Problem Formulation in Environmental Risk Assessment for Genetically Modified Crops: A Practitioner's Approach

Alan Gray
Centre for Ecology and Hydrology, CEH Wallingford, United Kingdom.
E-mail: ajg@ceh.ac.uk

Abstract

Problem Formulation, a tried and tested aspect of Environmental Risk Assessment (ERA), is increasingly being applied to assess the potential risks associated with the cultivation of genetically modified (GM) crops. The first step in the ERA, problem formulation is a way of focussing on those aspects of the environment which most need protection or are most at risk of harm, framing relevant scenarios in which they may be harmed and devising a plan to test whether such harm may occur. In the first phase, problem context, broad concerns about the environment, in the form of protection goals, are identified and utilised to select those entities of value in the environment which could be adversely affected by cultivation of the specific crop. In the second phase, problem definition, those postulated risks which warrant it are further analysed by constructing exposure scenarios which link the growing of the crop to a potentially adverse affect on the valued entity, and this link, or pathway, is then translated into a set of scientifically testable risk hypotheses. The testing of risk hypotheses forms the basis of the analysis plan for characterising identified risks. Operationally, problem formulation can be recast as a sequence of questions which a risk assessor is required to address: (1) What do we not want to see harmed? What must be protected? (2) Can we envision a way in which they could be harmed? (3) How can we assess whether they are likely to be harmed? and (4) Does it matter? What is the regulatory context? The first and last of these questions will be answered in the context of societal concerns and policy. Only questions 2 and 3 are amenable to evidence-based, scientific analysis. The issues most frequently raised in ERA for GM crops are the potential for the plant to become weedy or invasive, the problems which might arise from gene flow to wild or weedy relatives, the potential of the plant to adversely impact non-target species and the potential adverse effects on biodiversity. These topics, together with the potential of the GM plant to become a plant pest, are covered by the five environmental safety assessment criteria applied by the regulatory

Collection of Biosafety Reviews Vol. 6 (2012): 10-65

Padriciano, 99, 34149 Trieste, Italy
http://www.icgeb.org/biosafety/publications/collections.html

authority in Canada. Together with the four questions above, they provide a practical way of conducting problem formulation. A case study of herbicide-tolerant oilseed rape using this approach not only highlights the importance of the process of plant characterisation during the development of the crop in enabling several risk hypotheses to be corroborated or rejected without further data, but also demonstrates how problem formulation identifies what further information or experiments may be required to test such hypotheses. The case study also emphasises that 'harm' can not be defined by science, a potentially reduced population of weeds in arable fields (and a reduction in the arthropod populations which they support) being regarded as an adverse effect of growing herbicide-tolerant oilseed rape in one national regulatory context but as a beneficial effect in another.

Keywords: environmental risk assessment, genetically modified crops, herbicide-tolerant oilseed rape, problem formulation

Riassunto
La "formulazione del problema" (Problem formulation), un aspetto collaudato del processo di valutazione del rischio ambientale (Environmental Risk Assessment, ERA), viene sempre di più utilizzato per valutare i rischi associati alla coltivazione degli organismi geneticamente modificati (OGM). Il primo passo in un ERA, la formulazione del problema, è un modo di focalizzarsi su quegli aspetti dell'ambiente che più necessitano di protezione o sono maggiormente a rischio di essere danneggiati, inquadrando gli scenari più rilevanti nei quali essi potrebbero essere danneggiati ed elaborando un piano per testare se tali danni possano realmente verificarsi. Nella prima fase, il contesto del problema, un certo numero di preoccupazioni circa l'ambiente, sotto forma di obiettivi di protezione, vengono identificati e utilizzati per selezionare quei valori che nell'ambiente possono essere influenzati negativamente dalla coltivazione di una coltura specifica. Nella seconda fase, la definizione del problema, quei rischi postulati che lo garantiscono vengono ulteriormente analizzati con la costruzionne di scenari di esposizione che legano la crescita della coltura ad un effetto potenzialmente avverso sull'entità del valore stimato, e questo collegamento, o percorso, viene quindi tradotto in una serie di ipotesi di rischio scientificamente verificabili. La verifica delle ipotesi di rischio costituisce la base del piano di analisi per la caratterizzazione dei rischi identificati. Operativamente, la formulazione del problema può essere riformulata come una sequenza di domande che un operatore di analisi del rischio è tenuto ad indirizzare: (1) che cosa non vogliamo vedere danneggiato? Che cosa deve essere protetto? (2) possiamo prevedere un modo in cui potrebbero essere danneggiati? (3) come possiamo valutare le probabilità che vengano danneggiati? e (4) Ha importanza? Quale è il contesto normativo? Alla prima e all'ultima di queste domande sarà data risposta nel contesto delle preoccupazioni sociali e politiche. Soltanto le domande 2 e 3 sono adatte all'analisi basata su prove scientifiche. Le questioni più frequentemente sollevate nell'ERA per le colture GM sono le potenzialità della pianta di diventare infestante o invasiva, i problemi che potrebbero sorgere dal flusso genico verso piante simili selvatiche o infestanti, il potenziale della pianta di avere un impatto negativo su specie non bersaglio e gli effetti avversi potenziali sulla biodiversità. Questi temi, insieme al potenziale della pianta GM di diventare una pianta infestante, sono coperti dai cinque criteri di valutazione della sicurezza ambientale applicati dall'autorità competente nel Canada. Insieme alle quattro domande di cui sopra, forniscono un modo pratico di condurre la formulazione del problema. Facendo uso di questo approccio, uno studio

finalizzato della colza tollerante all'erbicida non solo evidenzia l'importanza del processo di caratterizzazione della pianta durante lo sviluppo della coltura nel permettere a varie ipotesi di rischio di essere confermate o rifiutate senza ulteriori dati, ma inoltre dimostra come la formulazione del problema identifichi quali ulteriori informazioni o esperimenti possano essere richiesti per verificare tali ipotesi. Lo studio finalizzato inoltre sottolinea che il "danno" puo' non può essere definito dalla scienza, poiché una popolazione di erbe infestanti potenzialmente ridotta nei campi arabili (e una riduzione della popolazione di artropodi che esse sostengono) può essere qualificata come un effetto avverso della coltivazione della colza tollerante all'erbicida in un contesto normativo nazionale, ma come effetto benefico in un altro.

1. INTRODUCTION

Although a newcomer to the literature might sometimes be forgiven for thinking so, the production and cultivation of genetically modified (GM) crops is not a novel form of agriculture. Since their first cultivation on a significantly commercial scale in 1996 they have been grown on a rapidly increasing area worldwide, and in 2010 had reached a cumulative area of 1 billion hectares (James, 2011). According to this annual survey of global GM crops, the 87-fold increase in hectarage over 15 years makes GM crops the fastest adopted crop technology in the history of modern agriculture. In 2010 a global total of 148 million hectares of GM crops were grown in 29 countries by an estimated 15.4 million farmers, the vast majority of whom were small resource-poor farmers in developing countries (James 2011). The first wave of GM crops, principally containing traits which confer herbicide tolerance or pest resistance, but increasingly with several traits in combination, have been widely adopted and are well established.

Despite this long history of safe use, the introduction and cultivation of GM crops continues to be subject to regulatory oversight. Whilst this oversight varies greatly from one country to another, both in terms of the amount of data required by the regulatory authorities and the legal framework within which it occurs, it invariably includes some form of environmental risk assessment (ERA). The ERA is normally made prior to the possible unconfined, large-scale, commercial release of a particular GM crop and benefits from the earlier stages of testing from laboratory and glasshouse through to field trials. These early stages generate much of the data needed to characterise the novel GM plant. In particular, they enable comparative studies to be made in which the performance and biology of the plant can be compared with those of a conventional counterpart. Such studies are frequently a key part of the ERA (Box 1). The main objectives of the ERA are to identify potentially significant risks to the receiving environment (which may be variously defined to include the 'natural' as well as the agricultural environment, and can include animal and human health), to estimate how serious the risks are (to gauge the level of risk), and to consider where and how such risks might be managed or reduced.

Cast within different regulatory frameworks, the ERA for GM crops has had a long and rather chequered history. Whether dealt with under existing legislation, as for example in the USA and Canada (the latter uniquely treating GM and prescribed non-GM crops together as plants with novel traits or PNTs),

or the subject of a dedicated body of new law as in the European Union, issues relating to the potential environmental impact of growing GM crops have become pre-eminent in assessing the biosafety of the agricultural products of biotechnology. Environmental concerns have arguably provided the biggest constraint to the further development and application of agricultural biotechnology, and, on a global scale remain the greatest challenge to the safe, and widely accepted, introduction of GM crops. The potential importance of such crops in helping to meet the burgeoning global demand for food in a way which is both sustainable and also reduces environmental impacts has frequently been highlighted in the past (e.g. Conway, 1999, 2004; Gregory *et al.*, 2002; Pretty, 2001) and recent reviews have reiterated and strengthened this view (Fedoroff *et al.*, 2010; Godfray *et al.*, 2010; Tester & Langridge 2010). The challenge of producing higher yields from the same, or a shrinking, area of farmland (dubbed 'sustainable intensification' [Royal Society, 2009]) involves not only halting the destruction of existing non-agricultural habitats, but also reducing the environmental damage associated directly with agriculture. The role of robust and science-based ERA in achieving these objectives is clearly crucial.

Although some general principles of ERA for GM crops were enunciated by ecologists and others more than 20 years ago (e.g. OECD, 1986; Royal Commission on Environmental Pollution, 1989; Tiedje *et al.*, 1989) universally agreed methods for conducting such ERAs have been slow to emerge. Guidelines, when produced, are frequently revised (e.g. EFSA, 2010) and ERA continues to place huge demands, especially in terms of data provision, on those who seek to release GM crops. Among the factors which may have contributed to this situation are the initial emphasis on the complexity and inter-relatedness of ecosystems (and hence on the many subtle and as-yet unknown ways in which they might be harmed [Gray, 2004a]) which led to an expanding research agenda aimed at filling the gaps in our understanding of ecosystem function and processes. The idea that policy decisions and assessment of risk are significantly hampered by a deficit of knowledge (the more we know the better our decision – the so-called 'deficit model' [Lawton, 2007; Raybould, 2010]) has also fostered the growth of research allied to ERA. However, as Raybould and others have pointed out (Raybould, 2006, 2010, 2011; Johnson *et al.*, 2007; Craig *et al.*, 2008), confusion about whether the research objectives are to support risk assessment and decision-making, or are to advance ecological knowledge and theory, has often led to ecological research which is largely irrelevant for an ERA.

BOX 1. PLANT CHARACTERISATION

By the time a complete Environmental Risk Assessment (ERA) is required for a GM plant, its biology and performance in a range of conditions is already well known to those who have been involved in its production and development. From the initial transformation and selection of a specific event in laboratory and glasshouse trials, through to contained field trials in different environments, the product developer has amassed a body of data which characterises the GM plant in relation to its non-transformed counterpart. This counterpart, or comparator, may variously be the host or recipient plant (an isogenic line) or a near-isogenic parental line or, for a broader comparison, the range of familiar varieties of that crop species. The important point, sometimes overlooked, is that the GM plant is usually being characterised against a background of long-standing familiarity with the crop and with the breeding of novel varieties by traditional methods. The two key questions which the process of plant characterisation is designed to answer are "have the intended changes occurred? (e.g. Is the gene stably integrated and inherited? Is the protein expressed in the target tissues? Does the plant function as intended – say, is it tolerant of a specific herbicide?)" and "Has anything else changed in the plant's biology and performance which was unintended?"

Both types of change, intended and unintended, are measured relative to a comparator and both will form part of the ERA. The ERA, and specifically the initial problem formulation process, will ask whether these changes have a potential to cause harm. A well-designed programme of plant characterisation should anticipate these questions by specifically measuring those attributes of plant biology which may alter the potential of the plant to cause harm. Some of these attributes are obvious and trigger a set of tests for specific harmful endpoints (e.g. laboratory tests of the effects of an insecticidal protein on a range of non-target organisms). Others are less obvious but are important indicators of a potential change in the plant's behaviour - for example changes in seed shattering or seed dormancy are known to be important in plant weediness and persistence in both agricultural and uncultivated environments. In practice a comprehensive range of compositional and phenotypic measurements are made, usually in multiple locations and over several years (molecular characterisation, commonly required as part of food and feed safety assessment, is arguably of less relevance to ERA). The choice of which traits to measure varies to some extent from crop to crop but is usually based on those characteristics commonly used to evaluate and select commercial varieties, and as mentioned above, those with the potential to significantly alter the plant's biology.

They will include a range of reproductive and survival biology parameters as well as observations on the plant's susceptibility to infestation by key pests and diseases. An example of the traits which may be measured in oilseed rape is given in Section 5.1.

Having established during its development that the GM plant functions as the genetic transformation intended that it should – it expresses an introduced protein in the leaves, shoots or roots, or tolerates applications of a specific herbicide, or is resistant to infection by a target virus – how does plant characterisation help us to assess whether other, perhaps unintended, changes have the potential to cause harm? The

process of seeking and assessing potential harm is discussed in detail below, but the first question to ask is "do the changes, measured as differences between the GM plant and a non-GM comparator, fall within the range of variability observed in the crop species as a whole?"

To help with this evaluation, and to give further context to the ERA, the OECD Working Group on Regulatory Oversight of Biotechnology, building on the concept of 'familiarity' (OECD, 1993), has produced a series of consensus documents describing the biology of major crops, concentrating on those aspects which make the crop familiar and those properties which are relevant for an ERA (http://www.oecd.org/document/60/0,3746,en_2649_34385_46720508_1_1_1_1,00.html). Examples include the crop biology document for oilseed rape (OECD, 1997) and for maize (OECD, 2003). Similar documents have been produced by Australia's Office of the Gene Technology Regulator (http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1); e.g. for oilseed (OGTR, 2008a) and maize (OGTR, 2008b). As well as describing the range of variation in the crop plant, these documents provide other data essential for a risk assessment such as the plant's breeding system, its history of use, the range and distribution of wild relatives, habitats in which the plant persists, the centres of origin and genetic diversity in the species, and so on.

