

An overview of general features of risk assessments of genetically modified crops

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Abstract The intentional introduction into the environment or market of genetically modified organisms (GMOs) is nearly always governed by a framework of science-based risk assessment and risk management measures. This is usually implemented through the integration of hazard identification and characterisation of all of the elements of risk associated with a new GM crop or derived product. Typical categories of hazards arising from the introduction of transgenic crops include: possible unintended negative health effects in a susceptible subgroup of the consumer (target) population; the evolution of resistance in the targeted pest/pathogen populations when the transgene confers resistance to a pest or pathogen; non-target hazards associated directly or indirectly with the transgenic plant or transgene product outside the plant; and those associated with the integration and subsequent expression of the transgene in a different organism or species following gene flow. The consequences of likely exposure to these and other hazards are considered in this introduction to the main issues

raised when evaluating the possible risks arising from the importation or cultivation of genetically modified crops.

Keywords Biosafety · GMO · GM crops · Risk assessment · Review

Introduction

Society is increasingly conscious of the harm that its activities can cause to the environment, and of the resultant impact it can have on wildlife and the quality of life of others. Lately, risk assessments (RAs) have been widely adopted by the business, regulatory and financial sectors in efforts to alleviate or avoid further harm. However, to date the definition of “environmental harm” has yet to be unequivocally agreed. A formalised framework of science-based risk assessment and risk management measures usually governs the intentional introduction into the environment or market of genetically modified organisms (GMOs). In this respect, the objective of an RA is, on a case-by-case basis, to identify and evaluate potential adverse effects of a GMO. The effects considered can have either a direct or indirect, immediate or delayed, impact on the environment or human and animal health (European Parliament 2001). An RA is also conducted with a view to determining if there is a need for risk management,

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and if so, the most appropriate methods to be used. The results of the RA can then be used by the relevant competent authority to make an informed decision regarding whether or not to give approval for import or cultivation.

The aim of this review is to provide an introduction to some of the main considerations made in the compilation and evaluation of RAs, as well as to indicate sources of useful information. This is not limited to the peer-reviewed scientific literature, but also includes documents published by national competent authorities and various international organisations. This review will focus on generic issues that will need to be addressed for commercial release of nearly all GM crops; of course much less information is required for small-scale pre-commercial field trials, and concerns that are specific to each transgene of interest will only be touched upon briefly. Although there is a broad worldwide consensus on the types of information upon which a RA is built, there is considerable national and regional variation in emphasis, and in the depth of information required by the competent authority. Certain competent authorities consider some of the issues presented here to be more properly questions of product quality rather than of safety, and are as such excluded from the RA. Nonetheless, for completeness, issues that may or may not be included in an RA are included in this review.

What is a risk assessment (RA)?

In practice, an RA is an iterative process that involves proceeding through several steps of assessment prior to obtaining results with an acceptable level of uncertainty. It should be noted that absolute certainty or zero risk in a safety assessment is never achievable, so uncertainty is an inescapable aspect of all risk assessment and risk management (NRC 1993; OECD 1993; Hill and Sendashonga 2003). The level of uncertainty that is acceptable may also depend greatly on social, economic and political factors (Johnson et al. 2007; Wilkinson and Ford 2007). The process usually consists of a series of pre-planned and prescribed sets of risk assessments of progressively increasing data and resource intensity (USA EPA 1998a; Hill and Sendashonga 2003; EFSA 2004). For example, when a certain potential adverse effect is considered in an RA, it is necessary to present a scenario describing the scientifically supportable

chain of causal events through which the specific effect might occur, an evaluation of the state of knowledge concerning the plausibility of each step of the chain, and finally a determination made as to whether the consequences are acceptable. In general, approaches (Box 1) are based either on a 'weight of evidence' (CAC 2003a; USA EPA 2005b; Prescott and Hogan 2006), hierarchical decision trees (FAO/WHO 2001) or a tiered system (Hill and Sendashonga 2003; Wilkinson et al. 2003a; EFSA 2004; Andow and Zwahlen 2006; Raybould and Cooper 2006).

RA specific to GM crops

The sequential steps in the risk assessment of a GMO begin with the identification of potential adverse environmental effects or hazards that could arise

Box 1 Theoretical approaches to risk assessment

- *Weight of evidence*—considers the sum total of the studies that comprise the safety and nutritional assessment of the GM crop to characterise risk. It provides plausible conclusions about risk made on the basis of available information, along with the scientific evidence supporting those conclusions, and describes major sources of uncertainty and alternative views. Additionally, it respects the objective, scientific basis of risks and procedures for making inferences in the absence of adequate data (CRARM 1997). Three principal categories of factors considered are: (1) the adequacy and quality of data; (2) the degree and type of uncertainty associated with the evidence; and (3) the relationship of the evidence to the risk assessment questions (US EPA 1998a).
- *Hierarchical decision tree*—consists of a flow diagram of decisions and their possible consequences, (including resource costs and risks) used to create a plan to reach a goal. It is a predictive model based upon a branching series of tests, with the result of each test used to determine the next test to apply, in order to help depict the information necessary to make decisions.
- *Tiered system*—the first step or 'tier' is typically conservative, and serves to narrow the scope of the assessment to the main issues of concern. It is usually representative of the "worst case scenario". The outcome of a given tier is to either make a management decision, or to request additional information to address issues of continuing concern, and from which a subsequent iteration of the risk assessment will follow (US EPA 1998a). For each tier, if the resultant level of risk is below that which triggers concern, then no further testing is necessary, otherwise a higher tier test ("less worse case") is required, which has a new trigger value to account for the greater realism introduced into the test (Raybould and Cooper 2005).

from its release, then an evaluation of the potential consequences, followed by a determination of the probability of them occurring. The risk posed by individual characteristics of the GMO can then be estimated, along with measures proposed to minimise or mitigate them. Generally, two types of hazards from the release of transgenic crop plants can be identified: (1) unintended effects in the *target* population, for example possible negative consequences in the health of the end-consumer, or the evolution of resistance in the targeted pest/pathogen populations if the transgene confers resistance to a pest or pathogen, and (2) unintended effects in *non-target* populations, for example changes in local biodiversity associated directly or indirectly with the transgenic plant or transgene product outside the plant, or those associated with the integration and subsequent expression of the individual transgene in a different organism or species following gene flow. However, a fully integrated approach to the hazard assessment and characterisation of all elements involved in producing a new GM variety is recommended, as variously outlined by, *inter alia*, USA APHIS (1996), ASEAN (2003), König et al. (2004), EFSA (2004), and CFIA (2005).

Information resources

There is a great deal of existing information that is relevant to risk assessment of GMOs, including basic ecological information and experience with both modified and non-modified organisms. This information needs to be carefully considered in RA (Box 2). Nevertheless, since RA is carried out on a case-by-case basis, the information needed to support it will vary, and there may often be limitations to information for particular types of organisms and particular types of receiving environments. In cases where certain information considered to be important for an RA does not exist, it may be necessary to generate further empirical data, for example through laboratory and/or field studies. This need may arise, for example, when considering risks in a new receiving environment about which there is limited basic biological and physical knowledge relevant for the RA.

Excellent sources of relevant information are the scientific literature and RA dossiers previously

Box 2 Examples of considerations for data presented or evaluated in RA dossiers

- Are the presented characterisation data adequate to describe the introduced trait for risk assessment purposes?
- Are the data relevant to the risk?
- Is the methodology appropriate? Is it up-to-date? Has it been validated?
- Is the study design adequate?—including the duration and sufficiency of the testing, whether or not there is a dose response curve, etc.
- Are the statistical analyses appropriate for the data sets and are the conclusions statistically justified?
- How reproducible are the data?
- Does the totality and the weight of the evidence support the conclusions made?

submitted to, or authored by, competent authorities concerning applications for the deliberate release of GM crop plants. Many of these latter documents can be found online at numerous sites, such as the Risk Assessment Search Mechanism (RASM; <http://www.icgeb.org/biosafety/db/rasm.php>) of the International Centre for Genetic Engineering and Biotechnology (ICGEB), or relevant regulatory authority websites or national biosafety clearing houses (Table 1). An examination of a number of these documents makes it apparent that the format of a “typical” RA dossier closely follows internationally agreed guidelines (UNEP 1995; OECD 2000a; CAC 2003a). In general, a dossier takes into account the biological properties of the donor and host organisms. It includes information on how the introduced gene(s) is (are) expressed in the GM plant and the nature of the gene product(s). Compositional and nutritional characteristics of the crop are considered, including information regarding the intended and any unintended effects caused by the modification, and any potential toxicity and allergenicity of gene products, plant metabolites and the GM plant as a whole. In addition, the agronomic characteristics of the GM plant are also taken into account, as well as its likely performance and impact in the environment where it is to be released. Further, information on the eventual use of the product is also provided, including the influence of processing on the properties of food or feed, any potential for changes in dietary intake, and any possible detrimental long-term nutritional impact. These issues are discussed more thoroughly below.

