasoning: A possible consequence of the random integration of transgenes in the plant DNA is the disruption of endogenous gene function due to the insertional mutagenesis process. At the site of insertion in the plant DNA chromosomal rearrangements linked to the T-DNA insertion are possible, including inversions and translocations. T-DNA integration into genes can cause mutations due to loss of gene function. This can result in phenomena such as gene silencing and the production of novel sequences. Little information exists on the likelihood of endogenous DNA disruption but extrapolating from similar

B) Insertion of extraneous DNA sequences – *Probability*: 0.01
Reasoning: The presence of backbone sequences (i.e. sequences belonging to bacterial plasmid outside the left and right borders) is often observed (De Buck et al., 2000), especially in the case of *Agrobacterium*-mediated transformation (Kononov et al., 1997). The genomic context of inserted transgenes has been investigated in some plant species and sequencing of flanking regions of T-DNA inserts has highlighted the recurring presence of motifs that flank transgenes, including retroelements, microsatellite sequences and tandem repeats (Cellini et al, 2004). Investigations in a limited number of species suggest the frequency of transfer of these non T-DNA sequences ranges between 0 to 31%. Here we have employed a cautious probability value of 0.01.

C) Rearrangement of transgene construct/target site – *Probability*: 0.01
Reasoning: Insertion of trangenes into plant chromosomal DNA can result in either single copy or repeated or multiple insertions, as well as inversion and translocations. Multiple insertions can occur in linked or unlinked sites and filler DNA of plant origin is often found between DNA repeats.

D) Deleterious mutation in P_1 – *Probability*: 0.03

Reasoning: The various probabilities of disruption of endogenous DNA, extraneous DNA sequences and rearrangement of sequences are added together to obtain the overall probability of a deleterious mutation in the plant genome.

E) Deleterious mutation possesses functionality – *Probability*: 0.01
Reasoning: In the vast majority of cases the unintended rearrangement of DNA sequences will not lead to functional alleles. However in a very few instances it can be expected that the functional alleles will be produced and these will be expressed to some degree. This expression may be in the form of altered phenotype, altered plant response or altered composition, and such alteration may be detrimental or beneficial for the plant. Such unintended effects may result from conventional plant breeding, however the probability that they will arise due to the genetic modification process is thought to be greater.

F) Deleterious mutation is recessive – *Probability*: 0.25
Reasoning: In order for the effects of the deleterious mutation to go unnoticed during the production of hybrid seed it is necessary that the allele is recessive.

G) Unintentional functional recessive allele in P_1 – *Probability*: 0.0008
Reasoning: Events D, E and F combine to give a probability for the occurrence of a deleterious functional allele in parent 1 that is recessive.

H) No detection of subtle unintended effect during screening – *Probability*: 0.01
Reasoning: During the development of a new transgenic plant variety assessments are carried out in the laboratory, glasshouse, and small field trial. Characteristics assessed include plant vigour, growth habit, yield, pest and disease resistance, composition and quality. During this evaluation process a large number of transformants/clones that do not perform to expectation will be discarded, thus the process will result in the elimination of major unintended effects. Subtle effects, however, that are less easily screened for, may remain undetected (Cellini, et al, 2004) due to the limitations of the screening process. The probability of detection depends on the subtly of the unintended effect.

I) P_1 parental breeding stock with deleterious allele – *Probability*: 0.0000008
Reasoning: Without detection of the unintended characteristic during small-scale screening the transformants are liable to be accepted as parent material for breeding. Currently most commercial varieties of maize are sold as hybrid seed, which will involve crossing the transformant with another maize line before larger-scale trials are conducted.

J) Dominant P_2 allele masks recessive P_1 allele – *Probability*: 0.75
Reasoning: If the deleterious allele is recessive in the transformed P_1 line and the P_2 parent possesses a dominant allele then the unintended effects will be masked and expression will not take place.

K) Production of F_1 hybrid with recessive deleterious allele – *Probability*: 0.0000006
Reasoning: F_1 hybrid seed production will result in the deleterious effect not being expressed in the offspring.

L) No detection of unintended effect during extensive trials due to 'silent' gene – *Probability*: 0.1
Reasoning: Due to the virtually complete homozygosity of the parental lines, F1 hybrids will have a very high level of heterozygosity. As a result any recessive alleles will be masked by dominant alleles with the result that unintended effects are not expressed in the hybrid.

M) Widespread commercialisation of crop – *Probability*: 0.00000006
Reasoning: With the absence of expression of the recessive alleles (and hence absence of the unintended effect) widespread commercialisation will be free to commence. This may result in large areas planted with the crop.

N) Production of volunteers with homozygous recessive deleterious alleles – *Probability*:
Reasoning: Maize volunteers are known occur in fields and by roadsides in the year following cultivation (e.g. Eastham & Sweet, 2002), although they are generally considered to be a minor weed problem due to the low germination rate and low viability. If the induced mutation increases the fitness of the volunteer plant (for example by enhancing its cold tolerance of reducing its susceptibility to pests) then more volunteers are likely to survive.

O) Manifestation of unintended effect – *Probability*:
Reasoning: Volunteers maize plants that are homozygous for the recessive allele will express the deleterious unintended effect in accordance with Mendelian ratios. This will result in expression of the unintended effect in the volunteer plants. It is also possible that the subtle unintended effects of the mutation could be enhanced by external stimuli, such as environmental conditions (global warming, for example) or attack by pests or diseases. It is also possible that crossing between different maize lines may enhance the expression of unintended genes. If the unintended effect confers a competitive advantage on the maize volunteers (e.g. enhanced frost resistance or insect resistance) they may be more difficult to control. If the mutation causes production of a toxin then there could be detrimental effects on insects that feed on the plant.

5. Assessing Likelihoods, Consequences and Risks

As stated, due to the hypothetical nature of the scenario outlined above it is difficult to ascribe substantiated likelihood values to the various events that are necessary to realise the hazard. The values that have been assigned may not be precise but nevertheless the exercise of applying the framework to this type of scenario is useful in that it clearly illustrates the steps that are needed to reach the top level event. Simply setting out these steps in a logical way provides a good basic indication of how probable an event is and provide the necessary incentive to obtain data in the areas where it is lacking.

6. Assess Uncertainties

In the case of this particular scenario many more investigations need to be conducted to determine the likelihood of insertional mutagenesis taking place.

7. Compare Risk Against Criteria

The release should be categorised as either acceptable or unacceptable when compared to the criteria set under Step 2. At present appropriate criteria needs to be developed for GM crops.

8. Identification and Evaluation of Mitigation and Monitoring Methods

Having carried out an assessment of risk for the identified hazard it is appropriate to consider the type and extent of monitoring and mitigation measures that could be put in place to negate or reduce that risk. Table 10 shows potential monitoring and mitigation measures for the various events resulting from insertional mutagenesis and the production of volunteer plants which exhibit unintended effects.

Table 10 – Potential prevention monitoring and mitigation methods

Event	**Possible monitoring methods**	**Possible mitigation measures**
Deleterious mutation due to transgene insertion	Profiling techniques such as genomics, proteomics and metabolomics could be used to help predict when to expect an expression of the recessive gene. Microarrays may help in early detection. Historic data from analogous cases may also help in prediction.	Detection would allow plants with unintended effects to be discarded at the early stages of the assessment process; Methods to enhance the targeting of T_DNA are currently being developed.
No detection of subtle unintended effect during initial screening	More rigorous screening methods may help to detect cultivars with subtle unintended characteristics. Such screening methods may include challenging the plants with different environmental conditions and pest stimuli.	Plants that do not perform as expected would be removed from the breeding programme.

No detection of subtle unintended effect during extensive trials due to silent gene	Silent genes are, by definition, not easy to detect. Generic surveillance monitoring of the environment or of the final products may help.	Product recall and a cessation in sales may be required if unintended effects are severe. A programme could be implemented to control maize volunteers.