Although the ERAs for GM crops in different countries vary in detail, Hill (2005) has pointed out that many have a shared framework derived from assessing the risks associated with agrochemicals (Hill & Sendashonga, 2003). Broadly speaking, this common framework (a version of which is shown later in Figure 1) comprises an initial stage of hazard identification (the "what could go wrong" step), followed by exposure assessment (the "how likely is it to happen" step) and consequences assessment (the "would it be a problem" step). These last two steps together allow the risk to be characterised (the "what is the risk" step) and, if appropriate, to be managed or reduced. Embedded in the first stage of the process, as articulated by the Environmental Protection Agency in the USA (USEPA, 1998), and extending its objectives beyond simply identifying the hazards, is an exercise called 'Problem Formulation'.

The purpose of this paper is to introduce and describe problem formulation in as simple a language as possible and to offer a method for embarking on ERA using a combination of questions derived by problem formulation and selected risk topics. It is not an academic review of problem formulation, of which there are some good examples, but relies heavily on one of these, namely Wolt *et al.* (2010), for background. A case study will be used to illustrate the approach.

2. WHAT IS PROBLEM FORMULATION?

It may be helpful to begin by saying what problem formulation is not; it is not environmental risk assessment, it is not a regulatory protocol, it is not a legal instrument, and it is not merely the identification of hazards. But it is, variously, an approach, a tool for environmental risk assessment, a first step, a concept, a method, a way of thinking, and an academic construct. At its very simplest, problem formulation is about framing questions relevant to the ERA and coming up with a plan to answer them.

The way in which problem formulation fits into the overall framework for ERA is shown in Figure 1, taken from Wolt *et al.* (2010). Here below, two aspects of problem formulation are distinguished and are labelled problem context and problem definition. The description of problem formulation under these two headings uses a number of terms which are essential for an understanding of the process. These terms, selected from a rather jargon-rich field of science and italicised on first mention below, are defined formally in the Glossary of Terms (Box 2), but are described in more detail in the text which follows.

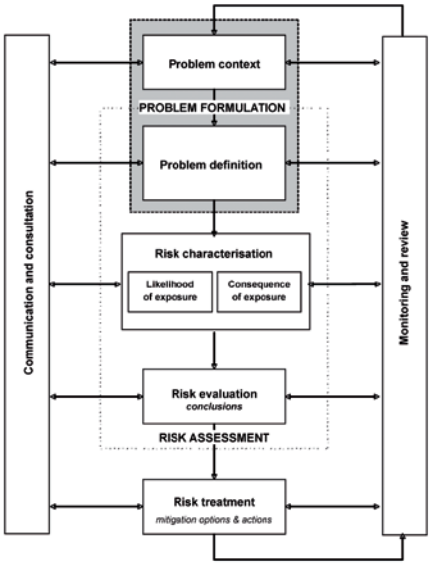

Figure 1. Problem formulation within the paradigm for environmental risk assessment (Wolt *et al.*, 2012; used with permission).

BOX 2. GLOSSARY OF SELECTED TERMS USED IN PROBLEM FORMULATION (based on Wolt *et al.*, 2010)

Assessment endpoint – An explicit expression of the environmental value that is to be protected. Operationally it is defined by an environmental entity of value that is susceptible to harm and an attribute that provides evidence of harm. For example beneficial insects are valued ecological entities, abundance within the agro-ecosystem is an important attribute; "beneficial insect abundance" constitutes an assessment endpoint.

Conceptual model – A way of describing a plausible scenario of how harm may arise from use of the GM crop in a way that enables characterisation of the risk. It may take the form of a simple statement, an outline of activities, a flow chart or a diagram, and sets out the relationship between exposure and effect.

Exposure scenario (risk scenario) – A particular set of circumstances describing the opportunity for harm to an environmental entity of value (can be in the form of a conceptual model).

Harm – A negative or unwanted outcome or effect of an action or event (= adverse effect).

Measurement endpoint – A measurable response to the changed attribute of the plant that is quantifiably-related to the assessment endpoint (USEPA, 1998).

Problem context – The activity that establishes the parameters for the risk assessment, including policy goals, scope, assessment endpoints and methodology.

Problem definition – The activity that leads to the identification of postulated significant risks that warrant further analysis for a specific ERA case and which leads to a specified analysis plan.

Problem formulation – The first step in ERA whereby policy goals, scope, assessment endpoints, and methodology are developed into an explicitly stated problem and an approach for analysis; comprised of the problem context and the problem definition.

Protection goals/management goals – The objectives of environmental policies, typically defined in law or regulations.

Risk hypothesis – A tentative explanation taken to be true for the purpose of argument or investigation. Not to be confused with scientific hypotheses which are specific testable postulates that will be part of the analytical phase of the ERA.

Risk assessment (Environmental Risk Assessment) – The process of identifying significant risks to the environment, estimating the level of risk, and determining those risks that require measures to reduce the level of risk (USEPA, 1998).

2.1. Problem context

In establishing the problem context for ERA the risk assessor is aiming to move from what may be very broad general concerns about environmental health and safety to a set of much more specific, and most importantly measurable and testable, propositions. The broad environmental concerns are very familiar; clean air, pure water, uncontaminated and sustainably high-yielding soils, biodiverse communities of plants and animals, and so on. Some of these concerns are enshrined in global legislation. For example a general objective in relation to biodiversity and ERA for GM crops appears in the Cartagena Protocol on Biosafety (CPB; Annex III [SCBD, 2000]) as "the objective of risk assessment….. is to identify and evaluate the potential adverse effects of living modified organisms on the conservation and sustainable use of biological diversity in the likely potential receiving environment…". Thus the 'conservation and sustainable use of biological diversity' is here a broadly-stated objective defined by global policy, an objective defined as a *protection goal*. Such protection goals (sometimes more appropriately described as *management goals*) may be very general, as in the CPB which is a legally binding instrument for over 135 countries and derives its definition of biodiversity from the original international Convention on Biological Diversity (CBD) in 1992, or they may be based on local law or policies devised to protect the human population and the environment from unacceptable risks. For example, in the United Kingdom there are very specific laws preventing harmful actions in designated areas of wildlife interest (draining wetlands, ploughing ancient grasslands etc.) linked to clearly prescribed targets defining the conditions in which such areas should be found. These targets, resulting from enactment of the CBD, are variously articulated as plans covering general biodiversity (Biodiversity Action Plans), particular habitat-types (Habitat Action Plans) and even selected valued species of plants and animals (Species Action Plans).

Unlike the UK's habitat and species action plans, most protection and management goals, whether they are explicitly defined in law, regulation, policy or guidelines or are unstated common-sense aims of best practice (e.g. to have as high-yielding, pest and disease-free crops as possible) do not necessarily identify those aspects of the environment which are valued (except perhaps human beings) and that we are seeking to protect. Nor do they always tell us what an adverse effect or harm might be. Nor, for that matter, do they clearly prescribe the environment of concern. These tasks are all part of the problem context stage of problem formulation.

First is the task of identifying the environmental values that are to be protected. In practice these will comprise specific entities to which value is attached by virtue of their posited susceptibility to harm (harm which may as yet be unidentified). Such entities, together with some attribute which might be adversely affected are described as *assessment endpoints*. The example given in Wolt *et al*. (2010) is that of 'beneficial insect abundance', beneficial insects being an acknowledged ecological entity of value and their abundance in agroecosystems an important, and potentially adversely-affected, attribute. Other assessment endpoints might relate to desirable or protected organisms and their abundance or establishment (which would be 'harmed' by a reduction) or to undesirable or pest organisms and their abundance and spread (where the 'harm' to the valued entity would be from their increase). Abiotic aspects of the environment can also give rise to legitimate assessment endpoints, as in, for example 'low soil salinity' or 'beneficial water table height'. The key point about an assessment endpoint is that the valued environmental entity is defined in terms of an attribute which will provide evidence of harm should it occur.

Before considering more carefully what is meant by 'harm' it is necessary on a case-specific basis to define the nature of the crop plant to be released, the environment into which it will be released (usually called the 'receiving environment') and which will be the theatre in which the risk assessment will be conducted, and the methodology to be adopted in the risk assessment. The first of these, as already mentioned, is the process of plant characterisation (Box 1). Most regulatory authorities have developed guidance on their data requirements in this area, particularly in respect of the biology of the host plant, the genetic material introduced, what happened during the transformation process, where the gene(s) are expressed and so on. From the standpoint of an ERA the key question to be addressed is whether the introduced trait has produced changes, both those intended and any others, which make the GM plant significantly different from the conventional counterpart and which may have the potential to cause harm. The unintended changes, if there are any, are almost certain to have been identified during the long process of plant development and trial. In fact experience suggests that unintended changes, say through pleiotropic effects, are rare and in practice ERAs are mostly concerned with the effect of the introduced trait on the biology and performance of the GM plant and whether this has the potential to cause harm. This immediately sets the parameters of the risk assessment – a plant differing from its counterpart

in expressing an insecticidal protein alerts us to the possibility that, among other things, non-target insects important for biological control could be affected, or the target insects could develop resistance and become more difficult to control. Although this may seem very obvious, dossiers aimed at ERAs for GM crops in the past have often attempted to rule out harmful effects by presenting data ranging over many quite irrelevant aspects of the plant's biology and performance.

How do we decide what is harmful? A working definition of 'harm' as 'a negative or unacceptable effect or outcome' helps only if everyone is able to agree what is 'unacceptable'. What is, or is not, unacceptable harm cannot be derived scientifically. But a definition of unacceptable harm is needed in order to assess the level of risk that harm might occur. Deriving such a definition from policy objectives or from legislation is facilitated by the identification of clear assessment endpoints. As discussed above assessment endpoints provide a possible way of measuring change which, on the basis of specific protection or management goals, has been identified as potentially harmful. For example a reduction in the abundance of a beneficial insect species or a valued bird species could be defined as unacceptable. It is even plausible, but rare, to be highly prescriptive – to define a threshold or trigger value (say the number or size of an insect's populations below which unacceptable harm can be said to have occurred). The task of the risk assessment then becomes more tractable as it seeks to predict the effects of cultivating the GM crop on the population sizes of the selected organisms (or on soil salinity or water table height and so on). Any measurable response to growing the crop that can be quantifiably related to the assessment endpoint is described as a *measurement endpoint* (Box 2).

Having used environmental policies and goals to derive assessment endpoints and define the specific purpose and scope of the risk assessment given the known properties of the GM plant and the receiving environment, the next step is to spell out in detail the possible ways in which harm might occur. This is the first part of the problem formulation process described as Problem Definition.

2.2. Problem definition

The main purpose of problem definition is to translate the broad concerns identified at the problem context stage into a series of specific, and verifiable, propositions about risk. Using the information about the plant and the receiving environment, including its entities of value, gleaned from the

initial scoping exercise, the risk assessor has now to identify those postulated risks which demand closer attention and those which can be put to one side as being either non-existent or negligible (a process described by Wolt *et al*. [2010] as a distilling exercise). Once identified as being reasonable (i.e potentially significant) and relevant, such risks may be cast in the form of a risk hypothesis or series of *risk hypotheses* (described in detail below) which are amenable to testing and measurement.

But the first, and absolutely essential, question to be asked of each and every postulated risk is 'what is likely to be the contact, if any, between the GM plant of interest or its attribute of concern and the entity of value which it may affect?' In other words 'what is the probability that the entity of value (e.g. a beneficial insect) will be exposed to (i.e. ingest) the presumed stressor (e.g. insecticidal protein) presented by the GM plant under the proposed conditions of cultivation?' Building the links between the GM plant and the entities which may be at risk from its cultivation is sometimes described as tracing 'pathways to harm'. For each potential risk the pathways to harm (there may be several) collectively constitute an *exposure scenario*. In constructing exposure scenarios it is customary to make worst-case assumptions, at least in the early stages. The search for exposure is vital since without exposure there is no risk (exposure is necessary but not sufficient for a risk i.e. not all exposure leads to risk, for example the beneficial insect may be unharmed by ingestion of a particular protein).

It is helpful to describe an exposure scenario for a particular risk in the form of a *conceptual model*. This can be structured as simple contingent steps in a sequence of events or situations which must occur if the entity of value is to be exposed to the potential hazard which is the GM plant. For example, if the presence of the insecticidal protein in a wild relative of the crop plant has been identified as potentially harmful (say to a rare or valued insect which feeds on the wild species) a simple conceptual model might include the following steps: GM crop produces pollen → pollen disperses to wild relative → pollen fertilises wild relative → transgene is stably introgressed → wild relative produces and expresses insecticidal protein → rare insect ingests protein → rare insect is sensitive to protein → rare insect populations are reduced. This ostensibly simple model linking the crop to an assessment endpoint (abundance of a rare or valued insect) is a very powerful tool in problem formulation (see Raybould [2011] for more examples of such models).

