GM SCIENCE REVIEW

FIRST REPORT



An open review of the science relevant to GM crops and food based on interests and concerns of the public

PREPARED BY THE GM SCIENCE REVIEW PANEL (JULY 2003)

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Foreword by Professor Sir David King, Government Chief Scientific Adviser

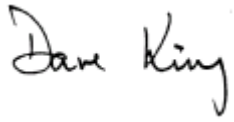
The GM Science Review was commissioned as part of the wider GM public dialogue by Mrs Margaret Beckett, the Secretary of State for the Environment, Food and Rural Affairs; with the agreement of the responsible Ministers in the devolved administrations. This report has therefore now been formally submitted to Mrs Margaret Beckett MP, Mr Allan Wilson MSP at the Scottish Executive, and Mr Carwyn Jones AM at the National Assembly for Wales to help inform Government decision making on GM crops and food.

The Review has endeavoured to take an open look at the science relevant to GM crops and food, and to do so in a way that recognises the interests and concerns of the public as well as the science community. So I am sure this report will be of widespread interest. **The Review Panel invites and welcomes your comments on the report. Over the Summer, our Review website[1] will be open to receive them. We also continue to welcome scientific contributions to the website. All contributions must be submitted by 15 October 2003**.

The Panel will then reconvene in late Autumn to consider these comments together with the report of the GM public debate "GM Nation?[2]". In the light of these, we will wish to consider whether there are any further issues we should address. We will also look to see if there have been significant developments in GM science over the summer that we should report on, and will consider the results of the farm scale evaluations of GM crops if these are available.

Those who attended our open Panel meetings will know that the Panel members cover a wide range of expertise and of views on GM. I would like to pay tribute to all those members who have given real commitment to the Review, expending a great deal of time and working extremely hard and cooperatively to ensure that the issues we have considered have been fully explored. Whilst respecting differences in views and recognising that Panel members do not individually cover all the areas of expertise, I am pleased to say that a really good degree of consensus was reached on the basis of the available science and that the Panel has collectively taken ownership of the review.

Finally, on behalf of the Panel, I would also like to thank all those who spoke at our open meetings around the country, those who hosted them and, of course, those who came along and took part. Our thanks go to the British Association for the Advancement of Science for organising this series of open meetings, as well as the Royal Society and the Royal Society of Edinburgh. We would particularly thank all those who contributed to the website; we have sought to take account of your submissions. We have also valued our contacts with the those running the public debate and with the Prime Minister's Strategy Unit who have produced the report on the costs and benefits of GM crops and food[3]. We are grateful to the Food Standards Agency and their advisory committees for their comments. And I am sure that the Panel would wish to acknowledge the dedication of the Secretariat, whose members have laboured mightily to bring this First Report to print.



21 July 2003

[1] http://www.gmsciencedebate.org.uk For guidance on how to submit comments.
[2] http://www.gmnation.org.uk
[3] Fieldwork: Weighing up the Costs and Benefits of GM Crops. http://www.strategy.gov.uk

Members of the Panel

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EXECUTIVE SUMMARY

BACKGROUND

Ever since the beginnings of agriculture, some ten thousand years ago, people have been selecting plants to develop into new crops. We now know that the process of plant breeding builds on changes brought about in a plant's genetic structure, with the information being encoded by genes (typically some 30,000 genes in each plant cell). Since the 1970s, it has become possible to modify the genetic information of living organisms in a new way, by transferring one or more gene-sized pieces of DNA directly between them. Such transfers have become an everyday tool in biological research and are already the basis of a considerable number of commercial applications in drug and food development that involve the genetic modification of micro-organisms such as yeast and bacteria. When applied to the production of crop plants, genetic modification can involve gene transfer from another plant species, or from a completely different organism such as a bacterium or virus. The process shares some common features with earlier plant breeding tools, as well as exhibiting unique differences.

World-wide, genetically modified (GM) crops occupy a relatively small proportion of the world's agricultural acreage. However, in 2002, GM crops were cultivated on some 59 million hectares globally. Almost all (99%) of this was grown in only four countries: USA (66%), Argentina (23%), Canada (6%) and China (4%). Three crops comprise 95% of the land under GM cultivation: soybean (62%), maize (21%) and cotton (12%). Traits achieved by genetic modification primarily involve herbicide tolerance (75%) and insect pest resistance (15%), or a combination of both in the same crop.

No GM crops are currently grown commercially in the UK although they are grown to a limited extent in some EU countries. There are, though, GM foods and animal feeds approved for consumption in the EU and these include processed products from GM herbicide tolerant soybean and maize, and oil from GM oilseed rape. Tomato paste made from slow-ripening GM tomatoes is approved but is not currently available, although it was widely sold in the UK in the late 1990s. Products made from GM micro-organisms are widely used in some sectors of the food industry (e.g. as a processing aid in cheese manufacture) and in medicine. However, the issues surrounding GM micro-organisms are not included in this science review which focuses specifically on GM crops and their products.

THE SCIENCE REVIEW

Many claims have been made about potential benefits available from GM crops. At the same time considerable reservations and concerns have been expressed. This review specifically addresses the science surrounding GM crops, with a focus on topics shaped by public questions and concerns. It differs from standard scientific reviews in its attempts to engage with the public and explore different viewpoints. For instance, the remit for the review mandated that the work be 'driven' by public interests and concerns and that deliberate attention be given to 'divergences of

view among scientists' and 'uncertainties, unknowns and gaps in knowledge'. Through a series of public workshops and meetings and through a website, we have solicited and considered concerns and interests of the public, whether or not professionally involved in science, agriculture or the food industry.

The review does not aim to be exhaustive in surveying all that is known scientifically about the various GM crops that have been developed to date. However, the review does aim to cover those areas where there is evident public concern. As a result of the public consultation exercise and input from the panel itself, seventeen topics were chosen for detailed analysis. In each case, the topic was considered within a framework that aimed to: (1) summarise the range, quality and degree of agreement of scientific studies that have investigated the issues; (2) ask whether the topic is unique to the processes and products of genetic modification or whether there are commonalities with crops bred conventionally; and (3) ask whether there are important scientific uncertainties. Two other components of the framework involved 'looking to the future', exploring relevant developments in scientific research, agricultural practice and also regulation.

The review panel included both specialist and non-specialist scientists and social scientists from a wide range of backgrounds. The institutions from which panellists were drawn included universities, specialist research institutes, research groups associated with biotechnology companies, and organisations with particular environmental concerns. Whatever their background and current employment and interests, all panel members acted as individuals in their own right, with a shared vision of producing a balanced, accurate and well-informed review. We hope that this review will enable debates and decisions to be informed by sound scientific evidence.

The review is specifically concerned with the potential use of GM crops in the UK. Assessing the implications of the adoption of GM technologies in other countries is beyond its scope, although issues with regard to the use of GM crops elsewhere, particularly in developing countries, were raised in the consultation exercise and discussed by the review panel. We hope that the approach we have used, and the scientific material we have brought together, may be of use in other countries in clarifying issues and generally informing debate.

THE SCIENTIFIC PROCESS

The bedrock of this Report is peer-reviewed published scientific literature in the relevant areas, but other sources of appropriate scientific evidence have also been considered where appropriate. Good scientific results have a sound basis in terms of existing knowledge and stand up to careful experimental and observational investigation. A good scientific paper explains clearly its claimed advance in knowledge and the evidence for it. When submitted for publication, the paper is read carefully by other experts in the field to see whether its conclusions are justified, and this process of 'quality control' is called *peer review*. A paper that passes this test is published in the scientific literature and becomes part of the public body of knowledge on which future scientific work can be based. No single peer-reviewed paper should be believed uncritically, and if a paper makes a surprising claim or a substantial advance, it becomes an obvious candidate for further scientific investigation. The aim of this whole system, which has grown up over more than three hundred years, is that knowledge should continually be challenged, refined and improved, through a

developmental process based on appropriate evidence, valid inference and the work of a large and open scientific community.