MON 810 maize has been cultivated in the EU for a number of years and as yet none of the detrimental effects discussed above are known to have materialised. However, it may be that the plants have not yet encountered the appropriate stimuli, or there has not been sufficient length of time for any effects to manifest themselves. The production of maize volunteers exhibiting an unintended effect due to insertional mutagenesis is one of a number of hazards that can be hypothesized for GM organisms in general. Such effects are not merely theoretical – such unexpected effects have been documented for various GM lines, including glyphosate resistant soybean exhibiting splitting stems and yield reduction at high temperature; glucose oxidase expressing wheat exhibiting phytotoxicity; and glycinin expressing potato with increased glycoalkaloid content (for a full review see Cellini et al 2004). To complete a full and comprehensive risk assessment it is necessary to assess the likelihood and consequences of each hazards and determine the overall level of risk. Based on this overall risk the decision as to whether the risk is tolerable would be made.

REFERENCES

Askew M F (1993). Volunteer potatoes from tubers and true potato seed. Aspects of Applied Biology 35, Volunteer crops as weeds, Cambridge, UK, 9-15.

Baker, H. G. (1965). Characteristics and modes of origin of weeds. In: The genetics of colonizing species, pp. 147-168. Baker, H. G., and Stebbins, G. L. (eds.), Academic Press, New York.

Becker, R., Augustin, J., Behrendt, U., Gransee, A., Hedtke, C., Luettschwanger, D., Mueller, M. and Ulrich, A. (2000). Oekologische Begleitforschung zum Anbau von transgenen Kartoffeln mit Veraenderung im Grundstoffwechsel. Landesumweltamt Brandenburg, Muencheberg.

Campbell LH, Avery MI, Donald P, Evans AD, Green RE and Wilson JD (1997). A review of the indirect effects of pesticides on birds. JNCC Report no 227.

Cellini, F., Chesson, I., Colquhoun, A., Constable, A., Davies, H.V., Engel, K.H., Gatehouse, A.M.R., Karenlampi, S., Kok, E.J., Leguay, J.-J., Lehesranta, S., Noteborn, H.P.J.M., Pedersen, J. and Smith, M. (2004). Food and Chemical Toxicology, 42, 1089-1125.

David Claessen, Christopher A.Gilligan, Frank van den Bosch. Which traits promote persistence of feral GM crops? Part 2: implications of metapopulation structure. *OIKOS* 2005;110:30-42.

Crawley MJ, Brown SL. (1995). Seed limitation and the Dynamics of feral oilseed rape on the M25 motorway. *Proceedings of the Royal Society of London Series B* 259:49-54.

De Buck, S., De Wilde, C., van Montagu, M., Depicker, A. (2000). Molecular Breeding 6, 459-468.Eastham, K. & Sweet, J. (2002) Genetically modified organisms (GMOs): the significance of gene flow through pollen transfer. Expert's Corner Series, European Environment Agency, Copenhagen.

Gasson, M.J. (2003). The Safety Evaluation of Genetically Modified Foods, *Institute of Food Research, Norwich, UK.* In: Food Safety: Contaminants and Toxins. D'Mello, J.P.F. (Ed.). Scottish Agricultural College, Edinburgh, UK. CABI 2003.

Hilbeck A, Moar WJ, Pustzai-Carey M, Filippini A, Bigler F. (1998). Toxicity of *Bacillus thuringiensis* Cry1Ab toxin to the predator *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Environmental Entomology* 27:1255-63.

Johnston, K.A. (1989). Biochemical mechanisms of resistance of potato cultivars to slug attacks. British Crop Protection Council Monograph 41: 281-288.

Kononov, M.E., Bassuner, B., Gelvin, S.B. (1997). Integration of T-DNA binary vector 'backbone' sequences into tobacco genome: evidence for multiple complex patterns of integration. Plant Journal, 11. 945-957.

Kumar, S. and Fladung, M. (2000). Transgene repeats in aspen: molecular characterisation suggests simultaneous integration of independent T-DNAs into receptive hotspots in the host genome. Molecular and General Genetics, 264, 20-28.

Krizkova, L. and Hrouda, M. (1998). Direct repeats of T-DNA integrated into tobacco chromosome: characterisation of junction regions. Plant Journal 16, 673-680.

Lips, J. (1998). Pleiotrope Effekte und genetische Stabilitaet transgener Pflanzen. In: Schuette G, Heidenreich B, Beusmann V (1998). Nutzung der Gentechnik im Agrarsektor der USA - Die Diskussion von Versuchsergebnissen und Szenarien zur Biosicherheit. UBA-Texte 47/98: 121-156.

SAC (1999). Technical Note - Potato volunteers and their management. Scottish Agricultural College. ISSN 0142 7695; ISBN 1 85482 651 4).
(http://www1.sac.ac.uk/info/External/About/publicns/TN/tn480.pdf)

Rich T. *Crucifers of Great Britain and Ireland Botanical Society of the British Isles*.

Appendix 7

Outline specifications for closing research gaps

IX.ii

7.1 Introduction

This Appendix outlines areas of future work that could help to close the research gaps identified within the study. For each suggested work package (WP) we have outlined:

- **Rationale:** why the work is needed
- **Objectives**
- **Scope:** limits and boundaries
- **Tasks:** what needs to be done to achieve the objectives
- **Outputs:** envisaged deliverables and benefits
- **Indicative timescale:** elapsed time
- **Indicative cost:**
- **Dependence on or interaction with other WPs**

The Work Packages have been grouped under three main themes:

- Scientific Basis for risk assessment (Section A7.2)
- Tools and Techniques for risk assessment (Section A7.3)
- Risk assessment as tool for Deliberation and Decision-making (Section A7.4)

This categorisation is purely for ease of reference. There will be interactions between the themes and WPs.

The costs given are intended only to give a broad idea of the likely range. The scope and depth of each WP can be adapted to reflect the EC's priorities, and any overlaps with other research in the environmental risk field. The costs indicated are costs to the EC (or other research sponsor). Costs to regulators or industry in implementing any resulting guidance have not been estimated, as the implications for them cannot be foreseen at this stage, and costs will depend on many factors - the specific national context, what releases come forward, how regulatory costs are charged and so forth.

7.2. Scientific Basis for Risk Assessment

WP1: Obtaining Baseline Data
Rationale: There is a need for good baseline data if monitoring is to be able to detect changes
Objectives: To advise on what baseline data should be collected
Scope: The main scientific question is what baseline data should be gathered, i.e. what are the best indicators of long term/ cumulative changes? As well as this scientific question, there are institutional and other practical aspects of implementation to be considered. For example, who should carry out the baselining, and would it relate to ecological baselining for other purposes? These issues could also be considered, at a high level, to provide guidance
Tasks: This WP would build on existing national research regarding indicators for short term effects, extending it to the consideration of long term and cumulative effects, and to the EU as a whole.
Outputs: Research report, giving guidance on what baseline data should be collected, and outlining how institutional and practical issues and how they could be addressed.
Indicative timescale: I year
Indicative cost: 50000 Euro
Dependence on / interaction with other WPs: Outputs from WP3 would inform the choice of indicators. There are also some common areas with WP6.

WP2: Ranking the Risks of Generic Crop-Trait combinations
Rationale: It could be more cost-effective, for society as a whole, if some risk assessment work were to be done at a higher level than the release-specific ERAs. If certain crop-trait combinations (or other types of scenario) could be ranked in broad order of inherent risk. This would help both regulator and industry to ensure proportionate levels of depth and detail in risk assessments. It might also lead regulators to issue guidance indicating any broad classes of combinations that would be unlikely to be authorised, preventing wasted effort by all parties.
Objectives: To establish whether certain crop-trait combinations are inherently riskier than others
Scope: the study should consider the range of releases for which applications can foreseeably be expected.
Tasks: • Define the scenarios to be considered – a set of crop-trait combinations spanning the likely range of releases • Apply the risk assessment framework, at a high level, to each scenario • Rank the scenarios in order of risk, and identify what characteristics contribute most to this ranking • Develop guidance – how can knowledge of what scenarios or characteristics are most risky be used to help ensure a proportionate and effective regulatory process?
Outputs: Research report describing the risk assessments of each scenario, and interpreting the findings to provide guidance to regulators
Indicative timescale: 6 months
Indicative cost: 40000 Euro
Dependence on / interaction with other WPs: This study could inform the choice of priority crop/ trait combinations to be studied under WP3. Or, conversely, outputs from WP3 could inform this study, by helping to identify combinations for which the risks of introgression and spread were greatest.