First it sets out a series of events which must happen for harm to occur. Each of the steps in this logical sequence has a different likelihood of happening. Each step can also be expressed in the form of a risk hypothesis, which is a (tentative) statement about the event or condition which can be used for argument or investigation. Risk hypotheses are very different from scientific hypotheses which, broadly speaking, are statements or assertions about the way we think the world is, as opposed to statements about the way the world would have to be for certain consequences not to occur. Thus risk hypotheses are always stated as negatives because, if rejected, they allow us to consider the next step in the causal chain of events leading to possible harm and if corroborated they suggest, with varying degrees of confidence, that harm will not result. In the rare insect example above, the risk hypotheses would be; GM crop does not produce pollen, pollen does not disperse to wild relative, pollen does not fertilise wild relative, and so on. If any one of those risk hypotheses can not be falsified (i.e. they are, for argument's sake, 'true') then the pathway to harm is disrupted and the risk can be eliminated from our assessment. The chain can be broken at any stage – near the beginning (say the crop can not fertilise the wild relative) or near the end (the protein does not harm the insect). In practice of course it often makes sense to test this penultimate risk hypothesis first because the inserted protein is likely to have specific targets, as in the case of the Cry proteins which are aimed at lepidopteran pests or beetle pests etc, and laboratory studies are both simpler and more powerful ways of falsifying a risk hypothesis than are most environmental studies (indeed the risk assessor should target the risk hypotheses that are most amenable to testing regardless of where they fall in the pathway to harm). If this hypothesis is falsified (i.e. the protein **does** harm the rare insect) it will be necessary to estimate the risk of this happening in the field. Probably the best-known example of a conceptual model for ERA, that of Sears *et al*. (2001; Figure 2), demonstrates that, whatever the effects of the protein on the larvae of the valued insect, in this case the Monarch butterfly, exposure in the field to this protein (lepidoptera-active Cry1 proteins expressed in maize pollen) depends on a range of variables, all measureable, relating to the crop, the ecology and behaviour of the butterfly and the occurrence and distribution of the food plant. Since these variables may be difficult, and expensive, to measure laboratory tests are the favoured first step or tier in an ERA where the risk hypothesis relates to the risks to such non-target organisms (see below and Garcia-Alonso *et al*., 2006; Romeis *et al*., 2008).

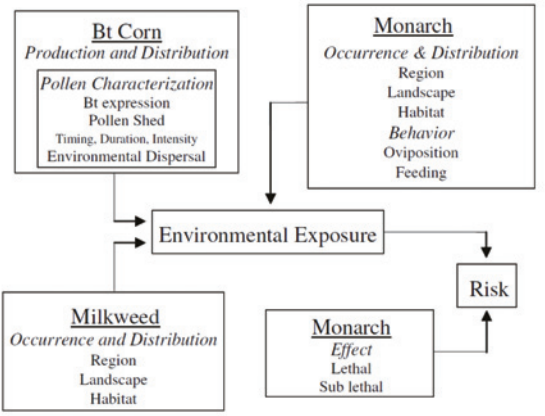

Figure 2. Example of a conceptual model for ERA (Sears et al., 2001; used with permission of derived source).

A second important feature of conceptual models, especially with the risk hypothesis or hypotheses articulated alongside each pathway, is that it forms the basis for any analysis plan. It tells you what information you may need in order to carry out a full risk assessment. In many cases the information will exist which enables the assessor to eliminate a risk as negligible and no further tests will be required. In the rare insect example you may know that the crop has no wild relatives which are key food plants or that the rare insect does not occur in the environment where the crop is grown. If it is known that wild relatives occur in contact with the crop but not whether the crop and its relative can hybridise or genes are stably introgressed into the wild relative populations (i.e. gene flow occurs), this leads the risk assessor to seek specific evidence in relation to a step in the pathway to posited harm. This evidence may exist from previous studies. Indeed it is becoming increasingly likely that useful evidence will exist as research aimed at informing ERA increases and relevant data are accumulated in widely-available documents (such as those on specific crops produced by OECD). Therefore this stage of problem formulation which immediately precedes the actual risk characterisation, may vary from a purely mental exercise (hugely valuable nonetheless in identifying risks which can be disregarded) to one which sets out in detail the data and experiments required to assess an identified risk. Knowing what information is required can also prevent the collection of information which is not necessary or informative for risk assessment.

Thirdly, once devised, an analysis plan based on falsifiable risk hypotheses provides a transparent process which can be inspected and if necessary refined by all stakeholders. It sets out precisely what will be done in order to characterise a potential risk, allowing, in principle, agreement to be reached in advance about both the validity of the process – whether certain data will or will not falsify a risk hypothesis – and, should none of the risk hypotheses be falsifiable, whether the outcome constitutes harm.

In practice the latter issue, what constitutes harm, is not straightforward. Used in a fairly loose way in the text above, *harm* can be defined specifically in the context of ERA to mean 'an adverse effect or outcome of an action or event' (see Box 2). This definition prompts the obvious question 'what is adverse?' In an ideal world agreed 'levels' of adverse effect would exist such that the risk assessor or regulator could pronounce that, if they are exceeded, harm has occurred. These thresholds which trigger regulatory concern (or decision criteria [Wolt *et al*., 2010]) are unfortunately uncommon in ERA for GM crops. They do exist; the effects of feeding the GM plant containing Cry protein to populations of the non-target insect in the example above can be defined in terms of a rate of mortality which is increased (or not) in comparison to that in populations fed on a non-GM equivalent. A biologically significant increase in mortality can here be defined as 'harm'. More frequently however harm has to be assessed in terms of a more qualitative comparison with a non-GM comparator. ERAs are then couched in the terms that 'the risk associated with the GM crop is no greater (or may be greater) than the risk associated with the comparator crop'. Versions of this general statement may be of the form 'the risks of the GM plant being invasive or more persistent in non-agricultural environments are no greater than those posed by the conventional plant'. To be able to make such bold statements the risk assessor must clearly be able to demonstrate where and how the evidence was obtained, how good and repeatable are the experiments or observations on which the evidence is based, and what criteria have been used to define harm and to make the assessment. If all these can be agreed in advance consensus about risk among the various stakeholders, including policy makers, is achievable; hence the importance of an agreed analysis plan and clear problem definition.

Defining harm is not the only aspect of ERA which may require discussion and agreement in advance. Just as harm cannot be derived scientifically, neither can assessment endpoints, which, as we saw above, are derived

from the protection and management goals which reflect societal concerns and policy. In fact the construction of pathways to harm and of exposure scenarios, along with the formulation of risk hypotheses are really the only parts of problem formulation which depend on the application of science (or at least on skills that rely on scientific training). A majority of people involved in ERA for GM crops, whether regulators, advisors, researchers or policy makers seem to have a background in some aspect of the biological sciences ranging from molecular genetics and biochemistry to ecology and environmental science, and the heart of the ERA is, quite properly, a science-based, evidence- based activity. But it is vital to acknowledge that the context and parameters for risk assessment are set by societal concern and locally-agreed policy.

This contrast between the science-based and policy-driven aspects of problem formulation is illustrated in the next section by restating the goals of problem formulation as four key questions.

3. FOUR KEY QUESTIONS

Operationally, problem formulation can be seen as a series of questions which the risk assessor might ask himself/herself to both establish the context and constraints of the ERA and to produce a tractable analysis plan to characterise specific risks. Offered below are four questions which represent key aspects of problem formulation and which it is hoped will provide a helpful introduction to the process.

3.1. Question 1: What do we not want to see harmed? What must be protected?

The first challenge in problem formulation is to identify as precisely as possible those aspects of the environment which it is thought will be impacted by, and potentially harmed by, the cultivation of the GM crop being assessed. As seen above these may be in the form of very general concerns to do with clean air, uncontaminated water, biodiversity and so on which comprise the broadly-stated protection goals for maintaining a healthy environment. In practice such concerns must be translated into a series of more definite and concise protection goals from which assessment endpoints can be derived. The extent to which explicit protection goals exist to facilitate this process varies enormously from country to country. Some developed countries have a large and often complex body of environmental law with precisely-stated goals (see the UK biodiversity protection laws mentioned above); others may have very little beyond

the general aspiration to sustain as safe and undamaged an environment as possible. Nevertheless, the identification of well-defined assessment endpoints from whatever protection and management goals are locally available is a crucial first step in the ERA.

The search for endpoints will be guided, on a case-by-case basis, by the biology of the GM plant (see Box 1) and the conditions under which it is proposed to cultivate it. Again this seems obvious; but it is not a principle that has been followed in all previous ERAs. Thus the presence of an insecticidal or anti-feedant protein in the plant alerts us to the possibility of one class of assessment endpoints, whilst a virus resistance gene or an herbicide tolerance gene will alert us to quite different classes, respectively. Having identified assessment endpoints the next step is to ask more specifically how they might be harmed.

3.2. Question 2: Can we envision a way in which they could be harmed?

Whereas Question 1 represents the 'problem context' stage of problem formulation dealing mainly with societal and policy concerns (Table 1), this second question encourages the risk assessor to clearly articulate the links between the cultivation of the GM crop and any potential harm to those endpoints which have been identified as susceptible or in need of protection. In problem formulation terms, we are requested to develop exposure scenarios – and if possible to construct conceptual models describing those scenarios.

This activity is more obviously science-based and in many ways is the most challenging part of the ERA. It is an activity which can benefit greatly from group discussions, especially 'brain-storming' sessions involving scientists from a range of backgrounds in biology and agriculture. The expertise brought to these sessions by molecular and population geneticists, plant and animal ecologists, entomologists, microbiologists, soil scientists, virologists, agronomists, modellers, and others with a range of related skills is a hugely important resource available to the risk assessor and regulator. The development of realistic and relevant exposure scenarios supported by clear conceptual models is a *sine que non* of effective risk assessment. Since there are probably a huge number of ways in which harm could arise, the more scenarios we can generate and assess as being plausible the better. The more informed and experienced the individuals involved in the process of imagining the scenarios and rejecting the wildly implausible ones the better. An inexact science, the

construction of exposure scenarios is nevertheless a scientific activity, and one that can benefit from repeated development and refinement. Since ERA is a finite process, necessarily limited in time and scope, it is important to identify those scenarios to which it is worth devoting resources.

Once the pathways to harm have been identified the next stage is to formulate risk hypotheses of no harm addressing the likelihood of the various steps in the chain being realised.

3.3. Question 3: How can we assess whether they are likely to be harmed?

In addressing this question one hopes not only to formulate a testable (potentially falsifiable) risk hypothesis, or series of hypotheses as in the rare insect example above, but also come up with a plan (the analysis plan) to test these hypotheses in a way which will help to characterise the risk.

The brain-storming and consultations used to search for and define potential sources of harm when addressing Question 2 should have enabled the general risk hypothesis that the GM crop will not be harmful to be restated as a set of pathways to harm, hopefully expressed as conceptual models. The models need not be complex. In fact simple models have more value as long as they logically trace pathways to harm in a way which enables testable (potentially falsifiable) risk hypotheses to be erected and allow the corroboration of any one of those hypotheses to break the link in the causal chain. In some cases conceptual models, as in the example of the Monarch butterfly (Figure 2), may be complex, the exposure of the valued entity to the potential hazard in the crop depending on an interacting set of factors in the test environment (maize fields across the USA). However, complex models are also amenable to analysis in terms of a sequence of risk hypotheses derived from the cause and effect pathways in the model (for the Monarch example the sequence might be something like: Bt maize does not produce pollen → the protein is not expressed in the pollen → pollen is not dispersed to the food plant → the food plant does not occur in or near the crop → larvae do not feed on these food plants → pollen is not available on the food plant when the larvae are feeding → larvae do not ingest pollen → larvae are not harmed by pollen → Monarch butterfly populations are not reduced. The ease with which any one of these risk hypotheses may be refuted or corroborated varies considerably, and selecting which ones to concentrate on is the task of the final part of the problem formulation process – devising an analysis plan.

The objective of the analysis plan, as discussed above, is to spell out, in detail if necessary, what information is required for risk characterisation and how that information will be acquired. It is important to re-emphasise that we are only concerned with information which will help us to corroborate or refute our chosen risk hypotheses. Other aspects of the GM crop's performance or biology, or, say, the ecology of its wild relatives, however interesting, are irrelevant for assessing risk. If data on the ecology of wild relatives or beneficial insects are needed to address a specific risk hypothesis they may be included in the plan's information requirements. However it is possible that corroboration of a more-easily tested hypothesis in the chain will make the collection of such data irrelevant. For example if the hypothesis that the insecticidal protein is not expressed in the pollen (or whichever part of the plant is consumed by the non-target insect) is corroborated, the pathway to harm is broken because of the non-exposure of the potential hazard, and knowledge about the behaviour and ecology of the insect is not needed. Choosing which of the risk hypotheses of no harm to test first is therefore an important part of the analysis plan. Those that are most likely to reveal an indication of harm should be tackled first. Experience has shown that they should be tested initially under conditions most likely to falsify them i.e. the conditions which are least likely to give false negatives (see Garcia-Alonso *et al*. [2006] and Raybould [2011] for a discussion of the principles of testing risk hypotheses using a tiered approach).

3.4. Question 4: Does it matter? What is the regulatory context?

Addressing the three questions above has hopefully helped to guide us through the first step of an ERA for GM crops using the problem formulation approach. Before embarking on the risk characterisation and evaluation stages of the ERA (Figure 1), it is often helpful to conduct a 'reality check' by setting the outcome of our analysis in the context of local policy and regulations. In other words we should check exactly what is required to comply with any stated regulatory requirements. It may be that there are quite specific decision criteria to assist with the analysis or, at the other extreme, that the level of concern is so low that costly data-gathering or experimentation is uncalled for. To return to the much-used non-target insect example, it may not actually matter that the populations of the insect are reduced in the receiving environment. Mortality there may be insignificant in relation to that in other parts of the insect's life cycle or to that in a comparator crop where insecticides are used (two statements incidentally which are true of the Monarch butterfly but which did not prevent extensive tests of their validity).

Decisions about whether an identified adverse effect, such as a reduction in the abundance of a non-target insect, matters (i.e. whether it constitutes unacceptable harm) are not, as emphasised earlier, derived scientifically. Even if the final risk hypothesis at the end of a causal chain has been falsified, indicating that there is a risk that the assessment endpoint will be adversely affected (rare insect abundance reduced), the decision whether this is an unacceptable risk relies on human judgement. Even where the final risk hypothesis of no harm has not been falsified (rare insect abundance is not adversely affected), our confidence in the conclusion that the GM crop presents a low risk will depend on how thoroughly we have been able to corroborate the risk hypothesis. Absolute freedom from risk of harm cannot be proved. Rigorous attempts to disprove no harm give us some confidence that the risk will be low.