Table 1 Relevant regulatory authority websites or national online biosafety clearing houses (BCH)

Country/Region	Authority ^a	Website address
Argentina	CONABIA	http://www.sagpya.mecon.gov.ar/new/0-0/programas/conabia/index.php
Australia	OGTR	http://www.ogtr.gov.au/
Australia & New Zealand	FSANZ	http://www.foodstandards.gov.au/
Brazil	CTNBio	http://www.ctnbio.gov.br/
Cambodia	BCH	http://www.cambodiabiosafety.org/
Canada	CFIA	http://www.inspection.gc.ca/english/toce.shtml
	Health Canada	http://www.hc-sc.gc.ca/index_e.html
China	BCH	http://english.biosafety.gov.cn/
Colombia	BCH	http://www.bch.org.co/bioseguiridad/index.jsp
Costa Rica	BCH	http://cr.biosafetyclearinghouse.net/
Europe Union	JRC	http://gmoinfo.jrc.it
	EFSA	http://www.efsa.eu.int/
India	BCH	http://www.indbch.nic.in/
Iran	BCH	http://biosafety.irandoe.org/BCH/domestic.htm
Korea	BCH	http://www.biosafety.or.kr/
Mexico	CIBIOGEM	http://www.cibiogem.gob.mx/
New Zealand	ERMA	http://www.ermanz.govt.nz/
Norway	Dir. for Nat.	http://english.dirnat.no/
The Philippines	NCBP	http://www.ncbp.dost.gov.ph/
Japan	BCH	http://www.bch.biodic.go.jp/english/e_index.html
Switzerland	SECB	http://www.umwelt-schweiz.ch/buwal/eng/fachgebiete/fg_efbs/index.html
Thailand	BCH	http://www.biosafety.biotec.or.th/?idgroup=-1519023173
USA	unified	http://usbiotechreg.nbii.gov/

^a *Acronyms.* Comisión Nacional Asesora de Biotecnología Agropecuaria (CONABIA); Office of the Gene Technology Regulator (OGTR); Food Standards Australia New Zealand (FSANZ); National Biosafety Technical Commission (CTNBio); Canadian Food Inspection Agency (CFIA); Joint Research Council Biotechnology & GMOs Information Website (JRC); European Food Safety Authority (EFSA); Mexican Comisión Intersecretarial de Bioseguridad de los Organismos Genéticamente Modificados (CIBIOGEM); Environmental Risk Management Authority New Zealand (ERMA); Norwegian Directorate for Nature Management (Dir. for Nat.); National Committee on Biosafety of the Philippines (NCBP); Swiss Expert Committee for Biosafety (SECB); US Regulatory Agencies Unified Biotechnology Website (unified)

Molecular description of the GM plant

The first section of an RA is usually devoted to the molecular description of the GM plant. Sufficient information on the process used to effect the genetic modification is required to enable an assessment of both direct and potential secondary effects (Box 3). The introduction of new genetic material into plant genomes has the potential to cause unintended changes in phenotype or composition due to pleiotropic effects or to alteration of the genome at the site of insertion (CAC 2003a; Cellini et al. 2004; Latham et al. 2006; Rischer and Oksman-Caldentey 2006). Specific examples of unanticipated effects arising in transgenic crops have been reviewed (Haslberger

2003; Cellini et al. 2004). Unintended effects may or may not prove to have relevance in terms of product safety, but must be taken into account when assessing risk. The type of information that can be requested by the competent authority will relate to the detailed molecular characterisation of the inserted DNA, as well as host genomic flanking sequences, e.g. the nature and source of vector used, including maps of all functional elements, restriction sites for probes, PCR primer positions and sequences, and a table of descriptions and functions, sources and construction, insertion site(s) and copy numbers of all inserted DNA (transgenes, regulatory sequences, vector backbone) (European Parliament 2001; UK ACRE 2001a; CFIA 2005). Many authorities require the inserted

DNA to be mapped to the level of the base sequence. Worth bearing in mind is that insertional effects are related to the specific transformation event, and not to the function of the inserted transgene, and that, as with random mutation, most unintended changes caused by deliberate insertions of genetic material will be, at best, neutral to the plant. It would be extremely rare for a significant improvement in ecological fitness or substantial compositional changes to result (Petersen et al. 2005).

Surprisingly, concerns have been raised with regard to any dietary effects on human or animal health derived from exposure to recombinant DNA (rDNA). All foods from plants and animal sources contain DNA, with humans consuming between 0.1 and 1.0 g of DNA per day (Doerfler 2000). By combining data concerning consumption and intake with that of recombinant DNA (rDNA) content in currently available GM crops, Jonas et al. (2001) have estimated that rDNA will comprise 0.00006% of a typical daily DNA intake of 0.6 g. To date, given

that there are no reports of DNA being toxic or allergenic, and the very long human history of DNA consumption from a wide variety of sources, it is concluded that such consumption poses no significant risk to human health, and that additional ingestion of rDNA, which is chemically indistinguishable from non-modified DNA, has no effect (OECD 2000; FAO/WHO 2000; Jonas et al. 2001; UK ACRE 2002; USA SOT 2002; Royal Society 2002; Van den Eede et al. 2004). Similarly, the risks to human health associated with the use of specific viral DNA sequences in GM plants are deemed to be negligible (Royal Society 2002). Nor are any deleterious environmental effects expected from the presence of rDNA itself in transgenic crops, as recognised by the United States Environmental Protection Agency (USA EPA) when it granted blanket exemption for all nucleic acids used in Plant-Incorporated Protectants (USA EPA 2001a).

Antibiotic resistance genes

Risk assessments of GM crops often include the evaluation of the impact of antibiotic resistance markers genes (ARMGs) used as selectable markers. The concern is that such markers, when introduced into food or into the environment, could drive the evolution of drug-resistant bacteria by functioning as sources of antibiotic resistance for the commensals (normal resident flora) in the body of humans or other animals, or for pathogens of humans or other animals. It is recommended (USA FDA 2001) that the use of ARMGs in crops be evaluated on a case-by-case basis, taking into account whether:

- the antibiotic is an important medication
- it is frequently used
- it is orally administered
- it is unique
- there would be selective pressure for transformation to take place
- the natural level of resistance to the antibiotic present in bacterial populations

If an evaluation of the data and information suggests that the presence of the marker gene or gene product in food or feed could compromise the use of the relevant antibiotic(s), it is recommended that the ARMG not be present in the finished food or feed.

Box 3 Examples of specific concerns regarding the transgenic insert include

- Is the actual insert the intended insert? Are any partial inserts present? Have any deletions occurred?
- Did any modifications occur that affect the product amino acid sequence? If 'yes', this will trigger an associated risk assessment.
- Are 5' & 3' flanking/junction sequences provided? This information is necessary for (a) GM detection & traceability, and (b) identification of any insertion/interruption into flanking host DNA, and any potential to produce novel chimaeric proteins.
- Are vector sequences present in the insert, especially origins of replication and antibiotic resistance genes? Concerns have arisen regarding the possibility of the generation of modified disease organisms through horizontal gene transfer of the vector sequences to known pathogens.
- Is (Are) the inserted gene(s) inducible or constitutive? For inducible gene(s), the inducing agent should be identified. Where there is an intention for restriction of expression of the inserted gene(s), detailed information on expression may be required. The mechanism whereby expression is restricted should be detailed, along with information assuring the stability of the restriction of expression.
- Are selectable markers present in the final product? If 'yes', they will also need to be evaluated for safety.
- Do(es) the insert(s) segregate as predicted by its (their) location?

Where particular antibiotics are the only drug available to treat certain clinical conditions (e.g. vancomycin for the treatment of certain staphylococcal infections), the recommendation is that marker genes that encode resistance to such antibiotics should not be used in transgenic plants.

The key issues relating to the biosafety of the use of ARMGs in GM plants have recently been reviewed (Bennett et al. 2004; Goldstein et al. 2005; Ramessar et al. 2007). After considering all bacterial DNA transfer systems and possible mechanisms of recombination that together might productively transfer ARMGs from GM plants to bacterial cells, no credible scenario whereby new drug-resistant bacteria would be created has been identified. It is therefore concluded that the risk of transfer of ARMGs from GM plants to bacteria is remote, and that the hazard arising from any such gene transfer is, at worst, slight. These findings are echoed by many competent authorities and international organisations (for example, NZ ERMA 2000; FAO/WHO 2000; OECD 2000b; USA FDA 2001; CAC 2003a; EFSA 2007).