WP3: Introgression into and Ecology of Wild Relatives
Rationale: Currently, the mechanisms, and hence likelihood, of introgression into wild relatives, are not understood in sufficient detail for robust risk assessment. Neither is there, in general, a good enough understanding of the ecology of wild relatives, and hence of how undesirable traits might spread in the environment.
Objectives: To improve the understanding of mechanisms of introgression or ecology of wild relatives in sufficient detail for risk assessment.
Scope: In order to keep the study within manageable bounds, it would probably be necessary to identify some priority crop – trait - wild relative combinations for which the mechanisms of introgression and spread were to be studied.
Tasks: • Identify priority crop – trait - wild relatives combinations to be studied • Desk study/ literature review to establish the state of knowledge about mechanisms of introgression and spread. • Explore potential for, and apply where appropriate, novel approaches, For example, what is the potential to learn from natural analogues, using historic or palaeontological evidence about the spread and impacts of new or introduced species? • Laboratory and field experiments to establish effects of traits on fitness, factors affecting establishment in various niches, etc • Analysis and synthesis of information from desk and experimental studies – possibly incorporation into a model.
Outputs: Research report updating the understanding of mechanisms of introgression or ecology of wild relatives.
Indicative timescale: 3 – 4 years
Indicative cost: 1 Million Euro
Dependence on / interaction with other WPs: WP 2 could inform the initial choice of priority crop/ trait combinations. Conversely, or at a later point, outputs from WP3 could inform WP2, by providing data on more specific crop-trait combinations with a high risk of introgression and spread. The ecological part of the work could be related to other ecological studies, outside the GM field.

7.3 Tools and Techniques for Risk Assessment

WP4: Improved hazard identification for long term and cumulative effects
Rationale: In the workshop and case studies, we found it difficult to identify hazards that were different in kind from those that can occur in the short term. In most cases, the hazards identified were the same as the short term ones, although the degree of risk associated with the hazard could be greater. Typically, given a longer time or more widespread planting of a crop, the likelihood and extent of undesirable effects may increase. Nevertheless, some genuinely different hazards were identified. These tend to arise from rather subtle and complex combinations of factors, which would only be manifested in the long term or with widespread releases and plantings. The process of identifying long term and cumulative risks could be made more efficient and effective if standard hazard-identification techniques (brainstorming, failure analyses, HHA etc) could be enhanced to focus more quickly on such genuinely long term and cumulative effects.
Objectives: To develop hazard-identification techniques that focus on genuinely long term and cumulative effects.
Scope:
Tasks: • Identify existing methods that could be adapted (brainstorming, failure analyses, HHA etc) • Develop novel, improved methods • Test, refine and demonstrate the improved methods in a case study comparison with traditional methods
Outputs: Research report describing the study, the improved methods and guidance on their application.
Indicative timescale: 6 months
Indicative cost: 50000 Euro
Dependence on / interaction with other WPs: WP3 could provide input regarding specific potential mechanisms that hazard identification would need to consider.

WP5: Tolerability Criteria

Rationale: The absence of explicit criteria in the Directive for judging whether a risk is tolerable, and the apparent variations in practice between Competent Authorities, point to a need to develop some more explicit guidance on criteria that will encourage well-founded, consistent and fair decisions. As well as defining criteria against which to judge the outputs of assessment, decisions about tolerability affect the level of assessment required. The more stringent the criteria, the more effort will need to go into demonstrating how they will be satisfied.

Objectives: To develop justifiable criteria for tolerable risk, and guidance on their application.

Scope: The main questions and areas for further development are as follows:

- What benchmarks or standards can be adopted? For example tolerable levels of risk could be defined by reference to a comparison with the risks from the unmodified crop in the current environment, or against the risks from the current crop allowing for normal bounds of variability and mutation in that crop and its environment.
- How is the precautionary principle to be interpreted? Is there a need for a formal requirement to minimise risks, such as the As Low As Reasonably Practicable (ALARP) principle? Should there be a 'bias in favour of safety'?
- Should the assessment take account of benefits as well as risks?
- Given the large and to some extent irreducible uncertainty in assessing long term and cumulative effects, what level of confidence in the assessment is required in order to make a decision? What are the appropriate rules for decision-making under uncertainty?
- How should tolerability criteria take account of ethical considerations such as environmental and social justice, the bounds of discourse, value judgements about the weight given to various benefits and risks, and issues of responsibility and choice arising from the potential for decisions taken now to affect future generations?

Tasks:

- Review the international literature on risk tolerability
- Consult with stakeholders in the GM crops domain and more widely
- Develop a range of potential criteria
- Illustrate how the criteria could be applied in practice, for example by means of case studies

Outputs: Research report describing the justification for the proposed criteria, providing guidance on their use and comments on advantages and disadvantages

Indicative timescale: 4 to 6 months

Indicative cost: 30000 to 50000 Euro

Dependence on / interaction with other WPs: Social and ethical aspects of risk will need to be considered in WP5. Dependent on timing, WP5 and WP7 could inform each other.

WP6: Tracking, Combining and Monitoring Cumulative Risks
Rationale: In considering cumulative risks, the EC will need mechanisms to track and combine the risk assessments from multiple, and in some cases apparently unrelated, releases.
Objectives: To explore the scientific and institutional issues involved in tracking multiple release assessments and consents and to develop guidance.
Scope: The study would consider both scientific aspects (how to identify any potential for cumulative risk) and institutional (processes and procedures, IT issues)
Tasks: • Desk studies and interviews to identify current procedures for tracking applications and releases • Analysis of findings • Development (in consultation with EC) of guidance
Outputs: Review of issues and guidance on implementing a programme for tracking and combining risks
Indicative timescale: 3 months
Indicative cost: 30000 to 50000 Euros (dependent on state of evolution and complexity of current processes)
Dependence on / interaction with other WPs:

IX.iii
7.4. Risk assessment as a tool for Deliberation and Decision-making

WP7: Stakeholder Engagement in Risk Assessment
Rationale: In other controversial debates about risk and the environment, there has been a growing realisation of the importance of considering social and ethical aspects. Stakeholder participation in framing the question and defining methods is being given serious consideration and has occurred in some cases, in addition to the more traditional model, in which stakeholders review and challenge assessment results and assertions. Risk assessment should be developed and used as a structured framework for discourse and decision-making under irreducible uncertainty, rather than as a technical and scientific exercise alone. Deliberative processes have been used in other domains, and this experience could potentially be adapted and applied to the GM domain.
Objectives: Explore how deliberative processes have been used in other risk domains, and how this experience could be applied to the GM domain.
Scope:
Tasks: • Collate literature and gather experience on deliberative processes • Identify/ adapt approaches that could be appropriate in the GM crops domain • (Optional) Conduct a trial application, linked to one or more current applications. The practicalities of conducting such a trial would require further discussion with EC • Develop guidance
Outputs: Research report, reviewing how deliberative processes have been used in other risk domains, and guidance on how this experience could be applied to the GM domain.
Indicative timescale: 2 months (significantly longer a if trial application were to be carried out)
Indicative cost: 10000 Euro (more if a trial application were to be carried out)
Dependence on / interaction with other WPs: Social and ethical aspects of risk will need to be considered in WP5. Dependent on timing, WP5 and WP7 could inform each other.

Appendix 8

Monitoring and mitigation plans

IX.iii.1.1.1.1.1.1
IX.iii.1.1.1.1.1.2

8.1. C/NL/98/11 Roundup Ready oilseed rape, event GT73 (Monsanto – updated version July 2003).

Trait: Tolerance to the herbicide glyphosate ('Roundup Ready').
Scope: The notification covers the importation and storage of GT73 oilseed rape and its use as feed as well as in the processing for feed, and its industrial uses as or in products.
Current status: European Commission decision 31 August 2005[12]; consent not yet issued.
Note: Originally submitted in 1998 under Directive 90/220/EC, which did not require elucidation of monitoring plans, these have been added in subsequent submissions to the lead competent authority.

IX.iii.1.2 Proposals for case-specific monitoring

The notifier claims that the results of the environmental risk assessment show effectively zero overall risk arising from the placing on the market of GT73, therefore case-specific monitoring is not considered applicable to the placing on the market of this product. It should be noted that several Member States commented that the prevention of seed spillage and the consequences of it happening have not been sufficiently taken into account, and more information is needed (see comment on this below).