Arguably the most difficult of the four questions to deal with is Question 2 which invites us to imagine ways in which entities of value in the environment may be harmed. Brainstorming and other group activities notwithstanding, sitting down with a blank sheet of paper and attempting to envision the many and various ways in which harm might occur from cultivating a crop is particularly challenging. One way of making the process less difficult is to group the possible sources of harm (or risks) which have been thought about in the past into particular topic areas, which can then form the basis of a more structured approach to the problem formulation. This is the subject of the next section.

Table 1. The relationship between the four questions and problem formulation stages

Question	Problem formulation stage
1. What do we not want to see harmed? What must be protected?	Identify assessment endpoints from protection and management goals
2. Can we envision a way in which they could be harmed?	Trace pathways to harm. Construct exposure scenarios (using conceptual models)
3. How can we assess whether they are likely to be harmed?	Formulate risk hypotheses and devise an analysis plan
4. Does it matter? What is the regulatory context?	Revisit protection goals and evaluate the acceptability of the risk

4. FIVE KEY TYPES OF RISK FROM GM CROPS

The objective of the ERA for GM crops is to provide the evidence on which decision-makers, usually regulators, can make a decision about the crop's cultivation. This decision will be based on the risks to the environment that cultivation may pose. In practice regulatory authorities request that the evidence on which assessments are to be made is organised in a way which enables them to consider, one-by-one, each of the most commonly identified types of risk. Ordering the ERA in this way helps to focus the risk assessment on specific topics which experience has indicated cover most of the potential problems which may arise from cultivating GM crops. Not all potential problems are necessarily covered by the specific topics, but most legislation (including that of the Canadian regulatory authority whose categorisation is adopted below) includes various 'safety nets' to ensure that risk issues which fall outside the topic areas are picked up in the assessment.

For the present purpose of concentrating the problem formulation exercise into a series of subject areas, the grouping of potential risks will be based on that recognised by the regulatory authority in Canada, the Canadian Food Inspection Agency (CFIA). The CFIA is responsible for assessing the environmental safety of Plants with Novel Traits (PNTs) which include those produced by recombinant DNA technology. They do so (under Part V of their Seeds Regulations) by tasking their Plant Biosafety Office with assessing the environmental safety of PNTs based on five criteria. These are (my italics):

- the potential of the novel plant to become a *weed of agriculture* or to be *invasive of natural habitats*
- potential for *gene-flow to wild relatives* whose hybrid offspring may become *more weedy or invasive*
- potential for the novel plant to become a *plant pest*
- potential impact of the novel plant or its gene products on *non-t arget species* including humans, and
- potential impact on *biodiversity*

The five Canadian environmental safety assessment criteria (or 'five pillars' as they are sometimes known) cover five areas of ERA which have, as a matter of fact, been those most consistently raised as potential risk areas. Whilst not exclusive, they provide here a convenient set of topic headings under which

to discuss the most commonly encountered types of environmental risk. (Other aspects of environmental risk are captured in the broader Canadian regulatory framework, specifically under the Canadian Environmental Protection Act). Some background to these topics, which will be revisited in the case study later, is given below. They are weediness and invasiveness, gene flow, plant pest potential, non-target organisms and biodiversity.

4.1. Weediness and invasiveness

Weeds (broadly defined as 'problem plants' or 'plants in the wrong place' [Naylor & Lutman, 2002]) and weediness (the attributes and biology of such plants) have attracted the attention of agricultural botanists and ecologists for a very long time. So too has the link between weeds of agriculture and plants that successfully invade uncultivated environments. The advent of GM crops has refreshed interest in both of these topics and the possibility that GM plants might become agricultural weeds or invade natural habitats was acknowledged from the beginning (Keeler, 1989; Crawley *et al.*, 1993). Approaches to assessing the risks of such a possibility have included attempts to predict how 'weedy' the GM plant might be by assessing whether the transformation has introduced traits commonly found in successful weeds (so-called 'Baker traits' [Baker, 1965]). Broad comparisons of GM plants with invasive plant species, particularly those introduced, accidentally or intentionally into new habitats or countries ('alien' species) have also been drawn in an attempt to detect weediness in advance. It turns out that neither approach has been very helpful. Baker traits have little or no power in predicting whether a species will be invasive (Williamson, 1996) and successful aliens (the majority of which tend to be thicket-forming woody perennials or aquatic species) have quite different attributes to the current GM crops, and are, in any case, difficult to characterise genetically (Gray, 1986). The only reliable indicator of whether or not an introduced species is likely to become invasive is whether it, or a close relative, has been invasive in other places (Veltman *et al.*, 1996).

More recently the prospect of GM plants with abiotic stress tolerances such as drought and salt tolerance has reignited interest in the potential for such plants to become weedy or invasive (Nickson, 2008; Wilkinson & Tepfer, 2009; Raybould, 2011). Emerging from these studies is a re-emphasis on the crucial importance of familiarity with the crop plant and the process of plant characterisation during the plant's development (see especially Nickson [2008] and Box 1 above). Comparison of the novel

phenotype with that of a comparable non-GM plant (as nearly isogenic as reasonably possible) remains the most powerful predictor of weediness potential. If those aspects of the GM plant's biology which might lead to weediness, such as seed germination patterns, early seedling growth, days to flowering, growth habit, seed production, and so on, fall within the range of parameter values of the comparator plant, confidence that a weedy character has not unexpectedly been introduced is increased. Even greater confidence (stronger corroboration of the 'no harm' hypothesis) is gained from comparing the GM plant and comparator under conditions where the potential differences between them are likely to be expressed; in say conditions of drought versus plentiful water (Nickson, 2008). Additional guidance in assigning levels of potential weediness when characterising the GM plant can be found in a number of national and local weed risk assessment guides (e.g. The South Australia Weed Risk Management Guide as used in Virtue & Melland, 2003). These can help to eliminate at a first screening those where the risk of harm from the plant becoming weedy or invasive is low or negligible, and to concentrate on those where more data may be required to confidently corroborate the 'no harm' hypothesis. For some crop species such as maize it is actually quite difficult to imagine how they could be modified to become weedy or invasive, whilst other, less domesticated, crops such as oilseed or forage crops already have a few weedy attributes.

4.2. Gene flow

The development of GM crops also gave great impetus to the study of gene flow. The possibility that genes might 'escape' from the crop and, by hybridisation with wild relatives, allow novel genes to spread in the environment, was regarded as an important uncertainty by early commentators on the environmental risks posed by GM crops (e.g. Royal Commission on Environmental Pollution, 1989; Tiedje *et al.*, 1989). Surveys of the extent to which different crop species were known to hybridise with wild relatives were undertaken on a national basis (de Vries *et al.*, 1992; Raybould & Gray, 1993) and globally (Ellstrand *et al.*, 1999; Stewart *et al.*, 2003). There have been extensive laboratory and field studies of hybridisation and rates of introgression in those species where gene flow is known to occur, such as oilseed rape (e.g. Wilkinson *et al.*, 2000; Warwick *et al.*, 2003), beet (e.g. Bartsch., 1999; Arnaud *et al.*, 2003) and sunflower (e.g. Burke & Rieseberg, 2003, Snow *et al.*, 2003). The topic of gene flow generally has amassed a huge literature and arguably

remains the most debated aspect of GMO biosafety. A comprehensive recent review of the subject as it relates to ERA is provided by Lu (2008).

The central concern around gene flow from crops to wild relatives is that the hybrid will become either a serious weed of agriculture or become invasive in the natural environment - in which respect it is a different version of the problem of weediness and invasiveness. However the transfer of a trait by gene flow to a wild relative presents, at least in theory, a different set of issues arising from the presence of the trait in a largely 'wild type' genetic background. Thus the envisaged mechanism for the plant's newly-acquired invasive properties is frequently that of 'ecological release'. Based on the assumption that populations of plants and animals in the wild are prevented from growing exponentially by factors such as competition, predation, herbivory and disease or by abiotic stresses such as drought, salinity or nutrient limitation, a situation of ecological release is one in which those individuals with the inserted trait are 'released' from a key limiting factor. For example, a population of plants normally limited by insect seed predation or virus disease might gain a selective advantage and spread if individuals were resistant to the insect or virus respectively. However, elucidating the role of specific pests or pathogens in limiting natural populations is a far from trivial exercise. A relative fitness advantage can only be established by complete life cycle studies, although in the case of virus infections in wild Brassica species (Raybould *et al.*, 1999; Gray *et al.*, 2003; Raybould & Cooper, 2005), insecticidal proteins in Brassica (Halfhill *et al.*, 2005) and sunflowers (Cummings & Alexander, 2002; Snow *et al.*, 2003), and disease-resistance in sunflowers (Burke & Rieseberg, 2003), insights into the effects of introduced traits have been gained by a combination of simulation experiments, exclusion experiments or the use of hybrids. Simulation modelling has also produced useful insights into those stages of hybrid plant life history which are most sensitive to change (Bullock, 1999; Hails & Gray, 2004). Underpinning these and other studies is the paradigm that genes will only spread in the environment where they confer a selective advantage. Evidence that past gene flow from agricultural plants has produced hybrids which invade natural habitats is very sparse (Gray, 2004b) despite extensive evidence that most crops hybridise with wild relatives somewhere in the world (Ellstrand, 2003). In contrast, several crop x wild relative hybrids have proved to be problems in agriculture or frequently-disturbed habitats, a contrast which highlights both the effects of genetic linkage (genes favoured under domestication are transferred together) and the power and ubiquity of natural selection.

Gene flow *per se* is generally not regarded as harmful (it may be the agent of harm) but may be seen as undesirable in situations where the genetic integrity of a particular population is itself an assessment endpoint. These include gene flow to wild relatives in centres of diversity or origin of the crop species, and crop-to-crop gene flow where the variation in traditional land races may be at risk, the quality or purity of a crop may be compromised (e.g. when adjacent crops are grown for food oils and industrial oils) or there is a desire to keep different agricultural systems separate (GM crops v organic crops). The ERA may also consider horizontal gene flow (as opposed to vertical gene flow which has been the subject so far), which is the introduction of genes into organisms by processes that are independent of reproduction – for a comprehensive review of this subject see Keese (2008).

4.3. Plant pest potential

The Canadian regulations on which the five environmental safety assessment criteria are based recognise the possibility that a novel plant may become a 'pest'. Defined in their Plant Protection Act 1990 as "any thing that is injurious or potentially injurious, whether directly or indirectly, to plants or to products or by-products of plants, and includes any plant prescribed as a pest" a plant pest is a bit more than a weedy or invasive plant – although those properties would characterise most cases of plant pests. The potential for injury (harm) to plant products or by-products alerts the risk assessor to possible downstream uses of the GM plant which lie outside of ERA. However, harm might occur indirectly if the plant harboured a pest or altered the potential to interfere with an existing pest or disease control measure. In the latter sense a GM plant with the potential to drive the evolution of resistance (say to insecticidal protein in a target insect pest) could be seen as having the potential to become a pest.

In practice most of the concerns within an ERA about the GM plant becoming a plant pest are subsumed under the assessment of its ability to become weedy or invasive. However, an assessment of potential changes to the disease and pest susceptibility and dynamics of the plant should also be made.

4.4. Non-target organisms

Arguably the most challenging area of ERA from an ecological viewpoint involves assessing the risks associated with the commercial release of crops engineered to be resistant to various animal, mainly arthropod, pests. The first of these, expressing delta-endotoxins isolated from the common soil

bacterium *Bacillus thuringiensis* (Bt), were among the earliest GM crops to be grown and insect-resistant varieties remain second only to herbicide-tolerant ones in total world cultivation (26.3 million hectares, 17% of the GM crop total in 2010 [James, 2011]). The challenge derives not only from the variation in these crops resulting from the diversity of intrinsic characteristics such as toxin specificity, expression levels, promoter effects, level of resistance, crop size and management, and so on, but also on the potential adverse effects which they might have on other animals, principally invertebrates, in the agricultural ecosystem (and in the surrounding countryside). Although these have included herbivores which feed in or near the crop, such as the Monarch butterfly, studies have been concentrated on agriculturally beneficial invertebrates which live in the crop. These include the natural, usually insect, enemies of crop pests (their predators or parasitoids), other beneficial organisms such as earthworms and some nematodes, and plant pollinators. Such non-target organisms may be affected directly by exposure to the toxin (by ingesting a part of the plant where the toxin is expressed or a prey animal containing active toxin) or indirectly by changes in plant quality, or prey quality or behaviour. Natural enemies can also be affected by reductions in the availability of prey and by different ways of managing the crop.

Fortunately these potentially complex interactions, termed tritrophic interactions because they involve the plant, the herbivores (both target and non-target), and the predators and parasitoids, have a history of targeted and careful research. Much of the research begins in the laboratory where the direct effects of an insecticidal protein can be measured on a range of arthropods, either as pure protein or when contained within a transgenic plant. From a risk assessment viewpoint laboratory conditions represent the worst-case exposure scenario. If no adverse effects are observed under such conditions, it is usually not necessary to undertake further tests, a risk hypothesis of 'no harm' being most confidently corroborated at this stage. If harm (i.e. a prescribed level of increased mortality in laboratory feeding studies) does occur, additional experiments can then be conducted which more realistically reflect the exposure in field conditions of the non-target organism to the toxin. Laboratory studies of non-target arthropods are a routine part of the plant characterisation of pest-resistant GM plants. The choice of species to test and the design of these studies, which is beyond the scope of this brief review, are covered in detail by Romeis *et al*. (2011). A comprehensive review of risk assessment for insect-resistant GM crops is given in Romeis *et al*. (2008).