Some of the questions asked about GM crops are purely scientific, whilst others are not of a scientific nature at all, but may be economic, social, ethical or even personal. For science, as for other areas, the answers given may often depend on the way the question is asked and be open to divergent interpretations. Accordingly, scientific issues represent only a part, albeit an important one, of the wider debate over GM crops. Being 'rational' is not enough to make a question scientific; the question and/or its potential solution must be amenable to objective testing. Of course, there are many questions that are not wholly or even primarily scientific, but are such that scientific understanding can make an important contribution to their resolution.

STRUCTURE OF THE MAIN REPORT

The first two chapters describe the scope and methodology of the review. Chapter 3 discusses the role of science in regulation. Chapter 4 discusses the reliability of GM plant breeding compared with conventional methods. Seventeen topics reflecting issues of public concern are then grouped into three chapters, broadly covering food, feed and animal safety, environmental impact, and gene flow.

HOW RELIABLE IS GM PLANT BREEDING? (CHAPTER 4)

Concern has been expressed that GM plant breeding is too unreliable and imprecise for crops to be grown and consumed safely, or at least without more extensive testing. One argument presented is that it is necessary to produce about 100 GM plants to obtain one that has the desirable characters for its use as a basis of a new GM crop variety. There is also evidence that genes introduced by genetic modification vary in their effects depending on precisely where they insert into the host plant's genetic material

To address such concerns it is important to place GM crop breeding in the context of non-GM crop breeding methods such as gene transfer by pollination, mutation breeding, cell selection and induced polyploidy. Most of these so-called conventional plant breeding methods have a substantially greater discard rate. Mutation breeding, for instance, involves the production of unpredictable and undirected genetic changes and many thousands, even millions, of undesirable plants are discarded in order to identify plants with suitable qualities for further breeding. The success of all methods of breeding relies on careful testing and evaluation and on rejection of plants with undesirable qualities. The rejection rate is substantially higher for most non-GM crop breeding methods than it is for GM crop breeding.

All plant breeding methods, however, have unique features and the main special feature of GM plant breeding is that it allows a wider choice of genes for modifying crops in novel ways. No other plant breeding technique permits the incorporation of genetic material from such diverse biological sources. Inevitably this raises the possibility that some new consequences of GM plant

breeding may be unexpected. This presents challenges for their regulation and management in the future that will need to be managed carefully and intelligently.

THE SAFETY OF FOOD AND ANIMAL FEED DERIVED FROM GM CROPS (CHAPTER 5)

A number of issues of public concern are considered in detail. Might GM crops result in more food allergies? Could GM foods be less nutritious or more toxic than their conventional counterparts? More generally, could DNA from GM crops harm people, either through being consumed directly in GM-derived food, or by entering the food chain through animal feed?

Possible nutritional and toxicological differences in GM food (5.2)

All novel food in the UK, which includes food produced by GM organisms, is subject to an EU-based and internationally determined regulatory regime, with procedures for safety assessment and risk analysis. The regime recognises that the consumption of food is not risk-free and requires any novel (including GM) food to be at least as safe and nutritious as any traditional food it replaces or complements.

To date world-wide there have been no verifiable untoward toxic or nutritionally deleterious effects resulting from the cultivation and consumption of products from GM crops. However, absence of readily observable adverse effects does not mean that these can be completely ruled out and there has been no epidemiological monitoring of those consuming GM food. Some reason that the absence of evidence of harm should not be treated as evidence of the absence of harm. This argues for greater reliance on scientific research and epidemiological monitoring. Others reason that the combination of testing by developers to demonstrate safety equivalence to commercial crops in order to satisfy regulatory requirements for clearance and extensive use around the world over long time periods and large exposed populations and absence of evidence of harm, does provide important experience of safety. The long-term assessment of the health effects for whole foods and feeds is considerably more difficult than the post-marketing monitoring and surveillance of a simple substance such as a single medicine. Countries are working to develop post-marketing surveillance to detect potential human health effects of food in general, but at present there is nothing yet available for GM foods in any country.

Safety assessment technologies such as screening and profiling techniques will need to continue to evolve, incorporating data on all possible entry-points for new hazards and to cope with uncertainties and gaps in knowledge. The complexity of the safety assessment process is likely to increase with the development of ‘second generation’ GM crops. These crops and their products aim to: decrease levels of anti-nutritional factors (e.g. toxins); increase levels of health promoting factors (e.g. antioxidants); and modify levels of macro or micronutrients (e.g. vitamins).

Food allergies from GM crops (5.3)

Changes in allergenicity during the breeding of conventional crops are not assessed in a regulatory framework and are not formally evaluated.

GM technology enables a particular gene construct for a new protein to be introduced, and the potential allergenic effect of that protein is a focal point for safety assessment. In addition, the regulatory process, with its case-by-case approach, must take account of possibly increasing exposure to a GM protein, especially if it is expressed in a diversity of different GM plants, and thus introduced into a diverse range of foodstuffs. In the hypothetical case, where an GM allergen was not recognised in regulatory screening, and its effects only emerged in the longer term, avoidance of the allergenic protein by the consumer could be difficult, because they would not be able to recognise its presence in the foodstuffs. The likelihood of this scenario is very low for a number of reasons. However, avoidance in a GM or non-GM case would depend on the relative effectiveness of labelling, traceability and recall systems and it would be for the regulatory system to ensure that any GM allergen once known, with a potentially significant effect on any consumer, should be labelled in a fail-safe way or withdrawn from the marketplace.

It is probably easier to evaluate the risk of introducing allergenic proteins and altering the allergenic composition of the target crops after use of GM than with some conventional breeding techniques.

There is an accepted approach, based on a standard set of safety tests, to the assessment of the allergic potential. But there is some contention over the value of specific tests and if, and how they can be improved. These tests are under continuous evaluation and improvements are considered in the scientific and regulatory literature.

The GM foods consumed at present (by large numbers of people for up to seven years) do not appear to have elicited allergic reactions. The same arguments for and against the significance of this are the same as for nutritional and toxicological effects (see 5.2 above). Our relative lack of knowledge about factors that are important in sensitisation and the elicitation of an allergic response suggest that we should continue to exercise caution when assessing all new foods, including foods and animal feeds derived from GM crops.

The fate of transgenic DNA (5.4)

The food we consume from conventionally bred crops contains large quantities of DNA, since DNA is a universal component of all living organisms and is not typically removed by the extraction and processing technologies used by the food and drinks industry. Some processes, such as sugar purification and the production of refined oils, remove most, sometimes all, of the DNA from a product before it is consumed. Other processes, such as heat treatment, whilst not removing DNA entirely, cause extensive inactivation and breakdown. The consumption of raw vegetables and fruits does, of course, mean that intact DNA is ingested.

DNA, like other large molecules in food, is very largely degraded (broken down to smaller molecules) in the gut, but this process of structural degradation whilst inactivating the DNA's genetic information, is not 100% efficient. Fragments of ingested DNA have been found throughout the digestive system and elsewhere in the body, including the blood stream. Our guts contain very large numbers of bacteria which help us to digest the food we consume. Whilst it is possible that these bacteria take up DNA from their environment (i.e. our digestive systems and the foods they contain) there are a series of well-established barriers in place to prevent the genomic integration and expression of foreign genes. This process is unlikely to be of biological significance unless: (1) the bacterial cells can use at least some of the genetic information that the DNA encodes; and (2) that information confers a selective advantage, leading to an increase in the proportion of the bacteria that contain this new DNA.