IX.iii.1.3 Proposals for general surveillance

The notifier will ensure that awareness of the GM crop is made widely available by providing key information, for example:

- The notifier will provide international traders with the necessary information to comply with statutory requirements relating to the placing on the market of the crop;
- Product briefings to selected networks;
- Technical literature (e.g. product-specific information, company contact details).

Further information and relevant legislation will be available from a number of sources, including industry and government websites, official registers and government publications.

Surveillance information will be collected from 1) feedback from selected networks, and 2) reported potential adverse effects and other relevant information received via direct contacts with the notifier. Where unanticipated adverse effects are confirmed they will be investigated to establish if there is a correlation between the observed effect and the GM crop, and if so appropriate remedial action will be carried out.

European Commission document 2005/637/EC[13] addressed concerns regarding potential for spillage of GT73 oilseed rapeseeds by advising: "specific technical guidelines should be added to the decision to place GT73 oilseed rape on the market

[12] Document number 2005/635/EC. OJ L228 pp 11-13 03/09/2005.
[13] OJ L228 pp 19-20 3/9/2005.

to prevent any damage to the environment in case of accidental spillage of the product."

Summary

- Short-term risks: consistent with other authorisations for import and processing.
- Long-term cumulative: key risks are associated with unmitigated spillage of GT73 rapeseed along roadsides, railways, waterways, leading to establishment of localised feral populations of GT73 and possibly gene transfer into sexually compatible species. Risks of reduced biodiversity in areas where this persists, and possible economic risks to commercial oilseed rape crops.
- Consent holder will be required to monitor spillage, and to encourage reporting of unexpected adverse effects as part of the general surveillance plan. How effectively this will work in practice away from the port environments is yet to be tested. Monitoring will only operate for the duration of the release.

IX.iii.1.3.1 Detailed assessment

Human toxicity and allergenicity

GT73 oilseed rape is modified for tolerance to glyphosate through the introduction of two genes, which encode CP4 EPSPS and GOX proteins. This was the first assessment of GOX for European market introduction. There are no antibiotic sequences in GT73 OSR. In the scientific assessment the notifier states that CP4 EPSPS protein has been demonstrated to be functionally similar to the EPSPS proteins typically present in food and feed derived from plant and microbial sources; there are reported to be no indications of toxicity from studies undertaken with mice and these studies are proposed to support the safety of CP4 EPSPS protein. The GOX enzyme has been extensively characterised and degrades glyphosate via a well-known degradation pathway. The notifier states that a number of studies have been undertaken that establish that the GOX protein and its enzymatic activity rapidly degrade in gastric and intestinal models, indicating that it will also degrade readily in the mammalian digestive tract. The notifier presents results of compositional analyses of GT73 OSR, in terms of protein and oil content, fatty acid profile, glucosinolate content and profile and proximate analysis and concluded that GT73 is substantially equivalent to the parental conventional variety. Both EPSPS and GOX proteins are heat and pH labile and are rapidly degraded on exposure to simulated digestive tract fluids, and are considered unlikely to have allergenic potential. EPSPS and GOX show no significant homology on the DNA level with known toxins and allergens. Acute toxicity studies did not point to any effects caused by expression of the proteins. It was concluded there are no reasons to assume that the CP4 EPSPS and GOX protein will have a toxic or allergenic effect upon incidental consumption or as use of feed of GT73, nor that the degradation products of the catalytic activity of the proteins will have a toxic effect.

Safety assessments have been in the form of standard short-term assessments, which are effective in uncovering acute toxicity but less valuable in determining chronic toxicity. The same theory applies in the case of allergenicity, where certain individuals (e.g. workers in contact with grain dust or animals being fed the meal) are more sensitive to certain compounds. Such predispositions may only become apparent over time, and sensitisation may increase with increased exposure to the

compound. It would seem prudent, therefore, to have a system of monitoring in place to confirm the results of the risk assessment in the longer term. Questions on this could be considered for inclusion as part of general surveillance monitoring.

In their assessment of GT73, EFSA noted recent reports that the GOX protein shares a 6-amino acid sequence present in a 9-amino acid epitope from the major shrimp allergen tropomyosin, which binds IgE in sera of shrimp allergic patients (Kleter and Peijnenburg, 2002)[14]; another publication describes the 9-mer peptide as potentially cross-sensitising (Ivanciuc et al., 2002)[3]. Given the presence of similar sequences in various other allergens EFSA concluded that since the presence of the GOX protein in GT73 derived oil meant for human consumption cannot be excluded, the relevance of such a finding should be assessed according to existing guidelines.

There are no potential risks from allergy to oilseed rape pollen as the crop will not deliberately be cultivated.

IX.iii.1.3.1.1.1 Potential to compromise human therapy

As there will be no direct consumption of oilseed rape meal by humans no direct or indirect effects were noted that might compromise human therapy. There are no antibiotic sequences in GT73 OSR.

IX.iii.2 Potential beneficial effect to humans

The crop will not be cultivated in Europe therefore any potential benefits, e.g. of reduced herbicide application will not be realised.

Animal toxicity and allergenicity

There will be no direct exposure of animals to the modified oilseed rape plants as the authorisation is not for cultivation. There are no antibiotic sequences in GT73 OSR. Animals will be exposed to the denatured EPSPS and GOX proteins in animal feed at low concentration. Feeding studies were carried out on rats, rainbow trout, quail, chickens for fattening and lamb (extrapolations were made to pigs and cows) and the scientific assessment found no evidence of acute toxicity from the CP4 EPSPS and GOX proteins. Note the comments above regarding relatively short-term feeding studies that do not identify longer-term/cumulative chronic effects.

Compromised animal therapy

The scientific assessment did not identify any risks. Some concerns have been raised over the possible effects on human and animal health of the possible presence of residues of glyphosate and its metabolites. The assessment of these risks is covered within the framework of a different EU directive 91/414/EEC concerning placing of plant protection products on the market.

Potential beneficial effect to animals

None are identified. An indirect effect of the availability of GT73 may be noted as a result of cheaper supply of rapemeal for inclusion in animal feed, this may lead to improved diet of otherwise poorly fed animals which may have concomitant health and welfare benefits.

[14] The EFSA Journal (2004) 29, 1-19.

IX.iii.3

Disease to plants:

As the crop will not be authorised for cultivation in Europe there should be no impacts on plant disease levels. Even if localised populations of plants containing the GT73 transgene are allowed to establish and persist over a long period as a result of spillage, there is not reported to be any alteration in the susceptibility of GT73 to pests or diseases.

Target organisms (incl. predators and parasitoids)

The crop will not be authorised for cultivation in Europe and it is not modified for insect resistance, therefore there are no target organisms. If localised populations of plants containing the GT73 transgene are allowed to establish and persist over a long period as a result of spillage, we are not aware of any reports suggesting that herbicide tolerance leads to altered palatability for herbivores, which may result in direct/indirect effects.

Effects on non-target organisms

The crop will not be authorised for cultivation in Europe and it is not modified for insect resistance, therefore there are no non-target organisms. The safety assessment states that analyses of seeds and derived factions (oil and meal) show that the chemical composition is not changed relative to the parental conventional variety. Direct or indirect effects on other organisms in the environment as a result of either feeding on spilled seed, or herbivory of plant populations that may establish following spillage has not been considered in the short-or long-term.

Effects of gene-transfer (incl. to soil organisms) and Potential for increased weediness

The crop will not be authorised for cultivation in Europe so potential for gene flow leading to hybridisation and gene transfer is not a major consideration in the ERA. However, should GT73 be grown illegally or inadvertently (e.g. due to spillage or adventitious presence), there is potential for gene transfer from *Brassica napus to B.rapa, B. juncea* and *B.campestris*, and to other crops of *B.napus*. Field trials have demonstrated that GT73 and its progeny are unlikely to be more persistent in the natural environment than the parental conventional variety. Possession of the herbicide resistant transgene will only result in a selective advantage for sexually compatible species in situations where glyphosate is in use; this is most likely to be semi-managed environments e.g. roadsides, railway lines, waterways, and in managed agricultural environments. In these situations, if alternative treatments were not employed to remove or control the spilled GT73 OSR seed or relatives containing the herbicide resistance transgene, it is possible that this may have direct impacts on the biodiversity of local plant communities and possible direct economic impacts on nearby conventional oilseed rape crops. Localised events such as these, if unnoticed in the short term could have more serious indirect effects on local natural communities if allowed to continue over the long-term and on a cumulative basis. The proposed monitoring programme recognises the potential for spillage, as does the European Commission. Currently much imported rapeseed arrives at European ports and is crushed in the vicinity of the port then transported as the oil. However, with the expansion of the EU member states it is not inconceivable that a number of

different commodity transport scenarios will arise, increasing the possibility for spillage and the longer-term consequences that this may bring.

Altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or new reservoirs or vectors
The crop will not be authorised for cultivation in Europe and it is not modified for pathogen resistance. There is no evidence that GT73 OSR has altered susceptibility to pests or diseases.

Horizontal gene transfer
As the crop is not authorised for cultivation, the conditions for horizontal gene flow should not arise. Both CP4S EPSPS and GOX proteins are derived from soil dwelling bacteria; while horizontal gene flow is considered theoretically possible, the safety assessment cites a number of studies have concluded that it is negligible (e.g. Smalla *et al* 2000, Simonet 2000). The safety assessment also states that there are reports of long term persistence of transgenes in soil, but no transfer to soil bacteria has been detected (e.g. Gebhard and Smalla 1999). Even if localised populations of plants containing the GT73 transgene are allowed to establish and persist over a long period, there is little evidence to suggest that this should specifically be monitored.

Effects on biogeochemistry, particularly carbon and nitrogen recycling through changes in soil decomposition
As the crop is not authorised for cultivation there should be no effects on biogeochemistry, assuming that monitoring and remediation of spillages of GT73 is effective. Even if localised populations of plants containing the GT73 transgene are allowed to establish and persist over a long period, both CP4 EPSPS and GOX proteins are derived from soil dwelling bacteria and do not demonstrate any known toxicity.

8.2. C/FR/96/05/10 - Bt11 maize (field or sweet maize) (Syngenta Seeds SAS)

Trait: Resistance to corn borers *Ostrinia nubilalis* and *Sesamia nonagrioides* using the Cry1Ab gene.
Scope: For use as any other maize, including cultivation in the EU. Bt11 also possesses herbicide tolerance but the scope of the notification has been limited to use without the application of glufosinate ammonium herbicides.
Current status: pending authorisation.

Case-specific monitoring
Based on the environmental risk assessment the notifier concluded that no case-specific monitoring was necessary; however, in order to delay insect resistance to Bt11, an Insect Resistance Management plan would accompany Bt11. The monitoring plan was divided into four parts:

- Maintenance of refuges (20% in the case of holdings with over 5 ha of maize);
- Monitoring of Bt resistance;
- Action plan in the event of resistance being detected;

- Training for producers.

Some Member States have commented that case-specific monitoring should also be undertaken to consider the impact of insect resistance on non-target insects

General surveillance

The proposed general surveillance plan includes an assessment of the impact of Bt11 on non-target arthropods and the impact of tolerance to the herbicide, but its implementation in practice has still to be finalized.

The original proposal for general surveillance was revised in May 2004. The revised proposal includes more detailed plans for surveillance 'modules' operating within existing surveillance 'nodes' in agriculture, the environment (agro-environment and wider environment) and the supply chain for living Bt11 maize grains. A detailed farmer questionnaire has been developed to collect relevant information from farmers relating to environmental and agronomic aspects of the GM crop (questions specifically cover agricultural practices, general observations of the crop and corn borer infestation). Farmer data will be collected from a structured subset of farmers within a representative geographical area and participant numbers will be sufficiently large to allow for meaningful data analysis. The exact network will be defined in advance of commercialisation of Bt11 maize in specific regions, Syngenta will coordinate and report the monitoring; all data will be compiled in a 'general surveillance database'. It still remains to be seen exactly how this general surveillance monitoring will work in practice and how the various networks involved will be compelled to participate.

Summary

- Short-term risks: currently proposals will only identify development of resistance in target organisms. Will not identify any indirect effects on non-target populations. Farmer questionnaire will provide information regarding on-farm effects.
- Long-term cumulative risks: key risks are likely to be associated with target and non-target organisms; on-farm monitoring may extend for 1 year beyond the life of the consent in the form of farmer questionnaire.

IX.iii.3.1Detailed assessment

Human toxicity and allergenicity

The scientific assessment of Bt11 maize included examination of the inserted DNA, the nature of the donor organisms and the characteristics and safety of the newly expressed proteins produced by the transgenic plants. In addition, a comparative analysis of agronomic traits and composition was undertaken and the safety of the product as a whole was evaluated with respect to toxicology and allergenicity. Following this assessment the notifier concluded that expression of the Cry1Ab protein would not lead to increased toxicity or pathogenicity of the recipient organism and consequently case-specific monitoring was not warranted.

It should be borne in mind that the safety assessments of Bt11 have been in the form of standard short-term assessments, which are effective in uncovering acute toxicity but less valuable in determining chronic toxicity. The same theory applies in the case

of allergenicity, where certain individuals (be they workers in contact with grain dust, or cows being fed the product) are more sensitive to certain compounds. Such predispositions may only become apparent over time, and sensitisation may increase with increased exposure to the compound. It would seem prudent, therefore, to have a system of monitoring in place to confirm the results of the risk assessment in the longer term. Questions on this could be considered for inclusion in the stewardship and farmer questionnaire schemes that Syngenta has developed as part of general surveillance monitoring.

Potential to compromise human therapy
There is no evidence that intact gene transfer to micro-organisms in the human gastro-intestinal tract can occur. If gene transfer were to occur the ERA considers that the Cry1Ab and PAT proteins would not be expressed because of the lack of bacterial promoters in Bt11. Furthermore, if expression of these proteins were to occur they would not confer any disadvantage and would not compromise therapy to human disease.

Potential beneficial effect to humans
Due to a reduction in secondary infections of maize plants by fungal pathogens due to the use of Bt11, the ERA considers there will be an increase in grain quality and a reduction in the levels of mycotoxins.

Animal toxicity and allergenicity
A large number of studies have been performed comparing the composition of Bt11 maize and conventional maize. Several feeding studies have also been conducted using various parts of the Bt11 plant, including studies employing poultry and milking cows, and whilst these have shown no detrimental effects it should be noted that the duration of most of these was no more than 14 days. Additionally, although Bt11 has been used for animal feed in the USA for several years, very few scientifically rigorous long-term feeding studies have been conducted, thus there is a scarcity of data on long-term animal exposure to Cry proteins (including any potential effects on gut micro-organisms) and on the nutritional or anti-nutritional properties of Bt11. In addition, despite numerous backcrosses, there is always the possibility that either the insect resistance trait or the herbicide tolerant trait could become unstable in the plant genome, leading to the production of novel proteins with unforeseen effects.

Compromised animal therapy
In the case of cattle fed with Bt11 there is evidence for the transfer of small fragments of predominantly non-recombinant DNA (<200 bp) in some tissues, indicating that transfer can occur from plant material to animal tissue. However the ERA concluded that there is no reason to expect such transfer to cause adverse effects, and no case-specific monitoring was proposed for this occurrence.

Potential beneficial effect to animals
Maize kernels from Bt-expressing crops have been found to have a lower level of *Fusarium* infection compared to kernels from conventional crops, and this is accompanied by a reduction in the content of the mycotoxin fumonisin, which can cause a variety of toxic effects in animals.

Disease to plants
Pathogenicity - detailed open reading frame analysis of the inserted DNA has found no evidence for proteins other than those for Cry1AB and PAT. Two components used to generate the transformation event are derived from plant pathogenic sequences, tNos derived from *Agrobacterium tumefaciens* and p35s derived from Cauliflower Mosaic virus, and whilst there has been a debate on the safety of using sequences derived from plant pathogens, the consensus of opinion is that they pose very little risk.

Target organisms (incl. predators and parasitoids)
Based on information from a number of countries currently growing Bt maize, the ERA concluded that a risk to beneficial arthropods and non-target species could not be identified and case-specific monitoring would not reveal any unanticipated effects. However, in order to delay insect resistance to Bt11, an Insect Resistance Management Plan (IRM) has been proposed by the notifier. This management plan will be based on non-Bt maize insect refugia, high dose expression of the Cry protein, and effective monitoring. The plan consists of the following elements:

- Maintenance of refuges (20% in the case of holdings with over 5 ha of maize);
- Monitoring of Bt resistance;
- Action plan in the event of resistance being detected;
- Training for producers.