Vertebrate 'non-target' organisms which might conceivably be adversely affected by a GM crop include animals which are higher in the food chain (e.g. birds which feed on the seed or on insects in the crop), as well as domestic animals and humans. These last two, including studies of potential allergenicity effects in humans, are usually dealt with under regulations covering the safety of food and feedstuffs. Birds and those impacted by higher-order effects are better discussed under the next topic; biodiversity.

4.5. Biodiversity

Biodiverse assemblages of plants and animals have fascinated biologists since before Charles Darwin first described the rich variety of the 'entangled bank' in *On the Origin of Species* in 1859. How such assemblages arise and are maintained, and the value and stability of their emergent properties continue to be hotly-debated topics among ecologists and evolutionary biologists today. Examples of contested issues include the relative importance of niche differentiation versus more random neutral processes in maintaining biodiverse communities, and the role of species richness (the number of different species) versus evenness (the relative abundance of species) in ecosystem function and stability. New explanatory paradigms, such as Hubbell's neutral theory of biodiversity (Hubbell, 2001), continue to emerge and be tested and the ability to routinely characterise genetic diversity in wild species, as well as the advent of computer-based modelling, has given recent impetus to biodiversity research. At the same time the word 'biodiversity' has entered the lexicon of policy-makers and environmentalists as a generic term for the rich variety of life at all levels (genetic, species, community and biotope). It is mostly in this sense that the concept of biodiversity and GM crops is debated.

The broadly-stated protection goal of the CPB (see Section 2.1. above) establishes as an objective the identification of potential harm to the conservation and sustainable use of biodiversity. In practice the possibility that growing GM crops might present a risk of harm to biodiversity provides a 'catch-all' category for most of the potential harms mentioned above. For example the creation of a weed or invasive plant might reduce the diversity of natural communities by the loss of plant species; higher-order effects of growing pest-resistant GM plants may include the loss of beneficial insects, and so on. Most discussions linking GM crops to biodiversity are couched in terms of species reduction or loss (or the loss of genetic diversity – Gray *et al.*, 2003). Although relevant,

the displacement or reduction in number of one or two species in natural habitats represents, on a global scale, a relatively minor threat to biodiversity compared to that from the three main forces of habitat loss and degradation, invasive alien species, and climate change. Of these, it may be possible to envision a pathway to harm from GM crop cultivation in situations where natural habitats are destroyed or degraded as a result – a trait such as salt tolerance, perhaps, enabling the crop to be grown where its conventional counterpart could not. Again however this scenario is much less likely than the continuing destruction of natural habitats to grow conventional crops (which, if they lead to increasing yields in existing agriculture, GM crops might actually help to circumvent [see the discussion in the Introduction and Gregory *et al.*, 2002]).

A special case of the potential impact of GM crops on biodiversity is provided by the example of growing herbicide-tolerant crops in Europe. Here, exceptionally, one of the envisioned harms is to the biodiversity in the crop and its immediate environs. Because the citizens of many European countries expect their farmland to deliver environmental goods as well as food, and farmland of one sort or another dominates most European landscapes, valued wildlife species which depend at some stage on agricultural land have, in countries such as the UK, become regarded as assessment endpoints. For example a reduction in the abundance of bird species which depend on weeds in and near the crop (or on the invertebrates which feed on the weeds), such as the skylark, can be classed as a harm. This example is further explored in the case study which follows.

5. A CASE STUDY - HERBICIDE-TOLERANT OILSEED RAPE

In order to illustrate the range of issues encountered during problem formulation, the case study below is discussed at a deliberately generic level – with respect both to the trait (tolerance to various herbicides) and the receiving environment (different agricultural systems and countries). It is not intended to be a protocol or template for a specific ERA (and of course is not a complete ERA) but solely to exemplify the sorts of questions that arise at each stage of the problem formulation process. For simplification the species discussed is *Brassica napus*, oilseed rape (OSR) or canola, although other *Brassica species*, notably *B. rapa* and *B. juncea* are also cultivated for oilseed (and called OSR and canola), and a tuberous form of *B. napus*, swede or rutabaga, is grown for food and animal feed.

5.1. Plant characterisation

Oilseed rape has been cultivated in Europe and Asia since ancient times and, whilst its history is confused by the failure to distinguish it from turnip rape (*B. rapa* ([previously classified as *B. campestris*)]), it spread within Europe reaching Britain in the 16th century. Initially grown mainly to provide oil for lamps and to feed the left over meal to animals, a period of decline in which the oil was mainly used as feed or an industrial lubricant preceded a phenomenal increase in the 1970s and 80s, principally in the cultivation of 'double low' varieties (with low levels of both glucosinolates and erucic acid) for cooking oils, margarine and fats. Today OSR is grown for oils, including biofuel, meal and forage in many countries, the major producers in 2009 being China, Canada, India and Europe (UNFAO, 2011). Both winter (sown August/September and harvested in July/August) and spring (sown March to May and harvested September onwards) varieties are grown, the latter generally in more northern latitudes.

B. napus is a tetraploid species of uncertain origin but which genomic analysis indicates has resulted from hybridisation between the diploids *B. rapa* and *B. oleracea* (U, 1935). It is unknown as a wild species but hybridises, with varying degrees of inter-fertility, with several wild relatives in the Brassica family (see below). A free-flowering annual, OSR is largely self-pollinating, with out-crossing by wind, insects and direct contact varying between 5 and 30%. The seed are readily released when the mature pods shatter, and will germinate to produce plants in subsequent crops, known as 'volunteers'. Outside the crop OSR occurs in peri-agricultural habitats such as headlands and the edges of farm tracks, and has become a common component of the flora of roadsides where its populations are maintained by seed spillage and/or frequent soil disturbance (Crawley & Brown, 1995; Saji *et al.*, 2005). Documents which review the biology and use of OSR include those produced by the OECD (1997), the OGTR (2008a) and Thomas (2003).

OSR has been genetically engineered to tolerate a number of herbicides but the two in greatest use commercially are glyphosate-tolerant and glufosinate-ammonium-tolerant varieties. In the first of these, tolerance to the herbicide glyphosate is conferred by transformation with the *c4 epsps* gene (5-enolpyruvylshikimate-3-phosphate synthase) from *Agrobacterium* sp. strain CP4, a common soil bacterium, enabling expression of *CP4 EPSPS* protein functionally equivalent to endogenous plant EPSPS enzymes apart from a reduced affinity for glyphosate (Franz *et al.*, 1997). The C4 EPSPS enzyme

continues to function in the presence of glyphosate, enabling the production of amino acids and other metabolites necessary for plant growth. Tolerance to glufosinate ammonium, which acts by disrupting the enzyme pathway for glutamine synthetase, thus preventing the formation of glutamine from glutamate (resulting in the accumulation of toxic levels of ammonia in the plant), is conferred by the insertion of one of two functionally equivalent genes; the *pat* gene originally isolated from *Streptomyces viridochromogenes* and the *bar* gene, from *S. hygroscopicus*. The protein encoded by these genes, phosphinothricin-N-acetyl transferase (*PAT*), acts by modifying the herbicide not its target. Both types of herbicide tolerance (HT) have been engineered into OSR (and several other crop species) and are grown worldwide. In Canada, where the HT technology has been enthusiastically adopted, HT canola has gone from zero in 1995 to almost 99% of total canola cultivation in 2009, with glyphosate-tolerant types comprising 48% of the total of 15.8million acres and glufosinate-ammonium-tolerant types 45%.(data from A. Roberts, *pers comm*.) In contrast, no HT OSR is currently grown in Europe.

For the purposes of this generalised case study we will assume that the HT plant is substantially equivalent to its comparator except for tolerance of a specific herbicide. As described elsewhere (Box 1), this will have been established by careful plant characterisation during the product development. In fact, it has been extensively demonstrated to be the case for the two HT plants discussed above – the HT plants fall within the range of their non-GM comparators for a comprehensive list of physiological, reproduction and growth parameters. In the case of OSR, the attributes of greatest significance are those which might affect the weediness and persistence of the plant such as seed shattering, dormancy and early growth rate. A list of the parameters routinely measured for GM OSR is given in Table 2.

Table 2. Parameters commonly measured in comparative trials of GM and conventional oilseed rape (based on data supplied by Monsanto and Pioneer Hi-Bred International Inc.)

Seed dormancy/germination in lab/field	Days to maturity
Days to emergence in field	Seed development, production and yield
Seedling vigour/early growth/stand count	Seed quality (green seed)/% moisture
Growth habit and morphology/plant height	Shattering (pre-and during harvest)
Days to first flowering/pollen shed	Population at harvest/final stand
Duration of flowering/pollen shed	Susceptibility to pests and disease
Pollen morphology and viability	Effects of selected abiotic factors (temp.)

5.2. What do we not want to see harmed? What must be protected?

In this first stage of problem formulation we are looking for possible harmful effects of growing HT OSR, in the form of adverse changes to assessment endpoints. Arguably the first concern will centre on the properties and safe use of the relevant herbicide. For the purpose of this case study however we will assume that the full range of very stringent environmental safety tests have been applied (a process which must clearly precede the engineering of the HT plant) and that the herbicide has been cleared for use in the crop (we will return to some general effects of the herbicide below). The scenario considered here is the deployment of HT OSR in rotational arable agriculture in conjunction with an herbicide which gives post-emergence control of a broad spectrum of broadleaved and grass weeds within the crop (and is not directly toxic to terrestrial invertebrates or soil microbial communities).

In very general terms the two answers to the headline questions above are: (i) agricultural production and sustainability, and; (ii) a biodiverse, or otherwise valued, non-agricultural environment. These provide two very broad assessment endpoints, sustainably high-yielding high quality crops and 'undamaged' non-agricultural environments. However these are clearly universal endpoints which apply in all cases of the introduction of a GM or

novel crop and are not operationally easy to measure. The degree to which they can be further refined, such that more specific assessment endpoints can be identified, and therefore adverse change or harm defined more precisely, depends both on the trait and on the regulatory environment within which they are introduced. For example, since weed control is the prime reason to introduce HT crops, a legitimate operational assessment endpoint would be 'low weed populations'. This now provides a basis for the next question – effectively, can we envision a way in which weed populations might increase? And of course we can. If HT OSR is more weedy or persistent and leads to more volunteers this could be an adverse effect. If the GM plant had expressed an insecticidal protein a different set of assessment endpoints would have been invoked.

In those countries where there is well-developed environmental law, the regulatory environment within which the HT OSR is introduced will furnish formal statements of the broad protection goals from which assessment endpoints are derived and may be further refined. As seen above, in Canada, where nearly all OSR is herbicide-tolerant, such plants are regulated under the Seeds Act and Seed Regulations as plants with novel traits. Two other environmental protection acts are also important in this context, the Canadian Environmental Protection Act already mentioned and the Species at Risk Act. The first provides protection goals related to the potential adverse effects of toxic or harmful substances in the environment, and the second identifies those species and the habitats in which they occur that are threatened or endangered in Canada, sustainable populations of which are likely to be specific assessment endpoints. Similarly in the UK, although GM crops are regulated under European Directive 2001/18/EC dealing specifically with their deliberate release and marketing, the presence of detailed environmental legislation and policy, such as Biodiversity Action Plans and Species Action Plans, provides a source of more explicit protection goals and hence assessment endpoints. For example the abundance of a wild relative of OSR legally defined as having conservation status would be an assessment endpoint which could be adversely affected if gene flow occurred between the relative and the crop (see below). Regulation of GM HT OSR in the USA falls under the Federal Plant Protection Act which aims to safeguard agriculture so as to ensure 'an abundant, high quality, and varied food supply'. Legislation covering its possible effect on the non-agricultural environment includes the Endangered Species Act, which prohibits any

action which may adversely affect an endangered species or habitat (see Raybould 2011 for a more detailed exposition of the USA legislation). Whether there is a specific body of legislation in place, as described in the countries above, or merely a general policy stating an aspiration to maintain a healthy and productive environment, the broad protection goals from which assessment endpoints will be derived generally divide into those which relate to the protection of agriculture and agro-ecosystems, and those which relate to the non-agricultural environment. Specific assessment endpoints derived from these broad goals will be considered in the next section which discusses ways in which they may be adversely affected.

5.3. Can we envision a way in which they could be harmed?

This question challenges us to identify pathways to harm by linking the cultivation of HT OSR to a potential adverse effect on our entity of value. In theory there is a virtually unlimited number of potential assessment endpoints which might be considered under this process. Since the risk assessment could not sensibly include all of these and since criteria are needed to evaluate their plausibility, this part of the problem formulation approach will, as mentioned above, benefit hugely from input from a wide range of experienced biologists. For example one could imagine, but instantly dismiss, that harm might occur to populations of a wild vertebrate, say deer, which happen to graze on the crop. This consideration is dealt with by compositional analysis during product development and if included in an ERA at all, should only be briefly mentioned. Certainly it is not necessary to explicitly trace the pathways to harm as a set of contingent events (e.g. HT OSR grown near woodland → deer in woodland → deer enter HT OSR field → deer graze on HT OSR → deer harmed by HT OSR ingestion (significantly more than by non-HT OSR ingestion) → deer populations reduced)! On the other hand it is extremely helpful to set out the pathways to harm for a scenario that links the cultivation of HT OSR to reduced crop yield or quality or to reduced biodiversity in a non-agricultural habitat (see below). Although the likelihood of such harms occurring may be seen to be low based on existing knowledge, to demonstrate that they have been considered and to describe the process by which the risk of harm has been assessed so that others may judge its value, is a crucial part of ERA.

Using the topics listed in Section 4 above, what ways can the growing of HT OSR lead to an adverse effect on the assessment endpoints broadly defined earlier? First, might the HT OSR be more weedy or invasive than conventional non-HT OSR? There are two scenarios to consider. The first is whether HT OSR

might produce more volunteers in following or in neighbouring crops and be more difficult to control, therefore causing harm to crop yield or quality, and the second is whether HT OSR might be more persistent or invasive in non-agricultural habitats. As we saw above these concerns can be rewritten as a series of conceptual models or exposure scenarios linking the postulated increased weediness of HT OSR to harm of an entity which must be protected, such as crop yield. In the case of increased invasiveness and persistence in non-agricultural habitats the entity of value could be biodiverse natural or semi-natural plant assemblages. Some examples of appropriate scenarios are given in Section 5.4 below where they are listed alongside the risk hypotheses which they generate. A series of scenarios related to potential weediness are given in Raybould (2011).