Protein coded by the transgene

Continuing the molecular characterisation of GM plants, concerns have been raised regarding the expressed GM-derived proteins (Box 4). For instance, expression of the introduced gene, through protein synthesis, may reduce the availability of amino acids used for synthesis of normal plant compounds. Although this is unlikely to be a factor for nuclear transformants, where typically the transgene-encoded protein is at maximum on the order of a few percent of total soluble protein (TSP), the question becomes of potential importance when the plastid genome is transformed, since this can lead to transgene-encoded protein accumulating up to 46 % of TSP (De Cosa et al. 2001), but more typically as 5–25% of TSP (Vitanen et al. 2004; Daniell et al. 2005). The production of normal plant compounds might also be affected if the expressed protein modifies the availability of substrates for important metabolic pathways. For instance, the complexity of the changes in carotenoids observed in “golden rice” was not entirely expected (Schaub et al. 2005). In addition, either the expressed protein or altered levels

of other proteins might have anti-nutritional effects (OECD 2000b). Addressing the possibility of the presence of host plant homologues to introduced transgenes, these can be identified using the Sequence Platform for the Phylogenetic Analysis of Plant Genes (SPPG, <http://www.bioinformatics.psb.ugent.be/cgi-bin/SPPG/index.html>; Flanders Interuniversity Institute for Biotechnology (VIB), Belgium), which combines expressed sequence tag (EST) sequence data with protein information from 32 different plant species (Vandepoele and Van de Peer 2005).

Biosafety considerations (Box 3) have been thoroughly discussed for a number of genes of microbial origin commonly used in plant biotechnology, for example selectable and scorable markers such as antibiotic-, herbicide- and insect-resistance genes. They have concluded that, *inter alia*, the genes encoding phosphinothricin acetyl transferase (*BAR*/

Box 4 Specific concerns regarding the GMO-derived protein include

Concerns indirectly related to the transgene-encoded protein

- Are any fusion proteins created between fragmentary inserted donor sequences and recipient sequences, and expressed? i.e. has the transgene been inserted into the coding region of a gene of the host organism, leading to a truncated or hybrid gene product whose function is altered, impaired or lost?
- Have overall gene expression patterns been altered? Possibly through the random insertion of the transgene into the regulatory region of a host gene, or by affecting the gene of a regulatory protein thereby affecting other genes.
- Is the introduced gene functionally equivalent to an existing host gene?—e.g. a herbicide tolerance transgene may be similar to a host gene involved in aromatic amino acid synthesis, therefore analyses of protein content and amino acid composition triggered.

Concerns directly related to the transgene-encoded protein

- Is the new protein homologous to any known allergens? Are there short (6–8) amino acid segments identical to known allergens? Is the protein predicted to be degraded rapidly in the digestive tract? If the host plant expresses allergens, is their level of accumulation different in the GM plant compared to the non-GM control?
- Is the introduced protein homologous to any known anti-nutritionals or lectins?

Concerns of importance when trait stability raises safety issues

- Is the new protein developmentally and stably expressed during the life cycle of the plant?
 - Is the introduced trait stable over a number of generations under representative environmental conditions?
-

PAT), various *Bacillus thuringiensis* (*Bt*)-derived toxins (*CRY*), glyphosate resistance (*CP4 EPSPS*), glyphosate oxidoreductase (*GOX*), β -glucuronidase (*GUS*), hygromycin phosphotransferase (*HPT*), neomycin phosphotransferase (*NPTII*), phosphomannose isomerase (*PMI*) and their products present little, if any, risk in terms of toxicity, allergenicity, pleiotropic effects, horizontal gene transfer, food and/or feed safety, and environmental safety (Miki and McHugh 2004; Van den Eede et al. 2004; Herouet et al. 2005; Kleter et al. 2005; Petersen et al. 2005; USA EPA 2005a). This is variously supported in statements by competent authorities and international organisations (see Table 2).

The stability of the trait may also be a safety concern. For instance, this would be the case for GM crops with reduced levels of allergens or anti-nutritional compounds. There are also cases in which changes in the concentration of the transgene-encoded protein may be of great importance in making an accurate ecological risk assessment. For example, growth conditions (Fitt 1994) and parental background (Adamczyk and Sumerford 2001; Kranthi et al. 2005) may cause sharp decreases in season-long *Bt* toxin expression in transgenic cotton, thereby impacting resistance management. In these cases, data should address how growth conditions affect expression of the desired trait. Stability may be affected by the physiological condition of plants producing transgenic proteins in regard to light quality and intensity, water and nutrient availability and presence of other stresses (e.g. heat, pests, diseases, etc.).

The need to consider safety issues at the earliest stages of GM product conceptualisation is being promoted to avoid or minimise the inclusion and expression of superfluous transgenes or sequences, and their possible dispersal in the environment (FAO/WHO 2000; UK ACRE 2001b; EFSA 2004). Considering these principles early in the development of a GM plant can help to refine the scope of the safety evaluation by eliminating unnecessary risk assessment. Likewise, avoiding expression of transgenes in parts of the plant where the gene product is not needed will reduce the potential for known or unanticipated effects on non-target organisms. Additionally, GM plants could be designed to reduce unwanted environmental risks by incorporating specific genetic features, for example the absence of

Table 2 Documents containing information and data relevant to undertaking risk assessments on the listed genes

Gene	Reference ^a
Herbicide resistance	
<i>bar/pat</i>	OECD (1999b); UK ACRE (2000); OECD (2002); CFIA (2004)
<i>epsps</i> $\pm$ <i>gox</i>	OECD (1999a)
Insect resistance	
<i>Bacillus thuringiensis</i> —derived insect control proteins	OECD (2007)
<i>cry</i> genes	IPCS (1999)
<i>cryIAb</i>	USA EPA (1996a, 2001b, 2005m)
<i>cryIAc</i>	USA EPA (1997a, 2001b)
<i>cryIAc</i> and <i>cry2Ab</i>	APVMA (2003)
<i>cryIF</i>	USA EPA (2001b, c, 2002a, 2005c, j)
<i>cryIF</i> + <i>cryIAc</i>	USA EPA (2005d)
<i>cry2Ab2</i>	USA EPA (2002b, 2004a)
<i>cry3A</i>	USA EPA (1995, 2001b)
modified <i>cry3A</i>	USA EPA (2005e)
<i>cry3Bb1</i>	USA EPA (2004b, 2005h, m)
<i>cry3Bb1</i> + <i>cryIAb</i>	USA EPA (2005i)
<i>cry9C</i>	USA EPA (1998b)
<i>cry34Ab1</i> and <i>cry35Ab1</i>	USA EPA (2002a, 2005f, k, l)
<i>vip3a</i>	USA EPA (2005g)
Virus resistance	
coat protein (CP) genes	OECD (1996)
Cucumber Mosaic Virus CP gene	USA EPA (1997b)
Papaya Ringspot Virus CP gene	USA EPA (1997c)
Potato Leaf Roll Virus orf1/orf2	USA EPA (1999b, 2000)
Potato Virus Y CP gene	USA EPA (1997d)
Watermelon Mosaic Virus-2 and Zucchini Yellow Mosaic Virus CP genes	USA EPA (1997e)

^a *Acronyms.* UK ACRE = Advisory Committee on Releases to the Environment, UK; APVMA = Australian Pesticides and Veterinary Medicines Authority; CFIA = Canadian Food Inspection Agency; IPCS = International Program on Chemical Safety; OECD = Organisation for Economic Co-operation and Development, Paris, France; USA EPA = United States Environmental Protection Agency, USA

undesirable selectable markers, inducible rather than constitutive gene expression, sterility, and reduced fitness (Snow et al. 2005).

Biological characteristics of the crop plant

The environmental effects of a transgenic crop will depend on the characteristics of the organism, the environmental system in which the organism is placed, and the skill with which it is managed. In short, whether a transgenic crop will benefit or adversely affect the environment may depend on the nature of the crop, as well as the location and the management of its cultivation (Ervin et al. 2000).

In determining any potential risks associated with the cultivation of a transgenic crop, the first step is acquisition of sufficient data to characterise the modified plant and permit a comparison with the unmodified counterpart (Stewart et al. 2000; Shewry et al. 2007). A sound understanding of the biology of an organism is required, as well as its relationship with the environment in which it is to be released. A number of online biology reference documents (Table 3) are available, specific to over 30 commonly cultivated crops throughout the world. Each document is intended to provide background information on the biology of a particular plant species, its centre of origin, its related species, the potential for gene introgression from the plant into relatives, as well as details on the organisms with which it interacts. Such species-specific information serves as a guide for addressing some of the information requirements of RAs (Box 5). Specifically, this information is used to determine whether there will be significantly different interactions with other organisms resulting from the cultivation of a GM crop, which could potentially cause it to become a weed of agricultural or natural habitats, or otherwise interact differently than their non-GM counterpart in the environment. An evaluation of toxicity in the environmental assessment is also undertaken, but is based on possible toxic effects due to incidental consumption or exposure to specific plant material (e.g. pollen), and is therefore different from the food safety assessment which looks at, among others, toxic effects in the case of normal (frequent) food consumption or exposure (addressed later).