In its advice of UK Advisory Committee on Releases to the Environment (ACRE) considered the IRM strategy and the monitoring of resistance to be adequate, however the Committee did state that it may be appropriate to consider the impact of the insect resistance trait not only on the primary targets *Ostrinia nubilalis* and *Sesamia nonagrioides*, but also on other potential maize or grass feeding Lepidoptera that might be affected. Such species could include *Agrotis ipsilon* (Noctuidae), *Helicoverpa* spp. (Noctuidae), *Mythimna unipunctata* (Noctuidae) and *Peridroma saucia*, all of which are minor pests of maize and thus can be seen as secondary target organisms rather than non-target organisms. In the long term and/or with cumulative exposure of these organisms to Bt11 maize

Effects on non-target organisms (Lepidopteran & non-Lepidopteran)
The ERA notes that butterflies and the majority of moths do not feed directly on maize plants, therefore the only potential route of exposure to the Cry1Ab protein would be by feeding on plants covered by maize pollen. The notifier points to a number of recent scientific studies that support the conclusion that butterfly larvae and maize pollen are unlikely to coincide, either temporally or spatially, and thus butterflies are unlikely to be significantly exposed to Bt pollen. In the case of non-lepidopteran insects the ERA acknowledges that some studies have found detrimental effects of Bt-maize on lacewings and forest gypsy months, but points to a large body of literature and field experimentation that indicates that any exposure which did occur will not have a significant adverse impact. Studies commissioned by Monsanto Company have looked at the toxicity of purified Bt proteins on honey bees (larvae and adults), lacewing larvae, parasitic hymenoptera and ladybirds, and have concluded that the protein is not toxic at concentrations present in the insect-protected maize plants and grain. The ERA points out that field studies and laboratory trials involving

non-target insect populations and soil organisms have been undertaken, but to date none have shown any adverse effects of Bt maize.

Regarding soil binding of the Bt-protein, the ERA notes that there is some discrepancy in the literature as to whether binding to soil particles affects insecticidal activity and whether such binding decreases the rate of bacterial degradation. Ledipopteran larvae are rare in the soil, although they do sometimes occur there. Studies conducted using earthworms, collembola, carabid beetles, the general arthropod soil fauna and soil micro-organisms have not shown toxicity of the Cry1Ab protein. The notifier highlights the fact that there are no indigenous species in the EU that rely exclusively on maize pests for survival, either as symbionts, predators or pathogens, thus it is unlikely that the reduction in numbers of target organisms will affect the population levels of these non-target organisms. Nevertheless, it must be remembered that the majority of observational studies on non-target organisms have been conducted in the USA which has a very different invertebrate fauna to the EU, and those field trials and laboratory studies that have been conducted in Europe have been on a relatively small scale compared to the scale that can be envisaged if Bt11 is commercialised.

For consideration of longer term and/or cumulative effects, it would seem prudent, therefore, to test the assumptions made in the ERA concerning non-target organisms, particularly with respect to impacts on the wider food web. In addition, rather than confining monitoring just to insects, there may be justification to monitor a wider range of non-target organisms, from soil-borne nematodes (which include useful indicator species) at the lower trophic level, to organisms that are nearer the top of the food chain, such as birds. Interestingly, even badgers are known to feed on maize, knocking over plants and feeding on the grain in dry summers when slugs and snails are in short supply.

Effects of gene-transfer (incl. to soil organisms)
Due to the lack of wild or weedy relatives of maize in Europe there is no opportunity for the transfer of the herbicide tolerance trait to wild relatives, however dissemination of pollen to other cultivated maize plants could occur, leading to cross-pollination. Theoretically there is then a degree of risk that the transgene could be passed to maize crops grown for seed, and thus become established in the maize genetic pool. However the majority of seed-producing companies are known to screen their seed for GM elements to detect and prevent adventitious GM presence. In addition, co-existence arrangements should minimise the risk of this occurring. With respect to the risk of gene transfer to soil micro-organisms, the ERA states that there is no reported evidence to suggest that intact gene transfer occurs from plants to micro-organisms under natural field conditions, however see the comment above with respect to monitoring non-target organisms.

Potential for increased weediness
Under current agricultural practices, maize volunteers are not considered to be an agronomic problem and where they do occur they are easily controlled by herbicide application or mechanical means. Maize is winter hardy only in parts of Southern Europe. The ERA acknowledges that expression of insect resistance may give a selective advantage to maize growing outside the managed agricultural environment,

but states that maize is incapable of surviving without human intervention. Similarly, expression of the pat gene may result in a selective advantage for maize plants sprayed with glufosinate ammonium herbicides, but again the risk of maize surviving outside the agricultural environment is remote. The ERA concludes, therefore, that the introduction of Bt11 will not result in increased persistence of maize volunteers and therefore has not proposed a case-specific monitoring plan for these factors.

Altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or new reservoirs or vectors

The ERA does not envisage that the use of Bt11 maize will result in altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or providing new reservoirs or vectors. The ERA did however conclude that Bt11 may lead to reduced *Fusarium* infection in the maize due to a reduction in corn borer damage which can lead to secondary fungal infections. No case-specific monitoring has been proposed for altered susceptibility to pathogens, or how, in the longer-term or on a cumulative basis, this may affect the incidence of fungal diseases on conventional maize. The farmer questionnaire developed for use in the general surveillance monitoring programme asks farmers to comment on disease incidence on the modified crop, in comparison to conventional maize cultivation.

Horizontal gene transfer

In addressing the possibility of horizontal gene transfer the ERA points out that no bacterial antibiotic genes have been used in the production of Bt11 maize. In addition, both the Cry1Ab protein and the PAT protein were derived from soil micro-organisms, thus the proteins are pre-existing and ubiquitous in soil. Transfer of genetic material between plants and soil micro-organisms is theoretically possible, but the ERA contends that if the transfer of the Cry1Ab gene or *pat* gene were to occur and resulted in successful gene expression it would not compromise prophylactic or therapeutic medical, veterinary, or plant protection treatments. No case-specific monitoring is therefore proposed for horizontal gene transfer, it is unlikely that the general surveillance monitoring plan would identify such effects if they occurred.

Development of insect resistance to Bt maize containing Cry1Ab

To date one species of insect, the Diamondback moth (*Plutella xylostella* (L.)), has been documented as showing resistance to Bt in the field, although over 10 species have been shown capable of evolving resistance, although not under field conditions. In the case of European Corn Borer, monitoring of Bt maize has been carried out in the USA since 1996, when it was first commercialised, and in Spain since 2000, and as yet there have been no reports of a decrease in susceptibility of ECB to Cry1Ab. Studies suggest that ECB larvae demonstrate incomplete dominance inheritance to preparations containing Bt proteins, and it has therefore been predicted that any ECB resistance genes for Bt11 maize would be at least partially recessive, making resistance more unlikely. Furthermore, studies of Bt-tolerant insects have shown reduced or neutral fitness associated with resistance, thus if ECB were to develop resistance to Bt11 maize it seems unlikely that the fitness would be altered such that damage to crop and non-crop environments is increased. However, where there is a high concentration of Bt11 maize being cultivated, the cumulative effects may lead to a different behaviour from that predicted.

If resistance to Bt11 were to occur in ECB or other insects feeding on maize, a number of consequences can be envisaged, including the possibility of compromised organic Bt pesticides, potential food chain effects (for example, would Bt-resistance result in a build-up of Cry proteins in the resistant insect causing toxicity to insect predators?), and agri-environment effects (e.g. higher use of 'conventional' pesticides). To prevent the build up of resistance to the Bt protein the notifier has devised an Insect Resistance Management Plan (see above). No timescale is specified for the duration of the IRM plan, although it seems reasonable to suppose it will be implemented for the life of the consent. Also, whilst refuges of 20% non-modified maize appear to be a requirement for all fields over 5ha, it is not clear how many fields will be exempted because they are below this size, and furthermore it is not clear whether monitoring will include all fields (unlikely) or just a few fields.