In GM food, the introduced DNA will have the same fate as DNA present in conventional food and will be inactivated and increasingly degraded as the food progresses through the digestive system. If the food originates from a GM crop in which bacterial DNA is part of the transgene, then, whilst still likely to be a rare occurrence, there is increased opportunity for that DNA to transfer into gut bacteria. This possibility makes it essential, in the achievement of maximum risk reduction, for the regulatory process to consider each GM crop as an individual entity with its own potential risks.

Antibiotic resistance is not only widespread as a consequence of antibiotic and feed additive usage, but because it is highly selected for in microbes in the wild. Bacterial genes conferring antibiotic resistance have been a commonly used tool for selection in GM technology, but alternatives have now been developed and it is possible to eliminate antibiotic resistance gene markers following GM plant construction. So, the presence of antibiotic resistance genes can now be avoided in GM plants intended for food use. The use of antibiotic resistance genes in plants remains controversial, with differing views on its potential impact. There is a scientifically well-supported argument that any rare resistance gene transfer event from a GM plant or food would have no impact as antibiotic resistance is already widespread as a consequence of antibiotic usage in medicine and animal feed.

The effect of GM derived feed on the food chain (5.5)

Animal feed is a major product of conventional agriculture, and of crops developed using GM technology. The processing of crops into animal feed often completely degrades such constituents as DNA and proteins, but this cannot be assumed always to be the case. Most DNA is degraded in the gut, but some survives and there is evidence that some DNA fragments from feed ingested by poultry and livestock can appear in the blood and other tissues.

However, food and feed safety studies have been unable to find introduced feed DNA or its gene products in milk, meat or eggs produced from animals fed GM crops. Many millions of people, particularly in the United States, Canada and Argentina, have for up to seven years been eating food products derived from animals fed on GM diets and no substantiated ill effects have been reported. There is a similarly lack of evidence for any adverse effects of GM feed on the health, welfare and productivity of livestock.

However, as mentioned in relation to nutritional and toxicological differences, the absence of readily observable adverse effects in humans or animals does not mean that these can be completely ruled out for any crop GM or non GM, existing or novel. For example, rare, mild or long-term adverse effects are not easy to detect and could in future be the subject of post-marketing monitoring and surveillance. The safety assessment of crops with significantly altered nutritional qualities will need careful consideration where there may not be historical knowledge of assumed safe use.

THE ENVIRONMENTAL IMPACT OF GM CROPS (CHAPTER 6)

There has long been concern about the ways that GM crops might affect the environment, and this was reflected in the public consultation exercise. In addition to direct environmental impacts, there could be indirect effects, for example in the ways that cultivation of GM crops might change agricultural practices and rural landscapes. The latter seems most likely and could bring benefits as well as risks. The great majority of all GM crops currently in cultivation are grown in the USA, Canada and Argentina. In each of these countries the crops tend to be grown on large-scale farms that are geographically quite isolated from wilderness areas. More recently, many smallholder farmers in China have also adopted GM crops. The circumstances are distinct from the many smaller-scale farms embedded in the countryside that are characteristic of the UK and the rest of Europe. These differences in scale and farming practices must be considered in harm/benefit analyses of the potential environmental impacts of GM crops in different parts of the world. It is also essential to compare the environmental impact of the GM crop with other current and evolving practices in conventional agriculture.

Could GM plants become more widely invasive or persistent? (6.2)

Notwithstanding the case-by-case approach taken by the regulatory authorities in evaluating invasiveness, there are two principal models that have been influential in considering the potential for GM crops to become more invasive of natural habitats than their conventional counterparts. One is the *alien species model*. The hypothesis is that roughly 0.1% of introduced GM plants would become pests, because that was the rate of invasive alien plants species (some 15 problem plants out of an estimated 15,000 alien species introduced into the UK). The other is the *crop model*, which argues that GM crops will behave in much the same way as conventional crop plants except for the GM trait that may influence fitness. Conventional annual crop plants generally do not prosper outside arable fields. Although escaped plants of crop species are found, they do not tend to increase in abundance but are replenished each year by fresh 'escapes'. Detailed field experiments on several GM crops in a range of environments have demonstrated that the transgenic traits investigated do not significantly increase the fitness of these plants in semi-natural habitats, and therefore they behave in a similar way to non-GM crops.

We do not have an exact understanding of what changes in a plant's life history will affect its invasiveness. More knowledge on the potential effects of releasing GM plants with traits such as pest and disease resistance and stress tolerance is required since these may significantly alter a

crop plant's ability to survive outside the agricultural environment. In particular, we need to know whether GM for fitness-affecting traits like growth rate, longevity, plant size, or survivorship in plant species with potentially more invasive life histories (e.g. woody plants, perennial grasses, thicket-forming herbs) is consequential.

Could GM crops be toxic to wildlife, and what might be the impacts? (6.3)

Crop breeding, whether through genetic modification or 'conventional' methods, has the potential to alter levels of plant toxins or create novel compounds that are toxic to some wildlife. Such effects are unusual but they are a key element of the risk assessment process for experimental and commercial release of GM crops. The principal risks arise for crops that have been deliberately bred to contain toxins to control key pests or diseases. GM pest- and disease-resistant crops are unlikely to be grown commercially in the UK in the near future. Nevertheless, evidence from the USA and China indicates that for some, but not all, GM pest-resistant crops there have been significant reductions in pesticide use. In every case when attempting to determine the effects of pest-resistance, it is necessary to judge the crop-pesticide combination as a 'system' rather than simply considering the ecological impacts of the crop in isolation

There is little scientific dispute about the fact that GM plants engineered to produce toxins can sometimes be toxic to non-target wildlife, since even in nature toxins are rarely species-specific. However, no significant adverse effects on non-target wildlife resulting from toxicity of GM 'Bt ' plants, for example, have so far been observed in the field. This suggests that Bt crops are generally beneficial to in-crop biodiversity in comparison to conventional crops that receive regular, broad-spectrum insecticide applications. Despite this, benefits would probably be restricted (or even negated) if Bt crops required insecticide applications to control target or secondary pests that were not sufficiently controlled by the Bt toxin. Studies on the impacts of GM crops on soil processes have shown some differences in soil microbial community structure, but so far there does not seem to be any convincing evidence to show that GM crops could adversely affect soil health in the long term. The differences in soil microbial communities observed beneath GM crops have been within the range of variation in microbial community structure and of the order of magnitude of the differences observed under different crops of even different cultivars of the same crop. However, almost all this data is drawn from small-scale, short-term studies and there is a need for larger, more agronomically realistic studies to be undertaken to demonstrate absence of harm to non-target organisms.

There tends to be scientific disagreement about the amount of information needed to demonstrate that growing GM pest and disease-resistant crops is environmentally sustainable in the long term. Some scientists argue that current evidence of reductions in pesticide use and increases in biodiversity compared to conventional crops are sufficient to demonstrate absence of adverse impacts, while others advocate the need for a greater fundamental understanding of the underlying processes.

Most of the possible negative impacts of GM crops on biodiversity are likely to be reversible, so small-scale field trials to test for impacts on relevant ecosystems are unlikely to pose any long-

term environmental risks. After a crop has been approved for commercial use, the monitoring systems required for GM crops grown in the EU provide a valuable mechanism to collect ecologically relevant data. This will be useful to enhance our understanding of the impacts of GM pest-resistant crops on non-target species.