The documents listed in Table 3 also provide descriptions of the characteristics of the plant species in question, such as habitat, fertility, dispersal, and endogenous toxins, as well as including information concerning the plant species' major interactions with other organisms in its production range (e.g.

Box 5 Examples of specific concerns regarding GM crop biology include

- Is there any increase in horizontal gene transfer potential?—due to the presence of bacterial sequences (origins of replication, homologous sequences, etc.) in the insert?
- Does the GM plant differ from the parental or near isogenic non-GM plant in its biology? (e.g. multiplication, dormancy, survivability, dispersal, outcrossing ability, stress tolerance, sensitivity to specific agents)—unintended effects may manifest themselves through gross changes to agronomic traits.
- Does it have increased weediness or invasiveness [due to characteristics of either GM crop or transgene(s)]—is a selective advantage conferred to the GM plant, e.g. are the characteristics of the GM plant altered in such a way that it becomes weedy in the agricultural environment or invasive in unmanaged ones?
- Are there changes in flower biology that could affect the ability to outcross? (extended flowering period, attractiveness to pollinators, change in fertility, etc.)
- Will gene flow by pollination of sexually-compatible relatives have an impact?—should hybridisation be possible, is a selective advantage transferred from the GM plant? Are there significant changes in the fitness or dispersal characteristics of the recipient organism that can lead it to become more persistent in agricultural habitats or more invasive in natural habitats?
- Will gene flow by seed dispersal have an impact? Is seed from the GM crop viable in nature? If so, can this be an avenue for transgene dispersal?
- Non-target organisms and biodiversity—will the GM crop cause adverse effects on populations of non-target organisms, e.g. by indirect effects on the population level of predators, competitors, herbivores, pollinators, symbionts, parasites and pathogens?
- Will there be changes in agricultural and cultivation practices? Do the required cultivation practices for the GM crop plant have direct or indirect, immediate or delayed negative effects on the environment?

predators, grazers, parasites, pathogens, competitors, symbionts and beneficial organisms, including humans, where appropriate). These documents are useful, but they are not always up to date. They may not provide sufficient information about the ecology of weedy wild relatives; therefore the competent authority may need information that is available in the scientific literature in order to evaluate questions about the consequences of crop-to-wild gene flow.

Plant ecologists and population geneticists have considered problems associated with traditionally improved crops to anticipate possible risks of transgenic crops. Those that have been most widely discussed are, *inter alia*, crop-to-wild hybridisation

Table 3 List of currently available online ‘Biology’ reference documents

Crop				
<i>Ananas comosus</i> var. <i>comosus</i> —Pineapple	✓			
<i>Beta vulgaris</i> (L.)—Sugarbeet		✓	✓	
<i>Betula pendula</i> Roth—European White Birch			✓	
<i>Brassica napus</i> (L.)—Canola/Oilseed rape	✓	✓	✓	✓
<i>Brassica rapa</i> (L.)—Canola/Rapeseed		✓		
<i>Carica papaya</i> —Papaya	✓		✓	
<i>Capsicum annuum</i> Complex Chili Peppers, Hot Peppers and Sweet Peppers			✓	
<i>Cucumis</i> , <i>Cucurbita</i> —Melon, Squash				✓
<i>Dianthus caryophyllus</i> (L.)—Carnation	✓			
<i>Glycine max</i> (L.) Merr.—Soybean		✓	✓	✓
<i>Gossypium hirsutum</i> —Cotton	✓			✓
<i>Helianthus annuus</i> (L.)—Sunflower		✓	✓	
<i>Larix</i> sp.—N. American larches		✓		
<i>Lens culinaris</i> Medikus—Lentil		✓		
<i>Linum usitatissimum</i> (L.)—Flax		✓		
<i>Lycopersicon esculentum</i> —Tomato				✓
<i>Medicago sativa</i> (L.)—Alfalfa		✓		
<i>Oryza sativa</i> —Rice	✓		✓	✓
<i>Picea abies</i> (L) Karst.—Norway Spruce			✓	
<i>Picea glauca</i> (Moench) Voss—White Spruce			✓	
<i>Pinus banksiana</i> —Jack Pine			✓	
<i>Pinus monticola</i> (Dougl. ex D. Don.)—Western White Pine			✓	
<i>Picea sitchensis</i> [Bong.] Carr.—Sitka Spruce			✓	
<i>Pinus strobus</i> (L.)—Eastern White Pine			✓	
<i>Populus</i> (L.)—Poplars			✓	
<i>Prunus</i> sp.—Stone fruits			✓	
<i>Rosa x hybrida</i> —Rose	✓			
<i>Saccharum</i> spp. Hybrids—Sugarcane	✓			
<i>Solanum tuberosum</i> subsp. <i>tuberosum</i> —Potato		✓	✓	✓
<i>Torenia x hybrida</i>	✓			
<i>Trifolium repens</i> (L.)—White Clover	✓			
<i>Triticum aestivum</i> —Bread Wheat	✓	✓	✓	
<i>Triticum turgidum</i> —Durum Wheat		✓		
<i>Zea mays</i> —Maize/Corn		✓	✓	✓

Sources. Office of the Gene Technology Regulator (OGTR), Department of Health & Ageing, Australian Government, Risk Assessment References (<http://www.ogtr.gov.au/pubform/riskassessments.htm>); Canadian Food Inspection Agency (CFIA) Biology Documents, Companion Documents for Directive 94–08, Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits (<http://www.inspection.gc.ca/english/plaveg/bio/dir/biodoce.shtml>); National Bureau of Plant Genetic Resources, New Delhi, India (<http://www.nbpgriernet.in/download/Document%20on%20Biology%20of%20Rice.pdf>); Organisation for Economic Co-operation and Development (OECD), Consensus Documents for the Work on Harmonisation of Regulatory Oversight in Biotechnology (http://www.oecd.org/document/51/0,2340,en_2649_201185_1889395_1_1_1_1,00.html); United States Department of Agriculture (USDA), Animal & Plant Health Inspection Service (APHIS), Biology of Crop Plants (<http://www.aphis.usda.gov/brs/biology.html>)

resulting in the evolution of increased weediness or invasiveness in wild relatives, the evolution of pests that are resistant to new strategies for their control, and the impacts on non-target species in associated ecosystems (Dale et al. 2002). To assess the potential ecological impact of field or commercial releases of GM crops in a given region (see Box 5 for examples), the likelihood and impact of gene flow by sexual out-crossing are taken into consideration.

Gene flow

Gene flow is not a phenomenon that is novel to GM crops, and has been exploited for millennia in the development of conventionally produced crops. It can occur concomitantly with reproduction of organisms, or can occur separately. The former is called vertical gene flow and the latter horizontal (or lateral) gene flow (Heinemann 2007). It is commonly accepted that vertical gene flow via seeds and pollen from crops is ubiquitous and evolutionary significant for the recipient populations for nearly all of the world's important crops. Hybridisation with wild relatives has been implicated in the evolution of more aggressive weeds for seven of the world's 13 most important crops (Ellstrand et al. 1999). Indeed, there is now substantial evidence that at least 48 cultivated plants mate with one or more wild relatives somewhere in the world (Ellstrand 2003). For many commercially grown GM crops, the question is not whether transgenic plants will outcross, because in many cases they will; it is whether the consequence of the resulting transgene flow will have an adverse environmental effect, which is a key point in the risk assessment (Lu and Snow 2005). If a transgenic crop is released in regions where wild relatives grow, it is expected that spontaneous hybridisation will occur unless the engineered plants are specially designed to limit gene flow (Lu 2003).

Additionally, unless those transgenes are deleterious or genetically tightly linked to deleterious genes, they may persist and introgress into the natural populations (Ellstrand 2003; Song et al. 2003a). Although this is mostly expected to have only minor effects, it could possibly either increase detectable genetic diversity in the wild species (Song et al. 2003a), or lead to the extinction of wild populations (Ellstrand et al. 1999). However, there are a number

of factors that will limit gene introgression from crop plants to related species, including various pre- and post-zygotic barriers, and the transfer of domestication traits such as seed dormancy or loss of seed dispersal to the hybrid plants (Conner et al. 2003). Moreover, hybrid establishment and the subsequent spread of the transgene will not only be affected by fitness, but also by unrelated factors such as recipient population size and density, pollen competition, overlapping flowering periods allowing gene flow between sympatric populations, and genetic drift (Wilkinson et al. 2000, 2003b; Song et al. 2001; Lu et al. 2003; Heinemann, 2007). In addition, dispersal as a result of agricultural activities such as seed spillage during transportation and machinery movements contributes to gene movement and the appearance of novel traits in plants at distances from their release (Barton and Dracup 2000; Saji et al. 2005; Heinemann 2007).