Effects on biogeochemistry, particularly carbon and nitrogen recycling through changes in soil decomposition
The ERA concluded that any effects on biogeochemical processes as a result of the use of Bt11 are likely to be the same as those effects resulting from the cultivation of conventional maize. No indication could be found that either protein breakdown or foliar decay would be altered in the Bt11 plant. Additionally, it was stated that it is highly unlikely that soil-dwelling non-lepidopteran species will be adversely affected by Bt11 maize, thus indirectly affecting biogeochemical processes. No mention is made in the ERA concerning potential differences in the management practices for Bt11 compared to conventional maize, which could lead to changes in soil structure, decomposition rates and/or carbon and nitrogen cycling. Such practices could include, for example, low till cultivation, altered herbicide regimes and altered organic and chemical nutrient inputs. The farmer questionnaire does ask questions regarding cultivation techniques, but these are not (currently) directed at associated observations of changes in soil structure etc. Some work has been conducted on the effects of Cry proteins on certain soil-borne species and taxonomic groups, but it may also be legitimate to question the potential effects of these compounds on the wider soil environment, including bacteria and fungi (including mycorrhiza) involved in nutrient cycling, in particular in the long term and under cumulative conditions.

8.3. C/SE/96/3501 Potato line EH92-527-1 (Amylogene HB)

***Trait*: Modified starch content.**
***Scope*: Cultivation of potato clone EH92-527-1 and use for the extraction of starch for industrial uses, and the use of the by-products of starch extraction in animal feed.**
***Current status*: Pending.**

Case-specific monitoring

The case-specific monitoring will focus on (1) any significant detrimental changes in the composition of the tubers and (2) a change in the presence of the bleomycin resistant protein associated with ORF4. Monitoring will comprise verification of the following assumptions of the risk assessment:

- the genes of interest remain stably inserted,
- the ORF 4 is not expressed at the protein level, and
- the starch composition and the glycoalkaloid content are stable.

The assessment report of the Swedish Competent Authority states that the notifier has developed methods and accounted for sampling and analysis. The suggested case-specific monitoring will continue for five years, after which the monitoring plan will be evaluated and may be extended or altered. The Swedish Board of Agriculture (SBA) concluded that in particular it is important to study how the glycoalkaloid content may vary depending on the place of cultivation or weather conditions. In addition, the SBA state there is very little reason to expect that the possible expression of a protein from ORF4 would result in negative health effects in animals that will eat the by-products of the potato. The SBA conclude that it is reasonable that monitoring is designed to fulfil the most important criterion in this context, that is to detect and avoid the unlikely scenario that ORF 4 causes a protein to occur in a significant share of the potatoes.

IX.iii.3.2 General surveillance

The general surveillance plan considers areas related to (1) growth characteristics of the plant (including time to flowering, abortion of flowers, and time to maturity), (2) general characteristics of the plants (including height, shape and colour of the foliage, size and shape of the plant), (3) susceptibility to disease and pests and (4) any changes in animals within and in proximity to the fields. Farmers who cultivate and/or use by-products of EH92-527-1 in feed will be required to note all possible unexpected effects. The notifier will supply the appropriate forms for this purpose. General surveillance will also monitor the spread of potatoes outside the field. Management of volunteer potatoes will be carried out according to standard agricultural practice. The general surveillance plan will run throughout the whole 10-year period of the consent. The base-line information consists of data that the notifier has gathered during previous years of cultivation and analysis. A general surveillance questionnaire to all growers and handlers of the potatoes has not been proposed.

IX.iii.3.2.1.1.1 Sampling plan

The applicant has a strategy for introduction of EH92 potatoes and has developed a clear plan for areas of production of seed potatoes and a sampling strategy at sites of production. Sampling strategies must ensure homogeneity and will be undertaken according to European (CEN) standards, further advice will be taken from the 'Ecological Monitoring and Assessment Network', and e.g. ISTA and the EC's Joint Research Centre.

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EH92 post market monitoring sampling plan:

Object	Parameters
Seed potatoes (80 pooled samples)	PCR- identity Absence or ORF4

Production potatoes (20 locations, 4 pooled samples each)	Starch composition Glycoalkaloid level Carbohydrates Protein
Seed potato production sites (5 per year)	Plant characteristics Susceptibility to pests and disease
Production sites (20 new sites per year, cumulative)	Persistence in field Persistence outside of the managed field
Production sites (all sites, verification by the growers)	Plant characteristics

Summary

- The scope of the notification includes cultivation and use for industrial purposes, and feeding of the by-products of processing to cattle. It is not intended for direct human consumption, but it cannot be completely excluded that humans will consume the potatoes. The post market monitoring plan includes a case-specific element to confirm the stability of the inserted genes (*nptII* and non-functional ORF 4) and to ensure that the content of starches and glycoalkaloids remain stable, general surveillance will include monitoring growth characteristics and pests and disease susceptibility. The notification has received favourable assessments for being very thorough and for the applicant's commitment to post market monitoring.
- The biological characteristics of the potato and the relatively benign nature of the trait make it difficult to anticipate long term or cumulative effects arising as a result of cultivation of this crop; the comprehensive post market plan is designed to identify any unanticipated effects of cultivation in the short term. Use of a farmer questionnaire may be advisable to identify any changes in management practice associated with cultivation of EH92, such as altered use of herbicides or pesticides.
- The main risks possibly lie in the co-mingling of EH92 with food potatoes, for which it is not authorised, however the strict Identify Preservation scheme should ensure against this and enable rapid management if co-mingling should occur.
- Longer-term cumulative impacts may lie in economic benefits, for example farmers may be able to secure long-term production contacts, or the increased supply of high quality raw materials may boost local industries with knock-on benefits to the local economy. There may also be potential benefits from the slight reduction in glycoalkaloid levels, which may improve the quality of EH92 pulp for cattle.

IX.iii.3.2.2 Detailed considerations

IX.iii.3.3 Human toxicity and allergenicity

EH92-527-1 potato (hereafter referred to as EH92) is modified by incorporation of the *gbss* gene (granule bound starch synthase) in antisense orientation to reduce the expression of the endogenous *gbss* gene. This has the effect of reducing the level of amylose starch and increasing the level of amylopectin, which is desirable for industrial uses. The potatoes also contain the *nptII* selective marker gene, which confers resistance to the antibiotic kanamycin. The environmental risk assessment (ERA) states that these two traits have a long history of safe use; the *nptII* gene is considered to belong to a class of antibiotic resistance genes that us acceptable for commercial release and has been documented to be safe even for human consumption,

and handling. The antisense *gbss* sequence has not revealed any indication of toxicology or allergenicity; this sequence does not produce a new protein but merely blocks the activity of a native enzyme, allowing the level of native amylopectin to increase. Other sources of amylopectin are known, and it has been found to be safe for human digestion. Amylopectin is, however, digested more readily than amylose and this could result in undesirable effects, for example in diabetics who could experience a swifter increase in blood sugar levels as a result of consumption of EH92 potatoes rather than normal food potatoes, over a prolonged period that this could affect insulin levels in these people. This would be a clear long-term cumulative effect of the release of these potatoes, if they were to become incorporated into the market for routine use a food item. Effective operation of the proposed Identify Preservation system will be essential to ensure that EH92 potatoes are not co-mingled with food potatoes.

Significant differences are reported in levels of sugar (elevated), glycoalkaloids (reduced) and vitamin C (elevated) in EH92 potatoes relative to the conventional parent ('Prevalent'), although these are within the normal variation for potatoes. Reduced levels of potentially toxic anti-nutritional glycoalkaloids and increased levels of vitamin C would be advantageous rather than a drawback. No changes in other compositional parameters were reported and the observed levels were demonstrated to be stable over several generations; these factors are, however, influenced by environmental factors.

Potato is not known to be a major allergenic crop; EFSA considered that over-expression of an endogenous protein that is not know to be allergenic would be unlikely to alter the overall allergenicity of the whole plant. Furthermore, there have been no adverse reports due to handling of the EH92 potatoes in glasshouse, field and processing activities during nine years of experimental trials.

No case-specific post market monitoring is proposed in connection with potential human toxicity or allergenicity effects and this has been accepted during the assessment process and is accepted by EFSA. Effective operation of the Identity Preservation system will be important to ensure EH92 potatoes do not enter the food chain.

IX.iii.3.4 Potential to compromise human therapy

EH92 expresses the *nptII* protein, but this is considered safe for use. There will be no direct consumption of EH92 potatoes by humans so no direct or indirect effects on human therapy are anticipated. If diabetic persons were to inadvertently consume EH92 potatoes over an extended period of time this may compromise treatment of the condition.