The second possibility to consider is whether gene flow to a wild relative might adversely affect crop yield or natural plant communities. How might the acquisition of herbicide tolerance by a wild relative cause harm? The first stage of tracing possible pathways to such harm is to ask whether gene flow is possible or likely. Oilseed rape, as *Brassica napus*, has several wild relatives with which it is known to hybridise, the presence of which in the wild will vary from country to country. In the UK for example, *B. napus* has been shown to produce spontaneous hybrids (by natural pollen transfer unassisted by man) with six wild species (*B. rapa, B. oleracea, B. juncea, Hirschfeldia incana, Raphanus raphanastrum* and *Sinapis arvensis*), although in all but the first two introgression is unlikely due to low hybrid fertility or genome incompatibility (Scheffler & Dale, 1994; Gray & Raybould, 1999; Gray, 2000). Whilst eleven other species have produced hybrids by manual pollination and/or embryo rescue (Gray & Raybould, 1999), the threshold of spontaneous hybridisation can be accepted as an appropriate indicator that gene flow might occur - even then it could be extremely unlikely for the reasons mentioned above. For example, although spontaneous hybridisation to male-sterile *B. napus* by *S. arvensis* has been observed (Lefol et al., 1996), attempted reciprocal crosses and an extensive search for hybrids in natural populations confirmed the extremely low probability of gene flow (Moyes et al., 1999). In contrast, hybrids between *B. napus* and *B. rapa* have been known for a long time (e.g. Davey, 1939) and despite the fact that the frequency of hybridisation and introgression is known to vary in different circumstances (Gray, 2000), the possibility that herbicide tolerance could be transferred to a wild or weedy population of *B. rapa* in the UK is one that we should consider in our risk assessment (a possibility which has been realised elsewhere - e.g. Warwick et al., 2003). None of the wild relatives of

OSR in the UK are species of significant conservation status such as important food plants for rare or valued insects. All six above are species which occur as feral populations (*B. oleracea* is also a probable escape from cultivation), as members of frequently disturbed habitats associated with agriculture, or in disturbed semi-natural habitats such as river banks and sea cliffs. That herbicide tolerance may be an attribute which confers an advantage in such environments seems, on the face of it, highly unlikely – unless, of course the herbicide is used as part of their management. In fact there is very good evidence to support the risk hypothesis that HT OSR is not more persistent than non-HT OSR in semi-natural habitats (see below) and this is likely to be true in the case of hybrids. However the presence of HT hybrids in agricultural environments may be a source of harm to yield or quality and should be assessed.

The possibility that HT OSR has potential as a plant pest is covered by the discussion above. It is also unlikely that HT OSR poses a risk to non-target organisms, at least directly. The proteins concerned with herbicide tolerance have been shown during earlier tests to have no toxic or anti-feedant effects on arthropods which feed on the plant or occur in the soil. However, and this is a rather special case confined to the EU as we shall discuss below, the more effective control of weeds in the crop, by removing the primary production on which a range of arthropods depend, may have an adverse effect on non-target organisms at higher trophic levels. This scenario and the contingent risk hypotheses are considered below. Finally it is difficult to envision any way in which HT OSR crops (or any crop-wild relative hybrid) might adversely impact biodiversity in habitats outside of the crop other than by the mechanisms discussed earlier of becoming more invasive and replacing valued species or significantly altering ecosystem function.

In summary, based on what we already know about the characteristics of HT OSR, we have come up with rather few ways in which its cultivation might harm the recognised broad assessment endpoints more than its non-HT counterpart. For those which have been thought of, the exposure scenarios can now be developed by formulating corresponding risk hypotheses and deciding how these can be tested.

5.4. How can we assess whether they are likely to be harmed?

The potentially adverse effects from cultivating HT OSR identified above were: (i) a possible reduction in yield or quality of the crop and/or of subsequent crops because of an increase in the weediness or invasiveness of HT OSR itself

(relative to non-HT OSR) or a hybrid between HT OSR and a wild relative; (ii) an adverse change in non-agricultural plant communities (measured as a loss of species diversity) because of the increased invasiveness and persistence of HT OSR, or a hybrid between HT OSR and a wild relative, in these habitats; and (iii) the rather special case of an adverse impact on species which depend in part on the presence of weeds in the OSR crop – this is an effect due to the management of the crop with broad-spectrum herbicide rather than the HT OSR per se. Some of the scenarios whereby these harms might occur, which were outlined above, are set out in Tables 3 – 7 below together with the relevant risk hypotheses.

First (Tables 3 and 4) are two examples of scenarios for harm through reduced crop yield from cultivating HT OSR resulting from increased weediness of the GM plant or a hybrid respectively. Additional, generic, pathways to harm resulting from increases in weediness are included in Raybould (2011), and it is perfectly possible to devise other scenarios, with fewer or more steps, which can form the basis for a series of risk hypotheses. Equally the necessary comparison with a conventional counterpart can be made at any stage in order to generate testable hypotheses. For example in Table 3, row 3 could be rephrased as 'significantly more HT OSR plants establish than non-HT plants'. This would produce a negatively-stated risk hypothesis which can be tested by observation and which, if corroborated, would provide evidence that the HT plant does not spread faster, persist or volunteer more than the non-HT plant. The question of what is defined as 'significantly more' is clearly of great interest here. Although significance in a statistical sense can be established by falsifying a null hypothesis of no statistically significant difference, such a difference indicates that the HT OSR has a potential to lead to harm, not that it causes harm. The subsequent risk hypotheses (rows 4 and 5) would need to be tested to establish the looked-for reduction in yield. On the other hand, corroborating such a statistical null hypothesis demonstrates no detectable difference in weediness potential and suggests that further tests are not needed.

Table 3. Scenario for harm through reduced crop yield caused by HT OSR crop

Exposure scenario	Risk hypothesis
HT OSR seed disperses to neighbouring crops/ HT OSR seed disperses to subsequent crops	HT OSR seed does not disperse to neighbouring crops/ HT OSR seed does not disperse to subsequent crops
Seed germinates in neighbouring crops /subsequent crops	Seed does not germinate in neighbouring crops/subsequent crops
HT OSR plants establish	HT OSR plants do not establish
HT OSR plants affect growth of the crop	HT OSR plants do not affect growth of the crop
HT OSR plants reduce crop yield more than non-HT counterpart	HT OSR plants do not reduce crop yield more than non-HT counterpart

Table 4. Scenario for harm through reduced crop yield caused by crop/wild relative hybrid. In this example the wild relative is *B. rapa* (Wild Turnip or Bargeman's Cabbage)

Exposure scenario	Risk hypothesis
HT OSR produces pollen	HT OSR does not produce pollen
Pollen disperses to populations of *B. rapa*	Pollen does not disperse to populations of *B. rapa*
HT OSR fertilises *B. rapa*	HT OSR does not fertilise *B. rapa*
Transgene is stably introgressed	Transgene is not stably introgressed
Reproductive HT *B.rapa* plant produced	No reproductive HT *B. rapa plant* produced
Seed of HT *B. rapa* disperse to crops	Seed of HT *B. rapa* does not disperse to crops
HT *B.rapa* produces more seed in crop	HT *B. rapa* does not produce more seed in crop
Higher seed production increases abundance of HT *B. rapa* in crop	Higher seed production does not increase abundance of HT *B. rapa* in crop
Increased abundance of HT *B. rapa* reduces crop yield	Increased abundance of HT *B. rapa* does not reduce crop yield

Similar scenarios to Table 3 and Table 4 can be constructed for a potential adverse effect of HT OSR on crop quality (possibly a more important impact in some situations). The scenario in Table 4 can also be applied to other species of wild or weedy relative. For most of these, one of the early risk hypotheses will be corroborated (HT OSR does not fertilise the wild relative, transgene is not stably introgressed, no reproductive HT hybrid is produced) based on existing information. In these cases there is no need for further analysis. However in the case of *B. rapa*, where existing information suggests that later risk hypotheses must be tested, we are now in a position to draw up an analysis plan to help to characterise the risk – which is the next stage of the ERA (Figure 1). If the risk is felt to be high, then a programme of experiments to test various risk hypotheses can be devised, or, as discussed in Section 5.5. below, rather than attempt to characterise the risk more accurately, one may decide to manage or mitigate the risk (say by agronomic practice).

Tables 5 and 6 present exposure scenarios and risk hypotheses for the possibility of harm from increased invasiveness of HT OSR in non-agricultural habitats, or from the HT trait being transferred to a wild relative growing in non-agricultural habitats.

Table 5. Scenario for harm through loss of a wild species or reduced abundance of a species of conservation value resulting from invasion of non-agricultural habitats by HT OSR (an adverse effect on 'biodiversity')

Exposure scenario	Risk hypothesis
HT OSR produces seed	HT OSR does not produce seed
Seed disperse to non-agricultural habitats	Seed does not disperse to non-agricultural habitats
HT OSR plants establish in non-agricultural habitats	HT OSR plants do not establish in non-agricultural habitats
HT OSR populations persist	HT OSR populations do not persist
HT OSR populations increase in abundance	HT OSR populations do not increase in abundance
HT OSR populations displace species or reduce valued species	HT OSR populations do not displace species or reduce valued species

Again existing information enables the 'testing' of risk hypotheses and indicates that further detailed analysis of risk is not necessary. For example in Table 5 we have to reject the first three risk hypotheses. OSR does produce seed, the seed do disperse to a range of non-agricultural habitats, mainly by spillage from harvesters and lorries but also by animal vectors, and populations of OSR do establish outside of agriculture, mainly in frequently disturbed environments. There are actually data to inform the next two risk hypotheses, that HT OSR populations may persist or increase more than non-HT OSR in non-agricultural habitats, from the experiments of Crawley et al. (1993; 2001). These demonstrated that there was no significant difference in the population biology of an HT and a non-HT OSR when introduced into a range of semi-natural habitats and confirmed that, whilst OSR can establish long-lived seed banks, their populations in non-agricultural habitats do not increase in abundance unless the ground is frequently disturbed. Thus HT OSR is not significantly different from non-HT OSR in its effects on these habitats. This of course is only true where no advantage is gained by HT OSR due to application of the relevant herbicide. Therefore any ERA must consider whether herbicide is used to control weeds in that environment and what the consequences of possible increased abundance of OSR might be. There are also data which indicate that the first five risk hypotheses in Table 6 can be safely invalidated – in fact that fully sexual HT *B. rapa* occurs via hybridisation with OSR (see references above). There is also evidence which corroborates the risk hypothesis that HT *B. rapa* is not more resistant to environmental stressors than non-HT genotypes (unless the stressor is herbicide application). However it is less important to discuss that here (it is in any case normally part of the next stage of ERA, namely risk characterisation) than to re-emphasise that for different crops and traits, the exposure scenarios will have similar features but there will be very different amounts of data enabling the risk hypotheses to be tested.

Table 6. A scenario for harm through reduced abundance or displacement of wild species resulting from increased abundance of HT hybrid *B. rapa*

Exposure scenario	Risk hypothesis
HT OSR produces pollen	HT OSR does not produce pollen
Pollen disperse to wild *B. rapa* populations	Pollen does not disperse to *B. rapa* populations
Pollen fertilises B.rapa	Pollen does not fertilise *B. rapa*
Transgene is stably introgressed	Transgene is not stably introgressed
Reproductive HT *B. rapa* produced	No reproductive HT *B. rapa* are produced
HT *B.rapa* more resistant to environmental stressor	HT *B. rapa* is not more resistant to environmental stressor
HT *B. rapa* has higher seed production (than non-HT genotypes)	HT *B. rapa* does not have higher seed production than non-HT genotypes
HT *B. rapa* abundance increases	HT *B. rapa* abundance does not increase
Increase in *B. rapa* displaces a species or reduces abundance of valued species	Increase in *B. rapa* does not displace a species or reduce abundance of valued species

Finally, in Table 7 a scenario in which harm to non-target species that depend on crop weeds is considered. As mentioned earlier this is a rather special case but is included here to underline the fact that risk is not defined by science but by cultural concerns, and that even extensive and costly experiments do not necessarily enable a risk hypothesis to be properly addressed. The non-target species are valued birds that depend at some stage in their life cycle (as juveniles or adults) on arthropod species which occur in OSR fields. The possibility that populations of farmland birds such as the skylark (*Alauda arvensis*), grey partridge (*Perdix perdix*) and corn bunting (*Emberiza calandra*), which have declined dramatically in the UK in the last thirty years (Fuller *et al.*, 1995), might be further reduced by the cultivation of HT GM crops led to public concern and eventually to a very large experiment aimed at informing this possibility. This experiment, the Farm Scale Evaluations (FSEs), conducted over four years and costing £5million, involved comparing the weed and invertebrate populations in a large number (c60) of adjacent paired fields of OSR (both spring and winter sown), fodder maize and sugar

and fodder beet. The paired fields comprised a herbicide-tolerant and a conventional variety of each crop. The results of the FSEs (mainly published as a special themed volume [358] of *Philosophical Transactions of the Royal Society Series B – Biological Sciences* in 2003), enabled the rejection of the null hypothesis that HT OSR and non-HT OSR crops (and other crop pairs) do not have significantly different arthropod populations. The significant decrease in weeds and (most) arthropods in HT OSR was sufficient to suggest to those responsible for the decision, that growing the crop could exacerbate the decline in valued farmland birds. However, as can be seen from Table 7, such a result only enables us to reject risk hypothesis 3 (HT OSR fields do not have fewer arthropods than non-HT OSR fields) with any confidence. The presumed link to the decline in bird populations has not been made (although modelling studies indicate some specific correlations between key food species and particular bird species [Watkinson *et al.*, 2000]) and the final risk hypothesis can not be safely rejected. Only the potential to lead to harm has been established. The next section considers the importance of addressing this potential in the context of the regulatory and policy environment.