Could GM crops lead to particular problems in the development of resistant insects, weeds and diseases? (6.4)

A key long-standing target of 'traditional' plant breeding, including some uses of genetic modification, has been the development of crop varieties that are resistant to pests and diseases. Widespread, uniform cultivation of these varieties, together with any agrochemicals applied to reduce the incidence of disease or to kill weeds, provide a strong selection pressure for the emergence/evolution of resistant *target organisms* (pests, pathogen and weeds) that can attack the new variety or survive the pesticide application. The time it takes for a resistant target organism to emerge depends on the nature of the toxins and how they are expressed, the ecology, genetics and mating behaviour of the target organism(s), the mode of action of the toxin, and on the effectiveness of the crop management techniques deployed by farmers.

Current widespread scientific opinion is that 'single dominant resistance gene' mechanisms are less durable than resistance controlled by several genes. However, some sources of GM resistance, including *Bt* genes that confer resistance to a narrow range of target insects, appear to be particularly robust. However, there is no *a priori* reason to suppose that resistance genes introduced by GM will be any less susceptible to 'breakdown ' than those introduced by slower conventional breeding methods.

Over 120 species of weeds have been recorded worldwide that have become resistant to various herbicides in association with herbicide-tolerant crops, irrespective of whether tolerance was obtained by GM or conventional breeding technologies. Weeds that are closely related and hybridise freely with the cultivated herbicide-tolerant crop variety have the added possibility of obtaining tolerance gene(s) directly from the crop. However, unless the weed is exposed to the herbicide in question, this does not pose any ecological or selective advantage.

Therefore, although resistance-breaking strains of pathogens, pests or weeds can be expected to emerge, there is no reason to expect different responses depending on whether a crop's resistance was introduced by GM or by conventional breeding methods.

However, since GM has frequently employed genes which confer resistance to common herbicides and pesticides (e.g. glyphosate and Bt) in its weed and pest control strategies, impacts on agriculture and possibly biodiversity could be significant if some target organisms developed resistance to these compounds. The extent and possible severity of impacts on the environment are difficult to quantify and subject to much debate.

Will herbicide-tolerant crops offer new weed control strategies and if so what are the likely positive and negative impacts? (6.5)

GM herbicide-tolerant (GMHT) crops enable new weed control strategies. The key possibility is the replacement of existing approved but persistent, toxic herbicides by those with a more benign environmental profile. They may also enable farmers to spray crops less frequently and to relax weed management practices for conventional crops at different stages in the rotation. Hence they are an attractive option for farmers wishing to simplify crop management. It may also be possible to delay the date of herbicide application, avoid pre-sowing weed treatments and so leave emerging weeds in the fields for longer. Such a result might have benefits for biodiversity, though this claim is largely speculative and is not strongly supported by the current small-scale experimental studies. Similarly, evidence from the USA indicates that tillage can be reduced in HT crops, which provides environmental benefits that may not necessarily be relevant to the UK.

Fifty years of agricultural intensification has undoubtedly led to a decline in farmland biodiversity, but the role of herbicides in this decline is unclear. Broad spectrum herbicides used in conjunction with GMHT crops are known to provide highly efficient and reliable weed control in comparison to many 'conventional' herbicide regimes, and if their use resulted in fewer weed seeds and further declines in weed populations then organisms depending on those weeds during part of their life cycle could be adversely affected. We do not yet have sufficient evidence to predict what the long-term impacts of GM HT crops might be on weed populations. An important uncertainty is how farmers will apply this technology in the field.

The publication of the UK farm-scale evaluations of GMHT crops will clarify some of these uncertainties. Inevitably others will remain. The question would become more complex if farmers were to grow two or more herbicide tolerant crops in rotation.

Apart from herbicide tolerance, what are the major new traits that might give rise to significant environmental impacts? (6.6)

Over the next ten years, there is the possibility of introducing GM crops resistant to attack by insects, nematodes, fungi, bacteria or viruses. In all cases, we would expect these to enable reductions in pesticide use. There are potential negative impacts on non-target organisms, but in the case of insect resistance, field studies on commercially grown Bt crops have failed to identify any adverse effects. In addition, subject to regulatory approval, there will be imports of GM food, feed and fibre, with improved shelf life or nutritional quality, but these are not expected to affect the UK environment.

Further ahead, it becomes more difficult to make confident predictions about the commercialisation of GM crops and their possible environmental impacts. The horizon scan has identified the paucity of baseline data and models at different scales, from field to landscape scale, which is needed as a basis for future assessment of large-scale environmental effects. Many of the issues foreseen are not unique to GM crops and will be driven by economic, social and political rather than purely scientific factors. Current research points to GM crops for certain non-food purposes: pharmaceuticals, speciality and bulk chemicals and biomass for energy. These

could provide renewable resources for industry, provide new medicines and could diversify rural landscapes and economies. Conversely, there could be undesired effects on wildlife caused by the way these crops might be managed and/or changes in patterns of land use. Another longer-term possibility is the development of traits aimed at improving crop production in marginal environments (e.g. tolerance of drought, heat or salt) with obvious advantages to certain growers in these environments. However, such crops could become more successful as weeds, there could be economic pressure to cultivate areas with wildlife and conservation value, and there might be adverse socio-economic and political consequences, for example with regard to optimal farm size.

Might GM crops change agricultural practice is the UK? If so, what might be the likely consequences? (6.7)

It is widely acknowledged that modern (non-GM) agriculture has already had significant negative impacts on biodiversity and the wider environment in the UK. Large changes over the last century, including recent decades, in the way farmland is managed have resulted in a decline in farmland plant, invertebrate and bird abundance and diversity.

The consequences of commercial growing of GM crops in the UK would depend on the nature of each individual technology and the decisions made by farmers, the public and policy makers. For example, some GM technologies could increase agricultural intensification, to produce more from the same area of land, while other niche and specialist GM crops could increase the diversity of the landscape. Some GM crops would lead to reduced agrochemical use while others would have the opposite effect.

Each potential agricultural application of genetic modification must, therefore, be examined on a case-by-case basis, taking careful account of the physical, social and political environments within which it would be deployed. There is a major need for policy makers to understand how these factors are likely to interface with the new technologies, to enable prediction of environmental outcomes and thus delivery of environmental targets because they will predict outcomes from the environment if targets are to be delivered.

What are the limitations of the science available to predict the environmental impact of GM plants? (6.8)

There are several approaches for determining the ecological consequences of GM crops. Examples include extrapolations from experience with comparable traits or with other crop varieties that are in some or all ways ‘equivalent’, laboratory and field experiments, experience of GM crops, and ecological modelling. In practice it is usually necessary to use a number of these methods in combination.

Most of the environmental issues raised by growing currently available GM crops do not differ qualitatively from conventional crops. In both the GM and conventional context, we are limited in our ability to predict ecological changes within complex systems. This applies to a wide range

of ecological issues and to many aspects of agriculture: modern intensive, organic or conventional. Important gaps in knowledge include the possible rate of uptake of GM crops in the UK; detailed knowledge of farmland ecology; soil ecology.

GENE FLOW, DETECTION AND IMPACT OF GM CROPS (CHAPTER 7)

Gene flow is the movement of genes from one organism to another, and is something that takes place in nature all the time. There are various mechanisms by which gene flow can occur and various natural barriers to minimise its effects. None of these mechanisms is specific to GM plants; therefore a great deal of evidence from conventional agriculture is relevant.