The most robust management strategy to limit gene flow is the establishment of safe isolation distances between populations, such as is used to separate cultivated forms from conserved wild populations in the context of in situ biodiversity conservation (Song et al. 2003a, b, 2004). Isolation distances are also critical for the co-existence of GM and non-GM crops (Rong et al. 2004; Messéan et al. 2006). The latter report also provides a decision table to determine the isolation distances necessary to keep adventitious GM presence due to gene flow below a desired threshold, for different field sizes and wind orientations, as well as demonstrating how isolation distances can be reduced when combined with non-GM buffer strips of different widths and/or with flowering time lags. Volunteers, feral populations and sexually compatible wild relatives may act as reservoirs for transgenes through time, with any risk assessment concerns being highly crop- and environment-specific (Cantamutto and Poverene 2007). For example, they are not considered to be an issue of any significance for maize, except in Mexico and Central America, since the crop does not produce volunteers nor establish feral populations, and there are no wild relatives elsewhere. However for oilseed rape in Europe and North America, the situation is complex, since both pollen and seed dispersal are issues (Hall et al. 2000; Saji et al. 2005). In addition, volunteer populations can establish, and out-crossing with related wild Brassicas can occur. However, it was

concluded that normal agricultural management practices were likely to maintain volunteer populations below levels that would contribute significantly to pollen transfer into nearby non-GM fields (UK ACRE 2004a).

The level of GM crop invasiveness and the location of compatible relatives can be determined by consulting the crop literature (access to which is provided by several databases: <http://www.icgeb.org/biosafety/db/biosafety.html>; <http://www.agbios.com/biblio.php>; <http://www.croplife.intraspin.com/BioTech/>) and local floras (e.g. <http://www.plants.usda.gov/>; USDA, NRCS 2005), as well as the online Global Biodiversity Information Facility Network (GBIF; <http://www.gbif.net/>; Copenhagen, Denmark). Decisions about potential invasiveness can be bolstered by determining the number of weediness traits carried by the crop and its conspecifics. Typical weed attributes include seed dormancy, phenotypic plasticity, indeterminate growth, continuous flowering and seed production, and seed dispersal mechanisms, all of which have been bred out of the most important crop plants over thousands of generations. These characteristics are not candidates for gene transfer back into crops because they would severely reduce the agronomic performance of a crop for modern farming practices. Additionally, data from a 10-year survey of GM crops in the United Kingdom showed that oilseed rape, potato, maize and sugar beet plants that had been modified for resistance to herbicides or insects had no more tendency to weediness than their unmodified equivalents (Crawley et al. 2001). The study showed that all the genetically modified crops had a poor survival record in the field and were eventually replaced by wild counterparts. Therefore, it was concluded that these GM crops are no more likely to become weeds outside farming situations than crop cultivars have in the past (Royal Society of Canada 2001; Conner et al. 2003). Specific attention, however, must be given to crops that already have weedy characteristics or in which added genes might be expected to improve crop or hybrid competitiveness in agricultural and natural habitats (Chen et al. 2004; Mercer et al. 2007; Warwick et al. 2007). This is supported by the occurrence of triple herbicide-resistant volunteer oilseed rape in Alberta, Canada which was explained by the accumulation of resistance genes resulting from two different spontaneous hybridisation events

between three different crop varieties, two GM and one conventional (Hall et al. 2000). Such is the concern that the agrochemical industry has now begun providing relevant information to delay the onset of resistance to their herbicides (e.g. Monsanto Company—<http://www.weedresistancemanagement.com/>; Syngenta AG—<http://www.resistancefighter.com/>).

It is also important to have information concerning the distribution and abundance of weedy relatives in both agricultural and unmanaged areas (derived from both conventional and GM agricultural practices) (Wilkinson et al. 2000; Chen et al. 2004). The Worldwide Herbicide Resistant Weeds Database (<http://www.weedscience.org/>) holds information concerning over 300 resistant biotypes, and includes reports of weeds resistant to those herbicides associated with GM crop cultivation; bromoxynil (e.g. Brominal, Buctril), glyphosate (e.g. Roundup, Touchdown) and chlorsulfuron (e.g. Glean, Telar). So far it has no records of resistance to phosphinothricin (e.g. Bialaphos, Basta, Liberty). The transfer of herbicide tolerance is, however, unlikely to confer any competitive advantage to hybrids outside agricultural areas (Dale et al. 2002).

Horizontal gene transfer

Horizontal gene transfer (HGT) is the non-sexual or parasexual transfer of genetic material between organisms belonging to the same or different species. Though actual evidence of its occurrence or feasibility (except among bacteria and fungi) is rare, the issue is taken seriously in the safety assessment of GMOs (OECD 2000b). Of particular concern are putative recipient micro-organisms in soil or in the digestive tracts of humans and livestock, which is especially relevant to the above discussion on antibiotic resistance genes. Recombinant DNA of high molecular weight has been found to be present in soil sites where free rDNA or GM plant material had been deposited and to persist in non-sterile soils for several months (Widmer et al. 1997). It is suggested that rDNA released from GM plants constitutes an extracellular gene pool which can be used by naturally competent bacterial cells that take up DNA and integrate it into their genomes (natural transformation) (de Vries et al. 2003). Natural transformation will therefore require the close presence of

naturally competent bacterial cells in the rhizosphere of GM crops, as well as rDNA homology to endogenous bacterial sequences (Dröge et al. 1998; Smalla et al. 2000). The low probability of integration of transgenes in recipient bacteria if DNA homology is not present has been confirmed in experimental studies that were unable to detect the transformation of *Acinetobacter* sp. strain BD413(pFG4) cells with transgenic sugar beet DNA in non-sterile soil microcosms (Nielsen et al. 2000). Laboratory-based studies have shown the possibility that transgenes can be transferred to native soil micro-organisms through horizontal gene transfer, although there is no evidence of this occurring in the soil. Specifically for the *nptII* gene, it has been concluded that natural soil conditions would rarely produce the selective pressure required for the fixation of possible transfers from transgenic plants into the recipient bacterium studied (Nielsen et al. 2000).

In the human gastrointestinal tract as well as that of husbandry animals, DNA may remain stable for some time, particularly in the colon. However, degradation already begins before the DNA or the material containing the DNA arrives at the critical sites for HGT, which are generally believed to be the lower part of the small intestine, caecum, and the colon (Van den Eede et al. 2004). Earlier studies have demonstrated that rDNA released from breached plant cells is rapidly degraded by nucleases present in ruminal contents (Alexander et al. 2007). Should rDNA reach the lower sites of the alimentary tract, it is most probably already in fragments smaller than the length of a complete gene. Sharma et al. (2004) have concluded that the uptake of rDNA fragments by ruminal bacteria is probably precluded or time-limited by rapid degradation of plant DNA upon plant cell lysis. Additionally, experiments using real-life conditions with human volunteers found no GM material to survive passage through the entire human digestive tract (Netherwood et al. 2002, 2004). Although some DNA survived in laboratory-created environments that simulated human or animal gastrointestinal tracts, complementary research has concluded that the likelihood of functioning DNA being taken up by bacteria in the human or animal gut is extremely low and will be dependent upon rDNA and the chromosomes of any naturally competent bacteria in the gut sharing regions of identical

sequences (Flint et al. 2002; Nordgård et al. 2007). Similar recent studies with insects (Brinkmann and Tebbe 2007; Ray et al. 2007) also support this conclusion. Thus, breakdown of DNA in the gut, combined with the breakdown of the DNA due to food processing, reduces the risk of dissemination (Van den Eede et al. 2004).

Non-target effects

Similar to conventional crops, all transgenic crops can have an impact on other organisms in their environment. In some cases, such as plants expressing resistance to a pest or pathogen, these organisms include the target of the transgene. In the case of insect resistance, enormous efforts have been devoted to studying the possible effects on non-target organisms, but in a broader sense, all GM crops can be considered to be associated with non-target organisms. For insect-resistant plants, they can be categorised into several overlapping functional groups (Snow et al. 2005; Andow and Zwahlen 2006; OECD 2007; Sanvido et al. 2007):

- pollinators and natural enemies of pests, and the wider category of beneficial species
- soil organisms
- non-target herbivores
- endangered and charismatic species, and any other species of conservation concern
- species which contribute to local biodiversity.

Although still poorly defined, any adverse effects on non-target species or ecological processes that affect biodiversity are considered as risks. Environmental concerns about the impact of GM crops on non-target organisms through tri-trophic interactions have been raised (i.e. the indirect effects of the GM plant on organisms not directly exposed to the GM plant but one or two steps removed in the food chain) and are becoming incorporated into revised guidelines (e.g. EFSA 2004). The most studied by far is the impact of *Bt* proteins in food webs (e.g. Ferry et al. 2007; Torres and Ruberson 2007), however, aside from their high selectivity, a recent careful evaluation indicated that decreased quality of the prey, rather than any direct effect, is responsible for the negative effects of *CryIAb* observed in some studies (Rodrigo-Simón et al. 2006). It is obvious that potential tri-

trophic effects arising from other transgenic proteins warrant further study on a case-by-case basis.