Table 7. Scenario for harm to farmland birds resulting from reduced food availability in HT OSR crops

Exposure scenario	Risk hypothesis
HT OSR crop managed using broad spectrum herbicide	HT OSR crop not managed using broad spectrum herbicide
HT OSR fields have fewer weeds than non-HT OSR fields	HT OSR fields do not have fewer weeds than non-HT OSR fields
HT OSR fields have fewer arthropods than non-HT OSR fields	HT OSR fields do not have fewer arthropods than non-HT OSR fields
Valued bird species feed extensively on arthropods in OSR fields	Valued bird species do not feed extensively on arthropods in OSR fields
Valued bird populations decline due to reduced food availability in OSR fields	Valued bird populations do not decline due to reduced food availability in OSR fields

5.5. Does it matter? What is the regulatory context?

Before embarking on a programme of data gathering or experiments to characterise the risk of potential harms which have been identified during problem formulation, it is useful to set these harms in the local (i. e. national) regulatory context. For example in the case of the UK FSEs referred to above, the potential to lead to harm indicated by the rejection of risk hypothesis 3 (HT OSR fields do not have fewer arthropods than non-HT OSR fields) was felt to be a sufficient basis for a decision to refuse approval to grow HT OSR. In the context of the earlier decline in farmland bird populations and the body of law protecting such birds, and against a background of affluence, high food security and the cultural value placed on birds in the UK (especially iconic species such as the skylark), it was not deemed necessary to test further risk hypotheses (which would have probably been an even more costly experiment). This conclusion was reached even though in the FSEs there were greater differences in arthropod biodiversity between different crops and between spring and winter OSR than between HT and non-HT pairs of the same crop. No weight was given to the possibility of mitigating potential impacts (e.g. setting aside areas farmed in a bird-friendly way) and other more damaging changes currently occurring in UK agriculture (e.g. ploughing grassland to grow forage crops) (Donald *et al.*, 2001). Thus the decision is particularly interesting in that it is not only different from that reached in countries where the yield-reducing effect of weeds is regarded as undesirable – i.e. low arable weed populations is an assessment endpoint - or additionally as in Australia, a potential source of invasive exotic species (CSIRO, 2003), but was derived from an incomplete risk assessment procedure and reflected the prevailing public attitude to farmland and wildlife. Above all it demonstrates very clearly the point made earlier that harm is not defined by science and is necessarily subjective.

Similarly the possibility that hybridisation between HT OSR and wild *B. rapa* might lead to a herbicide-tolerant weed problem – the scenario in Table 4 above – was sufficient for the authorities in several European countries to call for a ban on growing the HT crop when it was first evaluated (Gray and Raybould, 1999). In contrast, from the same science base the Canadian authorities concluded that hybridisation and gene flow which created herbicide-tolerant Brassica weeds was a risk that could be managed. Interestingly an early decision by CFIA did limit the growing of one particular HT plant, glyphosate-tolerant *B. rapa* event ZSR500/502 to western regions of Canada because of the presence of feral B. rapa in eastern Canada as a

weed of agriculture (CFIA, 1998). Today, as we have seen, almost 99% of the Canadian canola crop is herbicide-tolerant and the problem of herbicide-tolerant weeds and volunteers, including multiple-tolerant genotypes, is managed using a range of cultural practices including alternative herbicides (Beckie *et al.*, 2004).

The case study presented here, herbicide-tolerant oilseed rape, is an example of a familiar and well characterised trait in a widely-grown and familiar arable crop. HT OSR has been grown for at least 15 years and the assumption made at the beginning of this section, that it is substantially equivalent to non-tolerant OSR apart from its tolerance of herbicide, has been borne out around the world. Although the regulations pertaining to GM crops emphasise the value of a case-by-case assessment, it is important to utilise the huge database on this (and other) crops and to build on the vast experience gained from its cultivation. A problem formulation exercise for the introduction of HT OSR to a new country should therefore find that, actually, all or most of the information required to characterise the risk (the 'how can we assess whether they are likely to be harmed?' question) already exists. It is unlikely to be necessary to carry out more experiments to test specific risk hypotheses.

This will not be the case for many other crops and traits, and a problem formulation approach provides a way of identifying those data needed to test specific risk hypotheses. This is illustrated by Hokanson *et al.* (2010) in a case study of the possible introduction of a biofortified sorghum into Africa. Apart from the standard plant characterisation, which will help to address questions about the biology of the crop, the key problem was identified as the potential harm resulting from gene flow to wild and weedy relatives. Having postulated a series of risk scenarios from which risk hypotheses were generated, the study presents an analysis plan to compare the GM and non-GM sorghum for invasiveness (which could harm the genetic diversity of the wild relatives) and to compare GM and non-GM hybrids for key components of survival and reproduction (a GM hybrid having the envisioned potential to harm the crop and various valued wild plants and animals).

6. A FOOTNOTE TO PRACTITIONERS

In describing the process of problem formulation the question arises 'who should do it?' Is it the job of the regulator, problem formulation being a tried

and tested method used in other areas of risk assessment, or is it the job of the product developer or those who are applying to release the crop? The ideal answer is that both should be involved. However, since the sequence of the ERA for GM crops is very clearly: problem formulation → data collection → risk characterisation → risk evaluation (Figure 1), the onus to adopt such an approach from the beginning is on the product developer. The ideal situation is for product developers to begin a dialogue with risk assessors and other stakeholders as early as feasible in the development of the GM plant (notwithstanding the usual safeguards for commercial confidentiality). In this way problem formulation can help to prevent the costly collection and reporting of unnecessary data – that which does not inform the risk assessment – and to focus on those possible harms which are least understood. The value of utilising a wide range of expertise at this stage has already been emphasised, and is well illustrated by the sorghum study above. Arguably the most difficult stage of problem formulation is deriving specific protection goals and assessment endpoints from the broad environmental protection goals embedded in national and international legislation. Here the help of regulators and policy analysts is invaluable. Again ideally the assessment endpoints, and some way of characterising what would be harmful to them, should be identified before the risk assessment is made. However, as the HT OSR study illustrates, even in the most well-developed regulatory systems, agreed definitions of 'harm' are not always present or incontrovertible. For most practitioners the limitations to their risk assessment are less likely to come from the scientific process at the heart of problem formulation than from an inability to refine the definition of harm.

Acknowledgements

I have greatly benefited from discussing ERA and problem formulation over the years with a number of friends and colleagues, especially Alan Raybould, Wendy Craig, Paul Keese, Phil Macdonald, Morven Maclean, Jorg Romeis, Mark Tepfer and Mike Wilkinson. Information on plant characterisation was gained from Tom Nickson (Monsanto) and Don Mackenzie (Pioneer Hi-Bred International Inc.), the latter also helping me to understand the Canadian regulatory system. I am especially indebted to Andrew Roberts (Center for Environmental Risk Assessment, ILSI Research Foundation, Washington DC, USA) whose unpublished detailed case studies of oilseed rape in Canada were an excellent source of material for this paper. The approach and opinions in this paper, including the 'four questions', are my own and not necessarily those of CEH or any other organisation.

7. REFERENCES

Arnaud J-F, **Viard** F, **Delescluse** M & **Cuguen** J 2003. Evidence for gene flow via seed dispersal from crop to wild relatives in *Beta vulgaris* (Chenopodiaceae): Consequences for the release of genetically modified crop species with weedy lineages. *Proceedings of the Royal Society Series B – Biological Sciences* 270: 1565-1571.

Bartsch D, **Lehnen** M, **Clegg** J, **Pohl-Orf** M, **Schuphan** I & **Ellstrand** NC 1999. Impact of gene flow from cultivated beet on genetic diversity of wild sea beet populations. *Molecular Ecology* 8: 1733-1741.

Baker HG 1965. Characteristics and modes of origin of weeds. In: *The Genetics of Colonising Species*. Baker HG & Stebbins HG. Academic Press, London, UK. pp 147-172.

Beckie HJ, **Seguin-Swartz** G, **Nair** H, **Warwick** SI & **Johnson** E 2004. Multiple herbicide resistant canola can be controlled by alternative herbicides. *Weed Science* 52: 152-157.

Bullock JM 1999. Using population matrix models to target GMO risk assessment. Aspects of *Applied Biology* 53: 205-212.

Burke JM & **Rieseberg** LH 2003. The fitness effects of transgenic disease resistance in wild sunflowers. *Science* 300: 1250.

CFIA 1998. Determination of the Safety of Monsanto Canada Inc.'s Roundup® Herbicide-tolerant *Brassica rapa* Canola Lines ZSR500, ZSR502, and ZSR503. Canadian Food Inspection Agency (CFIA), Ottowa, Canada. Available at http://www.inspection.gc.ca/english/plaveg/bio/dd/dd9821e.shtml.

Conway G 1999. *The Doubly Green Revolution*: *Food for All in the 21st Century*. Cornell University Press, Ithaca NY, USA.

Conway G 2004. GM crops – An international perspective on the economic and environmental benefits. *Aspects of Applied Biology* 74: 3-24.

Craig W, **Tepfer** M, **Degrassi** G & **Ripandelli** D 2008. An overview of general features of risk assessments of genetically modified crops. *Euphytica* 164: 853-880.

Crawley MJ & **Brown** SL 1995. Seed limitation and dynamics of feral oilseed rape on the M25 motorway. *Proceedings of the Royal Society Series B - Biological Sciences 259*(1354): 49-54.

Crawley MJ, **Brown** SL, **Hails** RS, **Kohn** DD & **Rees** M 2001. Transgenic crops in natural habitats. *Nature* 409: 682-683.

Crawley MJ, **Hails** RS, **Rees** M, **Kohn** D & **Buxton** J 1993. Ecology of transgenic oilseed rape in natural habitats. *Nature* 363: 620-623.

CSIRO 2003. *Findings from the UK Farm Scale Evaluations of genetically modified herbicide tolerant crops – an appraisal of their implications for Australia*. Commonwealth Scientific and Industrial Research Organisation (CSIRO), Canberra, Australia. Available at http://www.csiro.au/proprietaryDocuments/CSIRO_FSE_appraisal.pdf.

Cummings CL & **Alexander** HM 2002. Population ecology of wild sunflowers: Effects of seed density and post-dispersal vertebrate seed predation on numbers of plants per patch and seed production. *Oecologia* 130: 274-280.

Davey VM 1939. Hybridisation in Brassicae and the occasional contamination of seed stocks. *Annals of Applied Biology* 26: 634-636.

deVries FT, **van der Meijden** R & **Brandenburg** WA 1992. Botanical Files. A study of real chances for spontaneous gene flow from cultivated plants to the wild flora of the Netherlands. *Gorteria*, Supplement No 1. Rijksherbarium, Leiden, Belgium.

Donald PF, **Green** RE & **Heath** 2001. Agricultural intensification and the collapse of Europe's farmland bird populations. *Philosophical Transactions of the Royal Society Series B – Biological Sciences* 268: 25-29.

EFSA 2010. Guidance on the environmental risk assessment of genetically modified plants. *EFSA Journal* 8(11): 1879. European Food Standards Agency (EFSA), Parma, Italy. Available at http://www.efsa.europa.eu/en/scdocs/doc/1879.pdf.

Ellstrand NC 2003. *Dangerous Liaisons? When Cultivated Plants Mate with Their Wild Relatives*. John Hopkins University Press, Baltimore (USA) and London (UK).

Ellstrand NC, **Prentice** HC & **Hancock** JF 1999. Gene flow and introgression from domesticated plants into their wild relatives. *Annual Review of Ecology and Systematics* 30: 539-563.

FAO 2011. Food and agricultural commodities production - Countries by commodity. Selected items: Rapeseed; 2009. FAOSTAT website. Food and Agriculture Organisation of the United Nations (FAO), Rome, Italy. Available at http://faostat.fao.org/site/339/default.aspx.

Fedoroff NV, **Battisti** DS, **Beachy** RN, **Cooper** PJM, **Fischoff** DA, **Hodges** CN, **Knauf** VC, **Lobeii** D, **Mazur** BJ, **Molden** D, **Reynolds** MP, **Ronald** PC, **Rosegrant** MW, **Sanchez** PA, **Vonshak** A & Zhu J-K 2010. Radically rethinking agriculture for the 21st century. *Science* 327: 833-834.

Franz JE, **Mao** MK & **Sikorski** JA 1997. Glyphosate: A Unique Global Herbicide. ACS *Monograph* 189. American Chemical Society, Washington DC, USA. pp 27-64.

Fuller RJ, **Gregory** RD, **Gibbons** DW, **Marchant** JH, **Wilson** JD, **Baillie** SR & **Carter** N 1995. Population declines and range contractions among lowland farmland birds in Britain. *Conservation Biology* 9: 1425-1441.

Garcia-Alonso M, **Jacobs** E, **Raybould** AF, **Nickson** TE, **Sowig** P, **Willekens** H, **van der Kouwe** P, **Layton** R, **Amijee** F, **Fuentes** AM & **Tencalla** F 2006. A tiered system for assessing the risk of genetically modified plants to non-target organisms. *Environmental Biosafety Research* 5: 57-65.

Godfray HCJ, **Beddington** JR, **Crute** I, **Haddad** L, **Lawrence** D, **Muir** JF, **Pretty** J, **Robinson** S, **Thomas** SM & **Toulmin** C 2010. Food security: The challenge of feeding 9 billion people. *Science* 327: 812-818.

Gray AJ 1986. Do invading species have definable genetic characteristics? *Philosophical Transactions of the Royal Society Series B – Biological Sciences* 314: 655-674.