Gene flow between crop varieties (7.2)

Genes can move between different varieties of the same species by the spread of seed and by cross-pollination. The complete genetic isolation of crops grown on a commercial scale, either GM or non-GM, is not practical at present. However, gene flow can be minimised, as currently happens in the case of oilseed rape varieties grown for food, feed or industrial oils. The levels at which gene flow can be maintained for different crop varieties are significant in determining whether co-existence of different types of agriculture is feasible. However, political decisions may ultimately affect whether co-existence is practical, in particular what thresholds are set for maximum GM presence in non-GM crops (and their products), whether conventional or organic. For some crops, maintaining thresholds of gene flow may be relatively straightforward, by employing separation distances and, more importantly, by reducing gene flow through seed. However, in other cases it may be difficult, if not impossible, to grow certain crops or use some existing farming practices (e.g. using farm-saved oilseed rape seed on farms where both GM and non-GM varieties are grown).

Gene flow from GM crops that have been approved for commercial release can be detected but unapproved GMOs present difficulties. Gene flow may be detected if commonly used transgenic DNA is present, but the actual source of the GM presence will be difficult, maybe impossible, to identify. Detection methods are very sensitive but they cannot guarantee a total absence of transgenic content. Equally, false positives may indicate that transgenic DNA is present when it is not.

‘Gene stacking’ is the accumulation of genes conferring a range of traits as a result of cross-pollination between different varieties. It is not unique to GM crops. However, if GM crops are to be grown commercially in the UK, assessments of the potential consequences of such gene stacking may well become a more prominent consideration for regulators. GM crops that produce non-food, non-feed products such as pharmaceuticals, bioplastics or biofuels pose different regulatory issues and would, as for all GN crops have to be judged on a case-by-case basis. In any case, such crops would (certainly, should) be designed and/or grown in ways that would preclude gene flow to food and feed crops.

More information is needed about the mechanisms and management of seed dispersal in agricultural systems, along with diagnostic and sampling methodologies for determining the extent of gene flow early in the production/supply chain. In the longer term, it is possible that gene containment systems will be developed that significantly reduce gene flow.

Gene flow from GM crops to agricultural weeds and wild relatives (7.3)

Gene flow can occur from GM crops to sexually compatible wild relatives and to agricultural weeds. Cross-pollination will occur to an extent that depends on the closeness of the relationship between the species and on other conditions. However, the key issue is whether any resulting hybrid plants survive, grow and reproduce successfully allowing the new gene to be introgressed (stably introduced into the new population). Hybridisation seems overwhelmingly likely to transfer genes that are advantageous in agricultural environments, but will not prosper in the wild. This general view is supported by specific studies on oilseed rape and on sugar beet, where there has been little or no detectable gene flow to semi-natural habitats even though there can be hybridisation within a field. Furthermore, no hybrid between any crop and any wild relative has ever become invasive in the wild in the UK.

Within current agricultural practice, more than 120 non-GM herbicide-resistant species have emerged worldwide in the last 40 years. In most, but not necessarily all cases, such plants are at a disadvantage away from agricultural conditions. This disadvantage has also been found in experiments carried out on GM plants. There have been some instances in Canada, where there is complete freedom to grow several herbicide-tolerant varieties, e.g. oilseed rape, of tolerance being transferred to weeds or stacked through hybrids in one variety. However, if herbicide-tolerant crops are carefully managed, this should delay, or even prevent, the emergence of any herbicide-tolerant weed problem.

Genes associated with resistance to pests and diseases have greater potential than herbicide-resistant genes to lead to the local expansion of a plant population. However, there are other natural constraints that could prevent an increase in population growth rates in such cases. Overall, genes for pest- and disease-resistance inserted into crops by conventional breeding have not produced invasions of wild relatives in semi-natural habitats.

However, there are gaps in our understanding of the potential consequences of gene flow, and the effect of particular traits on the fitness of the weed or wild relative, which may receive them, is an important target of ongoing research. In addition, several technological solutions to containing or reducing gene flow from GM crops have been proposed.

Can genetic material in GM plants transfer to soil microbes? (7.4)

Most plant DNA is degraded during the natural processes of decay, but there is a small possibility that genes in plant DNA could be acquired and expressed by environmental microbes. There is no evidence from complete bacterial gene sequences that genes from plants have successfully established during bacterial evolution, but bacterially derived transgenes in current use may have

a higher probability of transfer to soil bacteria than average plant DNA. No such transfer under field conditions has yet been observed. However, there are limited tools, and there have been limited attempts to test the phenomenon under field conditions.

Most current transgenes are of bacterial origin. They are therefore unlikely to have any significant novel effect on bacteria that have already been exposed to them by gene transfer from other bacteria, though their similarity to bacterial DNA may increase the chance that bacteria acquire them. Inserting transgenes in plastids (i.e. chloroplasts) may increase the chance of horizontal gene transfer (HGT) to bacteria because of the increased copy number (several 100 copies per cell instead of 1 or 2 copies of nuclear DNA) and closer relationship to prokaryotic gene structure. Careful design of transgenes can greatly reduce the potential for HGT to bacteria. In future, inserted genes may encode proteins not found naturally. Although these will be less easily acquired by bacteria, their effects may need to be explicitly tested in representative bacteria.

HGT to other microbes, (e.g. fungi and protists), has not been as well researched as for bacteria. As with bacteria, there is some indication that the rate may not be zero. Since these are eukaryotes, some further consideration should be given to the likelihood of incorporation and expression of the transgenic DNA used in GM plants, as the work directed at bacteria will not be applicable.

Initially, a gene transfer event affects a single microbial cell. It will have no ecological impact unless the transgene confers an advantage on its recipient that causes it to become widespread in the microbial population. For most genes that may be used in GM crops, this is unlikely. A potential transgene should be assessed by first asking whether it could be expressed in microbes and could confer an advantage on them. In some cases, this may require direct testing, and high-throughput methods could be used to scan for unexpected patterns of gene activity and metabolism. If the answers are positive, then consideration must be given to the potential wider consequences if the recipients became established, so that transgenes that can be predicted to cause harm if expressed in microbes can be avoided. There is inevitably some uncertainty associated with this assessment. Our current understanding of microbial ecology does not allow us to make detailed predictions of the effect of genetic perturbations, whether these are caused by natural genetic evolution events, by normal agricultural practices, or by the spread of a novel microbe. Experience suggests that microbial community functions are fairly resilient, but a better understanding of microbial ecology is clearly desirable.

It is important to reduce the potential for expression and transfer of genetic material from GM plants to soil microbes by removal of unnecessary vector DNA that may provide homology with soil microbial DNA, origins of replication and sites for transposition, and also by introducing non bacterial features (e.g. introns) where possible.

Can genetic material in GM plants transfer to viruses? (7.5)

Since 1986, thousands of GM plant lines have been made that contain a range of DNA sequences of viral origin, mostly short fragments that regulate the way in which other (non-viral) transgenes

are expressed. There have also been many hundreds of GM plant lines in which short viral DNA sequences have been introduced to confer resistance to viral diseases. This approach has proved to be a selective, measurable and environmentally sustainable method of crop protection. The conventional alternative is to use pesticides liberally to control the fungi and invertebrates that spread the viruses.

Several GM virus-resistant crops have been grown commercially on a large scale in several countries for at least seven years.

Laboratory and greenhouse studies, since 1994, have shown that defective mutant viruses with a range of genetic defects can be restored to their wild type phenotype by acquiring the necessary sequence from a suitable GM host plant through recombination. Detailed studies have been carried out to look for the transfer of genetic material from GM plants to viruses under field conditions. None has been detected. These studies have involved a number of commercial GM crops, including papaya, squash and sweet potato. If such transfer did occur, the potential consequences would have to be assessed on a case-by-case basis of each virus-resistant GM variety.