Guidelines have been developed on how to assess the wider indirect impacts of a GM crop (UK ACRE 2001c), and non-target and biodiversity risk assessments of transgenic plants are continually being improved. They require a comparison between the management of the GM crop and the equivalent non-GM crop, with an assessment of the potential impact on key indicator species typical of arable farmland and examples of impact mitigation are already beginning to appear (Pidgeon et al. 2007). While indicator species continue to be used in many RAs, there is a trend towards assessment of risks to non-target species that naturally occur in the local areas where the transgenic crops are to be cultivated (Arpaia et al. 2007; Faria et al. 2007; Marvier et al. 2007). Additionally, it is important to assess the effects associated with changes in agricultural practices and the impact of transgene flow to wild relatives on the non-target community associated with the GM crop. For example, on a sub-sample of fields used in the UK's Farm Scale Evaluation trials, differences in bird abundances and diversity were recorded between genetically modified herbicide tolerant (GMHT) and non-GMHT crops that were in accord with likely differences in food availability (Chamberlain et al. 2007). However, using farmland birds as a model system, Butler et al. (2007) have developed a generic risk assessment framework that accurately predicts the current conservation status of each bird species and population growth rate associated with past changes in UK agriculture. They concluded that replacing equivalent conventional crops in the current UK agricultural landscape with GMHT crops would only have a limited effect on the Farmland Bird Index (a measure of their biodiversity).

One of the most significant studies of non-target impacts has been on the effect of *Bt* insecticidal proteins on the Monarch butterfly in the United States (Sears et al. 2001). Contrary to the public outcry following the publication of demonstrated harm to Monarch butterfly larvae that had consumed leaves dusted with pollen from *Bt* maize (Losey et al. 1999), the subsequent USA EPA report suggested that the role of *Bt* maize in the elimination of pesticides may actually be beneficial to Monarch butterfly populations, and concluded that there is not sufficient

evidence to support the belief that there is significant risk to Monarch butterflies from *Bt* maize use (USA EPA 2001b). This report relied upon the detailed assessment of the specific non-target effects of these plants (Hellmich et al. 2001; Oberhauser et al. 2001; Pleasants et al. 2001; Stanley-Horn et al. 2001; Zanger et al. 2001).

In addition to birds and insects, concerns about the secondary ecological impacts of GM crops are starting to focus on soil ecosystems. Soil organisms are generally heavily exposed to GM plant material, either through leaf shedding, root exudates or decomposition (Royal Society of Canada 2001; Conner et al. 2003; Dunfield and Germida 2004). The potential impact of any GM plant on soil organisms will be mediated by factors including the potential toxicity to a range of organisms (e.g. of *Bt* toxins), the changes in plant metabolites that could affect soil organisms, and the likelihood of such products ending up in the soil. Studies have shown specific cultivated transgenic plants to have both variable and transient effects on microbial community biodiversity (Dunfield and Germida 2004). The effects observed so far however are slight in comparison to those associated with changes in the crop species or in agricultural practices.

In fact, quantitative field studies have shown that the environmental impact of changes in agricultural management of non-GM crops can be at least as significant as those associated with GM crops. For this reason, the UK's ACRE (2007) has suggested that a broader and more balanced regulatory approach is required to assess and manage more effectively the environmental footprint of agriculture as a whole. This approach would deal not only with GM crops but also with other novel crops and agricultural practices. It would allow the assessment of both environmental risks and benefits, and the development of rigorous and balanced decisions.

Stewardship and post-release monitoring

The release and widespread cultivation of GM crops with pest or disease resistance has also raised concerns that this will impose intense selection pressure on weed, pest and pathogen populations to adapt to the tolerance or resistance mechanism. This adaptation is not a new issue to agriculture, and is a

well-recognised problem by plant breeders and the agricultural industry, as demonstrated by the aforementioned Worldwide Herbicide Resistant Weeds Database, as well as the Database of Arthropods Resistant to Pesticides (<http://www.pesticideresistance.com/>). Additionally, the development of crop management strategies in attempts to minimise the evolution of pest/pathogen resistance to new cultivars is an ever-present phenomenon of modern agriculture, with the University of California State-wide Integrated Pest Management Program site (UC IPM; <http://www.ipm.ucdavis.edu/PMG/crops-agriculture.html>) offering insights into the diversity of current agricultural practices, as well as the development of pest management strategies that can also be adopted by those farmers growing GM crops.

Resistance management strategies associated with the release of GM crops are required by certain competent authorities (e.g. AUS OGTR 2005; CFIA 2005) to reduce the environmental impact of GM crops (Barton and Dracup 2000). They are well established for the commercial release of *Bt*-mediated insect resistance; for example the USA EPA has required the development of resistance management strategies for all approved *Bt* crops since 1995. The predominant approach has involved the combination of a consistent and high expression level of the *cry* gene (defined as “25 times the protein concentration necessary to kill susceptible larvae”; USA EPA 2001b) with the deployment of a refuge (an area of non-GM crop that is placed either within a crop as a seed mixture or as a separate block within close proximity) (Alstad and Andow 1995; NRC 2002). A structured refuge should provide sufficient susceptible adult insects to mate with potential *Bt*-resistant adult insects to dilute the frequency of resistance genes (USA EPA 1999a). However, it assumes that resistance to *Bt* is recessive and is conferred by a single locus with two alleles resulting in three genotypes: susceptible homozygotes (SS), susceptible heterozygotes (RS), and resistant homozygotes (RR). It also assumes that there will be a low initial resistance allele frequency as well as extensive random mating between resistant and susceptible adults (USA EPA 2001b). Stacked genes with dissimilar modes of action, high dose, and common pest spectrum will likely need smaller structured refuges to mitigate the development of resistance. The size of existing structured refuges may be

reduced in the future if new data (e.g. measurements of resistance gene frequency and gene expression) show that smaller refuges are adequate. Similarly, if new data (e.g. larval and adult movement, ovipositional and mating behaviour) suggest the location (or size) of a structured refuge should be altered, the proximity (and/or size) requirements for certain refuge options may be changed. Guidelines are available for the development of Insect Resistance Management (IRM) strategies suitable for *Bt* crops, with many of the principles transferable to the management of herbicide tolerance (USA EPA 2001b).

A general post-release plan to monitor for unintended or unexpected environmental effects of an authorised product is required in the EU as an integral part of a complete application for unrestricted or commercial release, and will be reviewed during the environmental assessment of the GM plant in question (EFSA 2006). The use of appropriate indicators to evaluate these effects should also be based on the characteristics of the GMO. A stewardship plan may be considered acceptable for post-release monitoring purposes (CFIA 2005), with the communication to growers and the availability of an efficient mechanism allowing growers to report problems to the applicant considered as integral components (e.g. Philippines Dept of Agric 2006). The enforcement of such a plan is extremely important, as a recent survey conducted by US maize growers and seed companies has shown that, in the year 2000, almost 30% of the farmers failed to comply with the refuge protocols designed to prevent or delay the emergence of insects resistant to *Bt* toxins (Dove 2001). This rate of non-compliance could well increase the risk of plant resistance breakdown, although to date, there is no reported evidence of insect resistance to *Bt* crops under field conditions in the countries where they have been deployed (Sivasupramaniam et al. 2007; Wu 2007).

Long-term and indirect effects of any new technology present a challenge to risk assessment. Post-release monitoring and general surveillance provide mechanisms for the early detection of any adverse effects (Wilkinson et al. 2000; UK ACRE 2004b; EFSA 2006) but the challenge for scientific committees, applicants and regulators is to identify the key areas of uncertainty and design appropriate monitoring and surveillance methods (Wilkinson et al. 2003b). Exacerbating the problem, Gaugitsch (2002)

has concluded that cultivation measures and traits cannot be assessed separately, and that the biological needs fulfilled by any of these measures must also be taken into account in environmental risk assessment. Small plots and laboratory studies are unlikely to prove useful in such an evaluation, therefore a call has been made for appropriate large scale monitoring, experimentation, and modelling to determine the impact on the landscape from GM trait characteristics (Firbank et al. 2005). The requirement for applicants to consider post-market monitoring and surveillance is becoming a key feature in new or revised biosafety regulations (e.g. European Parliament 2001; CFIA 2005). In this respect, detection methods and sequence tagging that improve traceability may be an important consideration in risk assessments (UK ACRE 2001b; Stewart 2005). Post-release GM crops should be monitored for disease, and disease incidence should also be recorded in crops following on from any GM crop. Unusual levels of disease incidence may be indicative of an effect on the soil organisms that suppress plant disease (UK ACRE 2006).