Gray AJ 2000. The transfer of traits to wild relatives. In: *Predicting Field Performance in Crop Protection. Symposium Proceedings No 74.* British Crop Protection Council. Farnham, UK. pp 165-174.

Gray AJ 2004a. Ecology and government policies: The GM crop debate. *Journal of Applied Ecology* 41: 1-10.

Gray AJ 2004b. Hybridisation between crops and wild plants in the age of genetic engineering – New risks or new paradigms? *American Journal of Botany* 92(4): 169-172.

Gray AJ & **Raybould** AF 1999. *Environmental risks of herbicide-tolerant oilseed rape – A review of the PGS Hybrid Oilseed Rape*. UK Department of the Environment, Transport and the Regions. Research Report No 15. London, UK.

Gray AJ, **Daniels** RE, **Raybould** AF, **Cooper** JI, **Maskell** LC, **Pallet** D, **Edwards** M-L, **Thurston** M & **Alexander** M 2003. The conservation of genetic diversity: Gene flow from agriculture. In: *Methods for risk assessment of transgenic plants. IV. Biodiversity and Biotechnology*. Ammann K, Jacot Y & Braun R (eds), Birkhauser Verlag, Basel, Switzerland. pp 105-110.

Gregory PJ, **Ingram** JSI, **Andersson** R, **Betts** RA, **Brovkin** V, **Chase** TN, **Grace** PR, **Gray** AJ, **Hamilton** N, **Hardy** TB, **Howdena** SM, **Jenkins** A, **Meybeck** M, **Olsson** M, **Ortiz-Monasterio** AI, **Palm** CA, **Payne** T, **Rummukainena** M, **Schulze** RE, **Thiema** M, **Valentin** C & **Wilkinson** MJ 2002. Environmental consequences of alternative practices for intensifying crop production. *Agriculture, Ecosystems and Environment* 88: 279-290.

Hails RS & **Gray** AJ 2004. Developing protocols for risk assessment. *Aspects of Applied Biology* 74: 169-181.

Halfhill MD, **Sutherland** JP, **Moon** HS, **Poppy** GM, **Warwick** SI, **Weissing** AK, **Rufty** TW, **Raymer** PL & **Stewart** CN 2005. Growth, productivity and competitiveness of introgressed weedy *Brassica rapa* hybrids selected for the presence of Bt *cry1Ac* and *gfp* transgenes. *Molecular Ecology* 14: 3177-3189.

Hill RA 2005. Conceptualizing risk assessment methodology for genetically modified organisms. *Environmental Biosafety Research* 4: 67-70.

Hill RA & **Sendashonga** C 2003. General principles for risk assessment of living modified organisms: lessons from chemical risk assessment. *Environmental Biosafety Research* 2: 81-88.

Hokanson KE, **Ellstrand** NC, **Ouedraogo** JT, **Olweny** PA, **Schaal** BA & **Raybould** AF 2010. Biofortified sorghum in Africa: using problem formulation to inform risk assessment. *Nature Biotechnology* 28(9): 900-903.

Hubbell SP 2001. *The Unified Neutral Theory of Biodiversity and Biogeography.* Princeton University Press, USA.

James C 2011. *Global Status of Commercialized Biotech/GM Crops: 2010.* ISAAA Brief 42. Available at http://www.isaaa.org/resources/publications/briefs/42/executivesummary.

Johnson KL, **Raybould** AF, **Hudson** MD & **Poppy** GM 2007. How does scientific risk assessment fit within the wider risk analysis? *Trends in Plant Science* 12: 1-5.

Keeler KH 1989. Can genetically engineered crops become weeds? *Biotechnology* 7: 1134-1137.

Keese P 2008. Risks from GMOs due to horizontal gene transfer. *Environmental Biosafety Research* 7: 123-149.

Lawton JH 2007. Ecology, politics and policy. *Journal of Applied Ecology* 44: 465-474.

Lefol E, **Danielou** V & **Darmency** H 1996. Predicting hybridisation between transgenic oilseed rape and wild mustard. *Field Crops Research* 45: 153-161.

Lu B-L 2008. Transgene escape from GM crops and potential biosafety consequences: An environmental perspective. *Collection of Biosafety Reviews* 4: 66-141. Available at http://www.icgeb.org/biosafety/pdffiles/Bao-Rong.pdf.

Moyes CL, **Lilley** J, **Casais** C & **Dale** PJ 1999. Gene flow from oilseed rape to *Sinapis arvensis:* Variation at the population level. *Gene Flow. Symposium Proceedings* No 72. British Crop Protection Council, Farnham, UK. pp 143-148.

Naylor REL & **Lutman** PJ 2002. What is a weed? *Weed Management Handbook.* Blackwell, Oxford, UK. pp 1-15.

Nickson TE 2008. Planning environmental risk assessment for genetically modified crops. *Plant Physiology* 147: 494-502.

OECD 1986. *Recombinant DNA safety considerations*. Organisation for Economic Cooperation and Development (OECD), Paris, France. Available at http://www.oecd.org/dataoecd/43/34/40986855.pdf.

OECD 1993. *Safety considerations for biotechnology: Scale-up of crop plants.* Organisation for Economic Cooperation and Development (OECD), Paris, France. Available at http://www.oecd.org/dataoecd/26/261958527.pdf.

OECD 1997. *Consensus document on the biology of Brassica napus L (oilseed rape). Series on Harmonization of Regulatory Oversight in Biotechnology No 7*. Organisation for Economic Cooperation and Development (OECD), Paris, France. Available at http://www.oecd.org/dataoecd/28/22/27531440.pdf.

OECD 2003. *Consensus document on the biology of Zea mays subsp. mays (maize). Series on Harmonization of Regulatory Oversight in Biotechnology No 27*. Organisation for Economic Cooperation and Development (OECD), Paris, France. Available at http://www.oecd.org/officialdocuments/displaydocumentpdf?cote=env/jm/mono(2003)11&doclanguage=en.

OGTR 2008a. *The Biology of Brassica napus L (canola). Version 2*. Office of the Gene Technology Regulator (OGTR), Canberra, Australia. Available at http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/canola-3/$FILE/BiologyCanola2011.pdf.

OGTR 2008b. *The biology of Zea mays L. ssp mays (maize or corn)*. Version 1. Office of the Gene Technology Regulator (OGTR), Canberra, Australia. Available at http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/maize-3/$FILE/biologymaize08_2.pdf.

Pretty J 2001. The rapid emergence of genetic modification in world agriculture: Contested risks and benefits. *Environmental Conservation* 28(3): 248-262.

Raybould AF 2006. Problem formulation and hypothesis testing for environmental risk assessments of genetically modified crops. *Environmental Biosafety Research* 5: 119-125.

Raybould AF 2010. Reducing uncertainty in regulatory decision-making for transgenic crops. More ecological research or clearer environmental risk assessment? *GM Crops* 1: 25-31.

Raybould AF 2011. The bucket and the searchlight: Formulating and testing risk hypotheses about the weediness and invasive potential of transgenic crops. *Environmental Biosafety Research* 9: 123-133.

Raybould AF & **Cooper** JI 2005. Tiered tests to assess the environmental risk of fitness changes in hybrids between transgenic crops and wild relatives: The example of virus resistant *Brassica napus*. *Environmental Biosafety Research* 4: 125-140.

Raybould AF & **Gray** AJ 1993. Genetically modified crops and hybridisation with wild relatives: A UK perspective. *Journal of Applied Ecology* 30: 199-219.

Raybould AF, **Moyes** CL, **Maskell** LC, **Mogg** RJ, **Warman** EA, **Wardlaw** JC, **Elmes** GW, **Edwards** M-L, **Cooper** JI, **Clarke** RT & **Gray** AJ 1999. Predicting the ecological impacts of transgenes for insect and virus resistance in natural and feral populations of Brassica species. In: *Methods for risk assessment of transgenic plants. III Ecological risks and prospects of transgenic plants*. Ammann K, Jacot Y, Simonsen V & Kjellsson G (eds), Birkhauser, Basel, Switzerland. pp 3-15.

Romeis J, **Bartsch** D, **Bigler** F, **Candolfi** MP, **Gielkens** MMC, **Hartley** SE, **Hellmich** RL, **Huesing** JE, **Jepson** PC, **Layton** R, **Quemada** H, **Raybould** AF, **Rose** RI, **Schiemann** J, **Sears** MK, **Shelton** AM, **Sweet** J, **Vaituzis** Z & **Wolt** JD 2008. Assessment of risk of insect-resistant transgenic crops to nontarget arthropods. *Nature Biotechnology* 26: 203-208.

Romeis J, **Hellmich** RL, **Candolfi** MP, **Carstens** K, **De Schrijver** A, **Gatehouse** AMR, **Herman** RA, **Huesing** JE, **McLean** MA, **Raybould** AF, **Shelton** AM & **Waggoner** A 2011. Recommendations for the design of laboratory studies on non-target arthropods for risk assessment of genetically engineered plants. *Transgenic Research* 20: 1-22.

Royal Commission on Environmental Pollution 1989. *The release of genetically engineered organisms to the environment*. 13th Report RCEP, HMSO, London, UK.

Royal Society 2009. *Reaping the Benefits: Science and the Sustainable Intensification of Global Agriculture*. Royal Society, London, UK. Available at http://royalsociety.org/uploadedFiles/Royal_Society_Content/policy/publications/2009/4294967719.pdf.

Saji H, **Nakajima** N, **Aono** M, **Tamaoki** M, **Kubo** A, **Wakiyama** S, **Hatase** Y & **Nagatsu** M (2005). Monitoring the escape of transgenic oilseed rape around Japanese ports and roadsides. *Environmental Biosafety Research* 4: 217-22.

SCBD 2000. *Cartagena Protocol on Biosafety to the Convention on Biological Diversity. Text and Annexes*. Secretariat of the Convention on Biological Diversity (SCBD), Montreal, Canada. Available at http://www.cbd.int/doc/legal/cartagena-protocol-en.pdf.

Scheffler JA & **Dale** PJ 1994. Opportunities for gene transfer from transgenic oilseed rape (*Brassica napus*) to related species. *Transgenic Research* 3: 263-278.

Sears MK, **Hellmich** RL, **Stanley-Horn** DE, **Oberhauser** KS, **Pleasants** JM, **Mattila** HR, **Siegfried** BD & **Dively** GP 2001. Impact of Bt corn pollen on monarch butterfly populations: a risk assessment. *Proceedings of the National Academy of Science USA 98*(21): 11937-11942.

Snow AA, **Pilson** D, **Rieseberg** LH, **Paulsen** MJ, **Pleskac** N, **Reagon** MR, **Wolf** DE & **Selbo** SM 2003. A Bt transgene reduces herbivory and enhances fecundity in wild sunflowers. *Ecological Applications* 13: 279-286.

Stewart CN, **Halfhill** MD & **Warwick** SI 2003. Transgene introgression from genetically modified crops to their wild relatives. *Nature Reviews Genetics* 4: 806-817.

Tester M & **Langridge** P 2010. Breeding technologies to increase crop production in a changing world. *Science* 327: 818-822.

Thomas P (2003). Canola Growers Manual. 2003 Edition. Canola Council of Canada, Winnipeg, Canada. Available at http://www.canolacouncil.org/canola_growers_manual.aspx.

Tiedje JM, **Colwell** RK, **Grossman** YL, **Hodson** RE, **Lenski** RE, **Mack** RN

& **Regal** PJ 1989. The planned introduction of genetically engineered organisms: ecological considerations and recommendations. *Ecology* 70: 298-315.

U N 1935. Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. *Japanese Journal of Botany* 7: 389-452.

USEPA 1998. *Guidelines for Ecological Risk Assessment.* EPA/630/R-95/002F. April 1998 Final. United States Environmental Protection Agency (USEPA), Washington DC, USA. Available at http://www.epa.gov/raf/publications/pdfs/ECOTXTBX.PDF.

Veltman CJ, **Nee** S & **Crawley** MJ 1996. Correlates of introduction success in exotic New Zealand birds. *American Naturalist* 147(4): 542-557.

Virtue JG & **Melland** RL 2003. *The Environmental Weed Risk of Revegetation and Forestry Plants.* DWLBC Report 2003/02. Department of Water, Land and Biodiversity Conservation, South Australia. Available at www.dwlbc.sa.gov.au

Warwick SI, **Sumard** MJ, **Legere** A, **Beckie** HJ, **Zhu** B, **Mason** P, **Seguin-Swartz** G & Stewart CN 2003. Hybridisation between transgenic Brassica napus L and its wild relatives: *Brassica rapa L, Raphanus raphanistrum L, Sinapis arvensis L, and Erucastrum gallicum (Willd.) OE Schulz. Theoretical and Applied Genetics* 107: 528-539.

Watkinson AR, **Freckleton** RP, **Robinson** RA & **Sutherland** WJ 2000. Predictions of biodiversity response to genetically modified herbicide-tolerant crops. *Science* 289: 1554-1557.

Williamson M 1996. *Biological Invasions*. Chapman & Hall, London, UK.

Wilkinson MJ & **Tepfer** M 2009. Fitness and beyond: preparing for the arrival of GM crops with ecologically important novel characters. *Environmental Biosafety Research* 8: 1-14.

Wilkinson MJ, **Davenport** J, **Charters** YM, **Jones** AE, **Allainguillaume** J, **Butler** HT, **Mason** DC & **Raybould** AF 2000. A direct regional scale estimate

of transgene movement from GM oilseed rape to its wild progenitors. *Molecular Ecology* 9: 983-991.

Wolt JD, **Keese** P, **Raybould** AF, **Fitzpatrick** JW, **Burachik** M, **Gray** AJ, **Olin** SS, **Schiemann** J, **Sears** M & **Wu** F 2010. Problem formulation in the environmental risk assessment for genetically modified plants. *Transgenic Research* 19: 425-436.