Containment of any newly emerging plant virus would be through standard and widely accepted control measures. Since the 1970s, an accepted and approved practice has been to intentionally infect highly susceptible, high-value crops such as glasshouse tomatoes with a mild strain of a virus to protect them against severe strains of the same or a related virus. This practice poses greater (and documented) opportunities than GM for genetic recombination to create new virus strains.

It is theoretically possible, but extremely unlikely and without precedent, that transfer of viral genetic material from a tested and approved GM plant would make an invading virus fitter. This is because that rapid mutation, selection, genome reassortment and switching of genetic material between naturally occurring viruses are common natural events. It is therefore reasonable to assume that any new genetic trait beneficial to the virus would already have been tried and selected through millennia of evolution, or during natural or artificial mixed virus infections.

Nevertheless, several practical recommendations can be made in the design of transgenes containing DNA derived from viral sequences that would minimise the theoretical risk associated with their use.

CONCLUSIONS

New technologies always bring uncertainties and generate new gaps in knowledge. Uncertainty and divergence of interpretation are a key part of scientific development, providing the stimulus for new scientific hypotheses to be formulated and tested out, for uncertainties to be reduced and for new insights to be developed. Part of science is the ability to be honest about uncertainty and to be able to judge the quality or strength of evidence for a particular conclusion. Challenge is central to the scientific process and so too is speculation. The way to resolve controversies, when

these are amenable to scientific resolution, is to do better science by going back to the real world and examining it with better tools and better ideas to improve understanding.

We cannot know everything and if we were paralysed by gaps in knowledge we would never get anywhere new. One of the paradoxes of science is that sometimes awareness of uncertainty grows as we learn more. At the same time, the lessons of history tell us that sometimes we have rushed forward incautiously to exploit new technologies, only subsequently to appreciate the medical, social, environmental or other costs. As individuals and as a society we have to be able to cope responsibly with incomplete knowledge and uncertainty.

We have conducted an issue-led, evidence-based review of the issues of concern to the science community and the general public. There are those who tend to state that, because GM is similar in many ways to conventional breeding, this is a useful baseline for comparison. There are others who reason that this approach understates the distinct differences between GM and non-GM and that, because the technology is relatively new, we know too little, the uncertainty is too great, and there are too many gaps in knowledge to pursue it safely at the current time. We have come face to face with both these arguments in our Panel discussions. However, we have progressed beyond this and we believe we have been helped in this by the framework we have developed and used. Absence of evidence of harm is not evidence of absence of harm. So what is the evidence for harm? And what is the evidence for the absence of harm? We have looked at this for each of the issues under review.

The reliability of GM technology is a feature of concern to many people. What is our response? It is clear that imprecision and unpredictability are features common both to conventional plant breeding and to GM plant breeding. In each case, testing needs to be adequate to ensure that plant varieties and the foods made from them are safe. For GM crops and GM food, it is important that testing also takes into account the potential unanticipated effects that might arise from the unique capability of placing genes into very different genetic backgrounds. It is appropriate and reassuring, therefore, that the regulatory system in place throughout the EU demands a high level of scrutiny in the testing of GM crops, and that powerful analytical tools are available to analyze GM plants with a degree of molecular precision impossible for all products of conventional (non-GM) plant breeding.

The current, and widely accepted view within the biological research and plant breeding communities is that the methods for evaluation of the current generation of GM crops for food and feed carried out within the European regulatory framework, are robust when consistently applied. There are those who are not so confident, and their challenge is an important factor in the improvement of the framework. Regulatory evaluation needs to keep pace with the challenges posed by developments in this technology and recognise progress in understanding and knowledge. It is important that research to ensure effective risk assessment is supported.

For human health, to date there is no evidence currently commercialised GM crop varieties or foods made from them, are toxic, allergenic or nutritionally deleterious. But what is the evidence for this? The principle arguments are that molecular tests done on products prior to commercialisation have been conducted, and that the combination of testing by developers to satisfy regulatory requirements for clearance, extensive use around the world over long time periods with large exposed populations, and the absence of evidence of harm, does provide

important experience of safety. Others are less convinced, pointing out that the techniques have limitations; for example we still do not have an exact understanding of what causes us to be sensitised to allergens (GM or otherwise), and that systematic surveillance and post–market monitoring is not conducted. On balance, we conclude that the risks to human health are very low for GM crops currently on the market. But GM does present certain particular potential challenges in risk management and the situation may prove to be more challenging in future, depending on the crops developed. There is a need, therefore, to continue to develop safety assessment technologies, effective surveillance, monitoring and labelling systems, and to have in place effective avoidance strategies.

Transgenic DNA and non-transgenic DNA appear, from the studies conducted, to share the same fate once ingested by humans, being very largely, but not entirely, degraded in the gut. There is an interesting but not yet proven possibility that, because transgenes may share sequences in common with bacteria present in the gastro-intestinal tract, this might permit 'horizontal gene transfer' to gut bacteria. From the few studies that have been carried out to date there is no compelling evidence that gene transfer occurs under natural conditions, and, for this to happen, a series of natural barriers would need to be overcome.With respect to GM-derived animal feeds several research studies have been unable to find transgenic DNA (or its gene products) in milk, meat or eggs produced from animals fed on GM crops.

Turning to the environment, the UK is characterised by a landscape in which many small-scale farms are embedded in the countryside so that farmland biodiversity forms an important part of the plants and animals that inhabit this country. We know that conventional intensive agriculture has provided benefits in terms of affordable food and predictable food supply, but at a significant cost to the natural environment. It is against this background that the commercial introduction of GM crops is contemplated in the UK, and because GM is tightly regulated, we know that the first ones, if introduced, are likely be herbicide-tolerant fodder beet, oilseed rape, and maize.

Detailed field experiments on several GM crops, including these three, in a range of environments have demonstrated that they are very unlikely to invade our countryside or become problematic plants, although HT oilseed rape and beet could become weedier in agricultural settings. Nor are they likely to be toxic to wildlife or to perturb soil structure in such a way that the functioning of soil communities is substantially affected.

We also know the extent and pattern of gene flow for these particular crops. Maize has no wild relatives in the UK with which to cross-pollinate. Beet and oilseed rape do. However, field studies indicate that there is very little gene flow from these crops to wild relatives living in semi-natural habitats. The frequency of genes in populations is dependent on whether or not they confer any selective advantage and, equally importantly, the frequency of hybridization: which in these cases is very low. However, for the future, the effect of particular traits on the fitness of the weed or wild relative that may receive them is an important target of ongoing research.

The few studies that have been carried out so far have been unable to detect evidence for horizontal gene flow between GM plants and either bacteria in the soil or viruses. If such horizontal gene flow does occur, then preliminary indications suggest that it is a very rare event. The possibility of horizontal genes transfer to other microbes, (e.g. fungi and protists), has not been well studied and is an important area for future research.

Agricultural intensification has undoubtedly led to a major decline in farm biodiversity in recent decades in the UK, but the role of herbicides in this decline is less clear. We do not yet have sufficient evidence to predict what the long-term impacts of GM herbicide-tolerant (GMHT) crops would be on weed populations and the wildlife that depends on weeds for food. Above all other concerns, this poses perhaps the most serious potential harm arising from these particular crops. An important uncertainty is how farmers would apply this technology in the field. The publication of the UK farm-scale evaluations of GM herbicide tolerant crops will clarify some of these uncertainties. We aim to consider these results in the autumn.