Crop and food product composition characteristics

Since their introduction, many millions of people have consumed foods derived from GM plants, mainly maize, soybean and oilseed rape, and to date no verifiable untoward toxic or nutritionally deleterious effects arising from this consumption have been discovered anywhere in the world (OECD 2000b, c; GM Science Review Panel 2003; USA National Research Council 2004). With regard to investigating the safety of GM foods, the key concept is the need to determine whether changes other than the intended new trait have occurred in the new crop (ILSI Task Force 2004). One approach taken by regulatory bodies is to obtain data on the composition of a GM food in relation to its conventional counterpart (OECD 2000b; Box 6). Compositional parameters are selected that are typical for the crop that is assessed and that are representative of the main metabolic pathways. Significant changes in these parameters are expected to be indicative of more fundamental changes in the crop that need to be evaluated for their potential to have adverse consequence to human health (König et al. 2004). In principle, this provides

a way to assess any new constituents introduced by the genetic modification process. It also permits an assessment of the degree of change in the amounts of the common constituents. After some initial uncertainty, the concept of substantial equivalence has now been clarified (FAO/WHO 2000; CAC 2003a) to refer to the methodology of comparative safety assessment, rather than to be the end point.

The ideal comparator is one that has a known history of safe use and is, in most cases, a near-isogenic variety of crop or food, genetically identical except for the presence of the novel trait (Cellini et al. 2004; König et al. 2004). Information obtained from food and feed crop composition studies is used to assess similarities and differences in important nutrients and anti-nutrients. Typically, these comparisons are made on the basis of the analysis of

- major metabolite groups (e.g. carbohydrates, proteins, lipids, fibre and minerals)
- nutritional components (e.g. essential macro- and micronutrients)

Box 6 Examples of specific concerns regarding GM crop composition include

In comparative assessments, do the component concentrations fall within the natural range found in the non-GM counterparts?—data presented from field trials in a range of geographic locations over more than one season, incorporating both GM crop & non-GM counterpart. Any consistent differences will require further assessment to determine their biological significance.

- Are the analyses appropriate to the intention of the genetic modification, and the considered nutritional value & use of the GM plant?
- Are there any changes (including intended) to the concentrations of any toxins, anti-nutritional compounds and allergens? If so, this triggers an RA to consider the newly expressed proteins, potential presence of other new constituents and/or changes in level of natural constituents.
- Are the characteristics of the end product modified by the applied processing and/or preserving technologies as compared with its non-GM counterpart?
- Will dietary changes occur in consumer exposure to the end product?—If so, an RA should be undertaken, and sections of the population sensitive to higher exposure of the end product should be identified.
- Post-market monitoring of GM food/feed—Is the product use as predicted/recommended? Are known effects & side-effects as predicted? Does the product induce unexpected side effects?

- known endogenous toxicants and anti-nutrients in specific species (e.g. digestive enzyme inhibitors, lectins, saponins, tannins, glycosides)
- the tendency to provoke allergic reactions
- data derived from feeding trials and toxicological studies in animals

and any other characterising components (USA IOM 2000; CAC 2003a; Cellini et al. 2004; ILSI Task Force 2004; Shewry et al. 2007). Any significant differences in composition between the GM crop that fall outside the range of natural variation in levels across a range of varieties of the same crop and that are reproducible then become the focus of further evaluation (Kok and Kuiper 2003). Key compositional information of crop species currently traded as transgenic commodities is available in documents (Table 4) and a publicly accessible database (Crop Composition Database [<http://www.cropcomposition.org/>], International Life Sciences Institute [ILSI], Washington, DC, USA). If a nutritional or other safety concern is identified by this analysis, the risk associated with it must then be characterised to determine its relevance to human health (CAC 2003b). The nutrient composition data requirements for a food safety analysis should also take into account the effects of storage, further processing and cooking. Attention should be paid to the particular physiological characteristics and metabolic requirements of vulnerable groups in society such as infants, children, pregnant and lactating women, the elderly and those with chronic disease (Royal Society 2002).

Currently, there are two basic analytical approaches available to detect compositional changes in food. Targeted quantitative analysis is the traditional approach, in which a method is established to quantify a predefined compound or class of compounds. In contrast, profiling methods involve the untargeted analysis of a complex mixture of compounds extracted from a biological sample with the objective of identifying and quantifying all detectable compounds present in a sample. Advanced chemical and genetic profiling techniques using molecular genetic, proteomic (analysis of complete complements of proteins), and metabolomic (global analysis of non-peptide small molecules) approaches are rapidly developing to produce technologies with the potential to provide an enormous amount of data for a given organism, tissue, or food product (Cellini et al. 2004; NRC 2004; Rischer and Oksman-Caldentey 2006). Application of such techniques to characterise differences between the GM crop and the appropriate comparator should help provide a rigorous scientific basis for hazard identification. However, much development work remains to be done, in particular to determine the utility of this approach in relation to the wide natural variation in composition between crops grown in different environments (Royal Society 2002). To aid evaluators, a framework has been developed to help identify appropriate scientific questions and methods for determining unintended changes in the levels of nutrients, toxins, toxicants, allergens, or other compounds in foods from GMOs, in order to assess potential short- and long-term human health consequences of such changes (NRC 2004).

Livestock feed safety is also determined in order to ensure that unsafe residues are not introduced into

Table 4 Currently available online Crop Composition documents

Crop	Source ^a
	
Key food and feed nutrients and anti-nutrients	
<i>Beta vulgaris</i> (L.)—Sugarbeet	✓
<i>Glycine max</i> (L.) Merr.—Soybean	✓
<i>Hordeum vulgare</i> (L.)—Barley	✓
Key feed nutrients, anti-nutrients and secondary plant metabolites	
<i>Gossypium hirsutum</i> —Cotton	✓
<i>Medicago sativa</i> (L.)—Alfalfa & other temperate forage legumes	✓
<i>Oryza sativa</i> —Rice	✓
<i>Zea mays</i> —Maize/Corn	✓
Key nutrients and key toxicants	
<i>Brassica napus</i> (L.)—Oilseed rape/Canola	✓
Key food and feed nutrients, anti-nutrients and toxicants	
<i>Agaricus bisporus</i> —Cultivated mushroom	✓
<i>Helianthus annuus</i> (L.)—Sunflower	✓
<i>Solanum tuberosum</i> subsp. <i>Tuberosum</i> —Potato	✓
<i>Triticum aestivum</i> —Bread wheat	✓

^aSource. Organisation for Economic Co-operation and Development (OECD), Consensus Documents for the Work on the Safety of Novel Foods and Feeds. http://www.oecd.org/document/9/0,2340,en_2649_201185_1812041_1_1_1_1,00.html

human food products via the ingestion of GM feed by food-producing animals. Submissions must demonstrate that new metabolites are degraded or denatured during processing of the feed, or through digestion of the feed in the animal. If digestion is incomplete, the metabolite must be shown to be non-toxic or non-allergenic. The nutrient composition and bioavailability of nutrients, and the introduction of toxicants or anti-nutritional factors, are also assessed (OECD 2000b; Flachowsky et al. 2007). The Royal Society (2002) has proposed that, during animal studies, any changes in tissue structure or metabolic function of various organs (liver, kidney, lungs, brain and cardiovascular organs) should be also assessed, and that in any necessary human studies, measures of general health, development and psychological well being should also be included.

Toxicity

A huge number of proteins in the normal diet are ingested without adverse effects, but a small number have the potential to affect health. As proteins and peptides have a wide range of functions in organisms, different possible effects have to be considered: for example, enzymatic activity or enzyme inhibition may influence the potential to synthesise toxic compounds or cause anti-nutritive effects by binding certain nutrients, and some proteins act as carrier molecules, hormones or toxins. Additionally, many plants naturally produce toxicants and anti-nutrients, often for defence against predators, and as such, it is possible that GM varieties could have altered expression levels of those compounds. A purely speculative possibility is that silent pathways for toxicant and anti-nutrient production could be reactivated by insertion or expression of the new genes (Kaeppler 2000). In the toxicity testing of specific proteins, current approaches are based on internationally approved acute or chronic tests in laboratory animals (rats or mice) or fast-growing domesticated species such as chickens. However no repeat dose tests with GM foods or feeds have yet been published (OECD 2000b).

A question frequently asked about food safety with regard to newly introduced traits concerns the effect of long-term exposure to new proteins. Chronic toxicity testing is used to address long-term exposure

to synthetic chemicals. However, for proteins (as opposed to other chemicals) there is a certain predictable metabolic fate in the human or animal gut similar to that for conventional dietary proteins. One method used to address this metabolic prediction is the *in vitro* digestibility assay, which indicates the likelihood that a protein will have characteristics that would be unusual in dietary proteins. It is assumed that all proteins will act like dietary proteins and break down under digestive conditions into their constituent amino acids. If a protein is shown to be resistant to typical digestive fluids, there may be added exposure to the intact protein or to large pieces of the protein. This digestive resistance would lead to a different analysis than if the protein were broken down as expected. However, there is still no consensus on resistant proteins being a significantly different risk if none of the other toxicity tests yields adverse results. This question may be partly resolved when we know more about the quantity and quality of our current dietary exposure to proteins resistant to digestive enzymes (OECD 2000b).