IX.iii.3.5 Potential beneficial effects to humans

There will be no direct consumption of EH92 potatoes by humans so no direct or indirect beneficial effects to human are anticipated, although reduced levels of glycoalkaloids and increased vitamin C would be considered beneficial in potato for food use.

IX.iii.3.6 Animal toxicity and allergenicity

Pulp produced as a by-product of starch extraction will be fed to animals; the main use of the pulp is as cattle feed in the autumn and as a nutritional supplement during periods of grazing and when stabled. The pulp is not reported to deviate in composition from conventionally produced starch potatoes and a pulp feeding study with heifers (8 weeks) confirmed that no significant changes had occurred that affected feed quality, and no significant differences in weight gain were detected when fed with pulp from EH92 or a conventional variety. Analysis of the pulp revealed that intact *nptII* was not detected in fruit juice and fruit water, but it was detected in the pulp itself, horizontal gene transfer in the gut could lead to development of kanamycin resistant bacterial populations in the gut. However, kanamycin resistant bacteria are naturally abundant e.g. in soil, and the risk of increased kanamycin resistant bacteria as a result of feeding potato pulp was considered negligible. The antisense protein was found to degrade very quickly in experiments simulating ruminant fluid.

The risk assessment concludes that there are no significant risks to animal toxicity and allergenicity. It is worth noting that feeding trials were conducted over a short time period and would identify any acute effects, whereas longer-term cumulative effects would not be identified. Given that the composition of the pulp does not differ from that for conventional varieties, except in benefiting from a reduced level of glycoalkaloid, chronic toxicity or allergenic effects due to the genetic modification is considered unlikely. Monitoring for any unanticipated effects of feeding the pulp could be done during the life of the consent as part of the general surveillance programme, e.g. as a questionnaire to farmers that utilise the pulp.

IX.iii.3.7 Compromised animal therapy

Kanamycin is not widely used in animal therapy so the release should not compromise animal therapy.

IX.iii.3.8 Potential beneficial effects to animals

Reduced levels of glycoalkaloids reduce the potential for toxic or anti-nutritional effects as a result of consumption of the pulp.

Diseases to plants

Official variety trials in Sweden, demonstrated that, in comparison with the parent variety ‘Prevalent’, EH92 did not demonstrate increased or reduced susceptibility or resistance to late blight (*Phytophthora infestans*), potato early blight (*Alternaria solani*) *Erwinia* rots, other bacterial diseases, potato cyst nematodes (*Glodobera* spp.), aphids or leafhoppers. Starch potatoes are currently only grown in countries that have been allocated a cultivation quota, this has a historical basis and all countries are located in Northern Europe; the environmental risk assessment is based on the assumption that EH92 will only be grown in these areas. The diseases listed are representative of those found in these regions. It should be considered that, consistent with development of a free market economy for agriculture in Europe, the quota system might be abolished in the future, this would widen the area in which EH92 would be considered for cultivation by farmers, particularly with current drives towards use of biodegradable packaging goods etc. If this were to occur, a review of the risk assessment should be considered as the compositional structure of EH92 is

reported to vary with different climatic conditions, in turn this may alter susceptibility to diseases, particularly if being cultivated outside of the northern European region. This shift in potential cultivation patterns would be an effect observed in the long-term, possibly as a result of long-term cultivation of EH92 in northern Europe with good results; it would be an economic market-driven effect.

Target organisms (incl. predators and parasitoids)
There are no target organisms for EH92 potatoes.

IX.iii.3.9 Effects on non-target organisms

A number of organisms, some pathogenic, feed on potato foliage; EH92 has not shown greater or reduced susceptibility to attack by aphids or leafhoppers than the parent variety, nor is any evidence presented to suggest that EH92 is eaten by larvae, worms or snails to any greater or lesser extent. Research has shown (e.g. Johnston, K.A., 1989) that potatoes with very low levels of glycoalkaloids are more appetizing to larvae and snails, however any effect of reduced glycoalkaloid levels has not been observed in EH92. There is no difference in EH92 and the parental variety in terms of susceptibility and resistance to potato cyst nematodes. Animals such as birds or rodents feeding on tubers left on the surface of the soil should not suffer adverse effects as compositional analysis shows the tubers to be essentially equivalent to the conventional parent. Long-term cumulative effects are, therefore, unlikely to be different from the conventional variety.

As already discussed, assessments of EH92 have been made on the assumption that it will only be cultivated in northern Europe. It is possible that exposure to a different suite of pests (e.g. as might be found in more southerly parts of Europe) would identify altered susceptibility to predation from non-target organisms, either as a result of a different suite of pests or due to climate-induced compositional changes. These would not be long-term cumulative effects, rather the result of changes to the basis on which EH92 was risk-assessed.

IX.iii.3.10 Effects of gene transfer (including to soil organisms) and potential for increased weediness

Horizontal transfer of *nptII* to soil bacteria is proposed to be negligible due to the inherently high levels of bacteria in the soil that already contain kanamycin resistant populations. It should be borne in mind that the tuber itself contains the *nptII* gene, a proportion of which will be left in the ground following harvest ('groundkeepers') and rot *in situ*, releasing the gene into the soil. In intensive potato growing areas this could significantly contribute to the pool of kanamycin resistant bacteria in the soil.

The potato has a low competitive ability in Europe outside arable land, particularly in northern Europe. It is mostly propagated vegetatively by tubers, but some varieties can form seeds; groundskeepers are easily destroyed by chemical treatment or by tilling, and are out-competed by the following crop in the rotation. The potato's wild relatives in Europe are *S.nigrum* and *S.dulcamara*, very low frequency genetic exchange has been reported with *S.nigrum* under artificial, forced conditions. Natural exchange of genetic material is only possible with other varieties of potato (*Solanum tuberosum*). EH92 (and its conventional parent) is reported to produce very little pollen, any pollen that is produced can only spread a short distance; the tendency to

shed flowers prematurely further weakens the competitive ability of EH92. In southern Europe, potatoes can occur as a weed outside of agricultural situation, if EH92 were to be cultivated in southern Europe, the possibility of gene transfer to wild relatives would be increased slightly, but effective incompatibility barriers make the occurrence of hybrids very unlikely. The likelihood of successful natural transfer between EH92 and other potato crops is considered very low, in addition it would confer no competitive benefit therefore hybrids would be unlikely to persist. There is no herbicide tolerance trait in EH92 so it will not present groundkeeper/weed management problems, in addition EH92 has not been found to possess increased frost tolerance relative to its parent. Its agronomic characteristics are reported to be essential unchanged from the parent variety. Given the biological characteristics of EH92, it is difficult to speculate what adverse long-term cumulative impacts could occur as a result of its cultivation. Farmers may derive long-term economic benefits by establishing contracts for provision of starch to industrial markets. This should not have a negative effect on the market for food potatoes, as production will be market-driven.

IX.iii.3.11 Biogeochemical processes

Potatoes are not known to play a prominent role in the cycle of chemical elements such as nitrogen fixation by the association of legumes with *Rhizobium*. The agronomic equivalence with the parental line means that any effects that would be seen would be similar to the conventional equivalent, and therefore not a candidate for monitoring. The slightly altered composition of the EH92 relative to the parent may lead to slightly altered decomposition in the soil, but this is not considered to likely to have any significant effects on soil processes.

IX.iii.3.11.1.1.1.1 References

Gebhard, F. and Smalla, K. (1999). Monitoring field releases of genetically modified sugar beets for persistence of transgenic plant DNA and horizontal gene transfer. *FEMS Microbiology Ecology* **28** (3) P. 261-272.

Smalla, K., Borin, S., Heuer, H., Gebhard, F., Van Elsas, J.D. & Neilson, K. (2000). Horizontal transfer of antibiotic resistance genes from transgenic plants to bacteria. Are there new data to fuel the debate? In: Fairbairn,G., Scoles, G., McHughen, A. (Eds) Proceedings of the 6th International Symposium on the Biosafety of Microorganisms p. 146-154.

Simonet, P. (2000). An evaluation of the possibility of transferring DNA from GM crops to soil bacteria. *Oleagineux Corps Gras Lipides* 7 (4) p. 320-323.