Looking further ahead, it is clear that complexity and uncertainty will increase as the range of plants and traits introduced increases. Gaps in our knowledge exist in the areas listed below:

We do not have a precise understanding of which changes in a plant's life history affect its fitness. We do not know whether fitness-affecting traits like altered growth rate, longevity, plant size, or survivorship in plant species with potentially more invasive life histories (e.g. woody plants, perennial grasses, thicket-forming herbs) will result in invasive and problematic plants as is true of such as Japanese knotweed and rhododendrons.

Genes associated with resistance to pests and diseases have greater potential than herbicide-resistant genes to lead to the local expansion of a plant population if transferred from a GM crop. However, there are other natural constraints that could prevent an increase in population growth rates in such cases. It may be significant that genes for pest and disease-resistance inserted into crops by conventional breeding have not produced invasions of wild relatives in semi-natural habitats. This may be related to linkage drag. That is, the hybrid 'crop-wild relative' inherits the transgene plus a set of all genes from the agricultural plant that reduces the competitiveness of the plant outside the agricultural environment.

'Stacking' of transgenes in crop plants or wild relatives is a distant future possibility in the UK. However, if it occurred (as it has with herbicide-tolerance genes in oil seed rape in Canada) it would involve plants with unintended and unstudied gene combinations. Predicting the ecological behaviour of such plants in advance of their accidental and unintended production will provide scientific challenges to the regulatory system.

There is an extensive 'tool kit ' to consider the environmental impacts of GM crops, but it must also be acknowledged that, given the complexity of ecology, we do not have all the data to make precise predictions, nor are we necessarily asking all the right questions. A case-by-case approach to making assessments on environmental impacts continues to be the appropriate approach.

To date, in countries that have the experience of growing GM crops, there have been no reports of them causing any significant environmental damage. This is an important point to recognise, but equally, we must be cautious in drawing general conclusions as these observations are based on relatively few field experiments. In addition, the findings may not be entirely relevant to the UK situation. This point about the difficulty in generalising confidently from one country to another also applies to evidence from the USA, China and India indicating that use of some, but not all, GM pest-resistant crops has resulted in significant reductions in pesticides, and the replacement of certain herbicides by others with a more benign environmental profile.

So what is the appropriate agriculture for the UK? We cannot answer this question fully, but clearly it will need to be sympathetic to wildlife, and allow co-existence of farming systems. Political decisions, market forces and other pressures will ultimately decide whether co-existence of different farming systems is practical, and in particular what thresholds are set for GM presence in crops and food labelled non-GM. Uncertainty surrounds the way in which different factors determining co-existence will combine at commercial scales (i.e. the real-life consequences of the combination of unintended presence in seed, cross-pollination, and the contribution of volunteers). For some crops, this may be relatively straightforward to manage, for others it may be difficult without significant changes to current practices. Tracing genes in supply chains is possible, but there are limits to reliability, which are, to a large extent, determined by the degree of sensitivity required. The issues of traceability, segregation and gene flow become potential health issues where GM crops produce non-food, non-feed products such as pharmaceuticals, bioplastics or biofuels. Such crops pose challenging regulatory issues and will also have to be judged on a case-by-case basis. In any case, such crops would (and certainly, should) be designed and/or grown in ways that would preclude gene flow to food and feed crops. The impacts (positive or negative) of GM plants will be largely dependent on how GM technology is deployed by farmers and this in turn may depend in part upon incentives to optimise a combination of productivity and environmentally friendly usage.

Genetic modification is not a homogeneous technology and to answer many questions each specific application of genetic modification must be considered on a case-by-case basis. Each product brings different potential benefits for different stakeholder groups; each may pose different environmental or health risks. In making judgments about GM crops, it is also vital to scrutinise the uncertainties as well as the potential risks and benefits and to make comparisons with non-GM crops grown in conventional, organic or other lower intensity farming systems. It is also important to recognise that non-GM plant breeding is becoming progressively more sophisticated and able to provide novel modifications to crops that can raise similar issues as those considered in this review.

There is a clear need for the science community to do more research in a number of areas, for companies to make good choices in terms of transgene design and plant hosts, and to develop products that meet wider societal wishes. Finally, the regulatory system in the UK should continue to operate so that it is sensitive to the degree of risk and uncertainty, recognises the distinctive features of GM, divergent scientific perspectives and associated gaps in knowledge, as well as taking into account the conventional breeding context and baselines.

Chapter 1

GENERAL INTRODUCTION

1.1 WHY HAVE A SCIENCE REVIEW?

Developments in science and technology invariably provide society with new opportunities, but also new challenges to apply them responsibly. As with many new technologies, people are keen to embrace many benefits but are concerned about the potential risks. The science of genetics[1] has developed considerably over recent decades, so that we can now fully understand the genetic make-up of many organisms and genetically modify crops and other living things in new ways. The UK is now at a crossroads about whether or not to accept the growing of genetically modified crops in agriculture. The aim of this review is to consider the evidence for both the real and perceived risks and benefits of GM crops from a scientific perspective. Before saying more about the nature of the review it is important to give some background.

In a sense, people have been genetically modifying plants (and many other living things) for thousands of years by breeding and selecting improved plants and by the domestication[2] of crops. Originally, this selection was done without any knowledge of the science of genetics. In the mid-1800s the monk, Gregor Mendel, working on peas established the basic laws of inheritance. In the early 1900s advances in the science of genetics led to a dramatic increase in our understanding of growth, development and inheritance in microbes, plants and animals.

Genes are made of the substance DNA[3]. The structure of DNA was worked out 50 years ago, and since then there have been dramatic advances in the subject of genetics. Working out the structure of DNA[4] was one of the most significant advances in genetics because it gave much better insights into both the structure of genes and how they work. These advances have already had profound impacts on our understanding of the fundamental processes of living things.

Throughout the mid-1900s there were important developments in the application of genetics for plant breeding and crop improvement. A wide range of plant breeding methods[5] has been used to contribute to a substantial increase in crop yields and food production, quality and safety across the world (e.g. the Green Revolution of the 1960s and 1970s in India which has been estimated to have fed over 1 billion extra people from the same area of land). Over the past 30 years, geneticists and plant breeders have been able to isolate and sequence DNA from different living organisms and to insert one or more specific genes into a wide range of important crop plants, worldwide. This new form of *genetic modification* (GM) presents opportunities to modify crops

[1] Genetics is the scientific study of heredity (how characters are passed on from parents to their offspring) and how genes control the development and behaviour of all living things.
[2] Domestication of crops involves selection by people of plants better able to provide food for people, feed for animals, materials for building and making things and medicines for treating illness.
[3] Deoxyribonucleic acid.
[4] Watson and Crick elucidated the structure of DNA at the University of Cambridge in 1953.
[5] The gradual evolution of plant breeding methods will be discussed further in Chapter 4.

in different ways, to make them resist pests and diseases, be more tolerant of drought and other stressful environments, and even produce vaccines and new medicines[6].

The ability to move specific pieces of DNA and genes into crops from different classes of living organisms has, in some countries, led to the widespread use of genetic modification in plant breeding and the extensive cultivation of the crops so produced (see Box 1.1). But there have been reservations and concerns expressed in the UK and in Europe about the possible impacts of cultivation and consumption of GM foods.

As genetic modification raises issues of significant public interest, Mrs Beckett the Environment Secretary announced, on 31st May 2002, that the Government would promote a public debate[7] on the future use of genetically modified organisms (GMOs) in the UK. She also announced two further strands of activity: a study into the costs and benefits[8] associated with growing or not growing GM crops, and a review of the science underpinning the GM assessment and approval process in the UK.