Several authors (FAO/WHO 2000; Royal Society of Canada 2001; USA SOT 2002) have concluded that conventional toxicological studies are of little value in assessing the safety of whole foods, including both traditional and genetically modified foods as well. Moreover, it is widely recognised that one of the pre-eminent safety concerns about food derived from genetically modified crops is the issue of potential allergenicity (USA SOT 2002). There are also still questions about the adequacy of the current toxicological approach to reliably predict potential adverse health outcomes resulting from exposure to genetically modified foods on the basis of the animal models currently available to the scientific community (FAO/WHO 2001; Royal Society of Canada 2001; USA SOT 2002).

Allergenicity

Food allergies occur in 1–2% of adults and 6–8% of children, although severe allergic reactions (anaphylaxis) to foods are relatively rare, occurring in approximately 3.2 individuals per 100,000 people per year (Royal Society 2002). Almost all known allergens are proteins, and as such, proteins from sources not previously used in human food present

particular difficulties in safety assessments. Allergenicity is determined by the antigenicity or foreignness of a molecule, and is influenced by its size, stability, the extent of allergen exposure, and the genetic predisposition of a host for inducing an allergic response (Bernstein et al. 2003). Two scenarios for potential allergenicity of a GM product may be assessed. First, a novel protein expressed in a GM organism may be assessed for the potential to cause new allergies or to cross-react with other allergens in sensitised patients. Second, the intrinsic allergenicity of the modified host organism may have been altered by the genetic modification (Kleter and Peijnenburg 2006; Prescott and Hogan 2006).

There is at present no evidence that the GM foods that are commercially available cause any clinical manifestations of allergenicity, and assertions to the contrary were not supported by systematic analysis (CDC 2001). However, it was noted that although this analysis is reassuring, methodological limitations make it less than conclusive (Kuiper et al. 2001), and it cannot eliminate the possibility that some adverse effects may have occurred that were not reported. No definitive tests exist for determining the allergenicity potential of proteins (CAC 2003a; Kleter and Peijnenburg 2006); therefore, currently used methods compare the new protein with known allergens and also test for heat stability and enzyme digestibility. If the new protein is heat unstable and easily digestible, its allergenicity potential is considered to be low, whereas heat stable proteins pose higher risks. (Lehrer 2000; FAO/WHO 2000, 2001). To elaborate, the following factors are considered in order to provide assurance that a protein is unlikely to elicit an allergic reaction (USA FDA 2002; CAC 2003a; Kleter and Peijnenburg 2006; Silvanovich et al. 2006):

- whether the source of the gene is associated with any reports of allergenicity
- amino acid sequence comparison with known allergens, both overall similarity and stepwise contiguous amino acid searches
- biochemical properties of the protein, including *in vitro* digestibility in simulated gastric fluid (SGF), heat stability, and glycosylation
- prevalence in food
- specific serum screening (e.g. for proteins with sequence similarity with a known allergen).

Of particular interest is the availability of three online search mechanisms, Allermatch (<http://www.allermatch.org/>; Wageningen University and Research Center, Wageningen, The Netherlands), the Structural Database of Allergenic Proteins (<http://www.fermi.utmb.edu/SDAP/>; the University of Texas Medical Branch, USA) and the FARRP Protein Allergen Database (<http://www.allergenonline.com/>; University of Nebraska, USA), which allow the comparison of the amino acid sequence of a protein of interest with sequences of allergenic and isoallergenic proteins, using bioinformatics approaches as recommended by the Codex alimentarius and FAO/WHO to predict the potential allergenicity of proteins.

In addition to the investigation of the novel proteins, an obligatory investigation of whole plants has been proposed (Spök et al. 2004), to comprise a two-step procedure of an IgE binding study applying sera from allergic patients and immunisation studies in mice. The development of suitable animal models, the identification and characterisation of food allergens, and the establishment of well-defined clinical serum banks are amongst the many identified research needs for the prediction and/or post-market surveillance of allergy to novel proteins in foods (Royal Society of Canada 2001; Ritter 2002; USA FDA 2002; CAC 2003a; Selgrade et al. 2003; Kleter and Peijnenburg 2006; Prescott and Hogan 2006). Until validated tests become available for reliable evaluation/prediction of a novel protein's allergenicity, the record of reported allergic reactions in relation to actual intake of the novel food would be a useful tool to guarantee complete safety for consumers.

Post-market surveillance

Consumer groups and the governments of several OECD countries have called for the establishment of a post-market surveillance mechanism for products that have been approved as novel foods, following a safety assessment. Some parties advocate that this mechanism should follow up and reinforce the initial risk assessment, as an integral part of the risk analysis process; others recognise the many technical challenges involved in implementing such a mechanism and therefore favour proceeding in a step-wise

manner, beginning with a feasibility study (OECD 2000b).

Post-market monitoring of nutritionally improved food products may be useful to verify pre-market exposure assessments or to identify changes in dietary intake patterns, and they should be conducted with a scientifically valid testable hypothesis as a basis, or to verify pre-market exposure assessments (König et al. 2004). Concern has been raised, however, that in the absence of a valid hypothesis, post-market monitoring for undefined hypothetical adverse effects from foods from a GM (or non-GM) crop is not feasible, and adds nothing to the pre-market testing results, while potentially undermining confidence in the overall safety assessment process (ILSI Task Force 2004).

Conclusions

Empirical-based scientific RAs of GMOs are undertaken to assist competent authorities make an informed decision regarding whether or not to give approval for import or cultivation. In the decade since the first authorisations for commercial release of GM crops, there has been an enormous increase in the amount of data generated by scientific studies that relates to RA. If this trend continues, we run the risk of the competent authorities being submerged by excessively large amounts of data that may be of questionable pertinence to verifiable safety questions. It is worth emphasising that the role of an effective RA is not to completely understand a natural phenomenon, but to focus only on data necessary to make a sound judgement. Superfluous data often confuses decision-making, diverting time and efforts from the more serious of the identified risks, thereby slowing down the procedure and increasing associated costs (Raybould and Cooper 2006; Andow and Zwahlen 2006). In this regard, structuring the RA process using decision trees and/or tiered approaches should be seriously considered as a tool for focusing on the essential issues.

Many countries (and regions) have, or are currently putting into place, a framework for undertaking RAs of the cultivation or production of GM foods, and as such, it represents a significant opportunity to work towards international harmonisation on many levels. For example, it is becoming increasingly

important to reach a consensus on safety assessment approaches. Specifically, scientific consensus has not been established concerning for example, what constitutes a proper toxicity and allergenicity assessment for GM foods, as well as how to apply the concept of substantial equivalence (Spök et al. 2004). The entire risk assessment process requires the use of scientific data of the highest quality and the application of established scientific procedures. While research and scientific studies continue to provide answers needed to make informed decisions in risk analysis related to hazards in food, uncertainty and unresolved questions still cause concern to decision-makers (Van den Eede et al. 2004).

On another level, regulatory harmonisation for GMOs is promoted and underpinned by international agreements such as the Cartagena Protocol on Biosafety (CPB) and the World Trade Organization agreements. This is no easy task, as demonstrated by the fact that the two most ambitious multilateral harmonisation efforts in this policy area, the CODEX Alimentarius Commission (<http://www.codexalimentarius.net>) and those in the context of the UN Convention on Biodiversity Commission (<http://www.biodiv.org>), have been deadlocked and are likely to remain so for the next few years (Bernauer 2005 and references therein). Nevertheless, the orthodox assumption is that a globally-uniform standard can eventually be set for the health impact of a GM food, but is highly unlikely regarding environmental considerations of GM crops due to the diversity of habitats across the globe (Millstone and van Zwabenberg 2003). Additionally, it is essential that care is taken to accommodate diverse national and local priorities and realities that go beyond ecological differences, which is especially important for developing countries. Public consultation is required under the CPB but few countries (including developed countries) have yet undertaken the type of consultations which are necessary in order to determine what levels of risk are considered acceptable by the public, and consequently what measures are appropriate to achieve the desired level of protection (Mackenzie and Glover 2003). It is anticipated that dialogue between experts and civil society will contribute over time to further clarify and structure risk analysis strategies to improve the pertinence of assessments to address concerns of policy makers and the public (König et al. 2004). This is echoed by

Kapuscinski et al. (2003) who describe a “safety first” approach, involving public–private partnerships for “transparent development of proactive safety standards that anticipate and resolve safety issues as far upstream of commercialisation as possible.” The authors are optimistic about such a process because it should benefit multiple stakeholders, including biotechnology companies and those concerned with food and environmental safety.

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