1.2 WHAT HAS THE SCIENCE REVIEW INVOLVED?

The science review, along with the economics and public debate, marks a new venture in public engagement in the UK. The science review has involved taking popular concerns and questions about GM crops and foods and considering the evidence for the salient scientific issues they raise. The issues considered were identified from several activities including a series of public workshops to determine views about GM crops[9]; the public meetings held in association with the Science Review; the Science Review website[10]; and topics highlighted by the Science Review Panel itself. Where possible we have tried to adhere closely to concerns and questions in the way the public have expressed them. The concerns and questions have been grouped into seventeen scientific issues. These issues have then been grouped into four Chapters in the review.

It is important to note that the review is not intended to cover all scientific issues relevant to the assessment of GM crops and foods. For over a decade several independent Government advisory bodies[11] have considered the underlying science relating to production and use of GMOs in their

[6] Examples of research on a wide range of crop genetic modification is summarised in Chapter 6.7 on Horizon Scanning.

[7] The Public Debate is a programme of deliberation with issues for debate framed by the public and conducted at arms length from Government by an independent Steering Board that will report to Government in September 2003. Its focus will be on public views, particularly at grass roots level, to inform Government decision-making. http://www.gmnation.org.uk

[8] An analysis by The Prime Minister's Strategy Unit (SU) of the nature and distribution of costs and benefits that could arise under different scenarios with or without the commercialisation of GM crops in the UK. The SU report was published in July 2003. http://www.number10.gov.uk/output/Page4131.asp

[9] The Foundation Workshops were run independently by Corr Willbourn in the early stages of the Public Debate to establish the principal concerns and questions raised by members of the real public randomly selected. A copy of the Report can be found on the GM Science Review website.

[10] GM Science Review website: http://www.gmsciencedebate.org.uk

[11] The main statutory advisory Committees are the Advisory Committee on Releases to the Environment (ACRE); The Advisory Committee on Novel Foods and Processes (ACNFP) and the Advisory Committee on Animal Feedstuffs (ACAF).

statutory risk assessments on food, feed and environmental matters. There have also been extensive and numerous research programmes to assess the possible risks, benefits and characteristics of GM crops in the UK, EU and worldwide for almost two decades. We have deliberately concentrated on those issues raised that are of particular concern to the public. We have endeavoured to analyse scientific knowledge relevant to those concerns, to acknowledge where there are gaps in scientific understanding, and how these gaps can be dealt with in decision-making and in defining further research. We have also considered what lessons can be learned from comparisons with so-called conventional plant breeding and modern agricultural practice.

While this Science Review is principally designed to aid Government decision-making in the UK, it is acknowledged that any decisions on the future cultivation of GM crops in the UK will be noted across the world, including in developing countries. With the current extent of international trade in a wide range of crops, agriculture in one country frequently impacts on other countries. The work of the economics strand, carried out by the Strategy Unit, has focussed some of its analysis on the ways in which the UK and EU decision on GM crops could impact on decision-making in developing countries. The Science Review Panel felt it important that all countries, including developing countries[12], should carry out their own independent evaluation of the cultivation of particular GM crops; not least because the demands of agriculture, and the societies it supports, vary too much across the world to be able to reach simple generalisations. It is clear that GM technology does offer new approaches to old problems in some agricultural systems, and these have been adopted in some parts of the developing world (Garg *et al.*, 2002; Huang *et al.*, 2002; Pray *et al.*, 2002; Pretty, 2002; Conway, 2003; Nuffield Council on Bioethics, 2003). However, non-GM approaches have also been pointed-out that might also be used as alternatives (AEBC, 2002; Nuffield Council on Bioethics, 2003).

The Science Review process has been open and accessible to the public in various ways through: a dedicated website[13], through public Science Review meetings, and through public attendance at Science Review Panel meetings[14].

Discussions during the Science Review process have suggested that while practising research scientists are familiar with how scientific knowledge is acquired, communicated and validated; there is far less familiarity of these processes in the wider community. We thought it would be helpful, therefore, first to give the reader some insights into the scientific process.

1.3 HOW IS SCIENTIFIC KNOWLEDGE ACQUIRED?

Scientists are usually people who are fascinated by learning about how things work. Plant biologists are interested in how plants grow and develop, resist pests and diseases, produce many different products and compete with other plants. They are often intrigued by how plant species

[12] The role of GM crops in developing countries was raised in the Corr Willbourn Foundation Workshops and in contributions to the Science Review website.
[13] GM Science Review website: http://www.gmsciencedebate.org.uk
[14] The public was invited to observe all full Science Review Panel meetings. The first set of drafting subgroup discussions was without the public, but later the public was invited to observe.

have evolved and how plants can be selected for different purposes (food, feed, health, energy, raw materials etc). Interest in how things work has existed for as long as humankind has been evolving. Particularly during the past two centuries or so, people have engaged in scientific observation and experimentation on an expanding scale. This has led to significant changes in society. Most, but certainly not all these changes could be described as beneficial. For instance, 150 years ago average human life expectancy was around 40 years, it is now close to 80 years. Science, in its various forms has undoubtedly made a significant contribution to this change.

The development of scientific knowledge is affected not only by the interests of individual scientists, but also by the allocation of resources to research. This reflects complex economic and institutional issues and raises questions of priorities and the distribution of benefits and risks. Where there are uncertainties, these may often be open to legitimately divergent interpretations. Such scientific judgements on uncertainty may in turn be informed by wider social and economic perspectives. For instance, modern intensive farming methods have yielded many economic benefits but have, also been linked to a decline in certain forms of wildlife. Science and technology cause environmental damage and it is our wish to understand the risk of damage from new developments.

Over the past two centuries, international science has evolved a set of working principles based on the accumulation of evidence, assessment of that evidence and communication by publication, so that the global scientific community can benefit from shared knowledge. A fundamental part of the assessment of science is peer review by fellow scientists with relevant and complementary experience and expertise. Over this time, there has also been an evolution in the formal methods of the scrutiny of scientific evidence to provide the so-called ‘scientific method’ we have today.

The process can be illustrated as follows. A scientist, interested in how a particular plant is able to resist a disease, studies various features of the disease and the way the plant avoids or actively resists infection. In modern science this usually involves carrying out experiments to answer certain questions about the disease-causing microbe and the plant host. Eventually the scientist will develop a hypothesis to explain the evidence observed. A hypothesis (or model) is often used because it helps to identify the gaps in knowledge and the questions to be answered by further research. Experiments to find information to fill these gaps are then designed and carried out. Proper controls and statistically valid results are critical. Scientists frequently communicate with colleagues during the course of the investigation to learn from their experience. Eventually when they have gathered a sufficient amount of new knowledge about the subject they decide to publish the results.

A report on the research is then prepared as a scientific paper and sent to a Journal specialising in relevant areas of science (e.g. plant diseases). The Journal editor sends the paper out to fellow scientists or referees (usually anonymously) for comment. Peer reviewers’ comments are then communicated back to the author who must modify the paper accordingly or provide a convincing justification why not, before the scientific paper can be published. Scientists new to the peer review process often find it unnecessarily critical and negative. But peer review and the refereeing process are vitally important for the evidence-based evolution of scientific knowledge. The scientific method we currently have is by no means infallible, not least because we can never know ‘everything about everything’; but it is an approach that has stood the test of time, is objective and is the best method we have.

In this science review we agreed at the outset that we would rely principally on evidence from refereed publications that have passed through a rigorous peer review process. However we also agreed that scientific evidence from conference proceedings, specialised technical reports and other publications that have not gone through a comparable peer review may also contain information valuable to the review process. We have peer reviewed this non-peer reviewed literature ourselves and selected what we think is reasonable. The quality of the evidence surrounding GM crops varies considerably. Some claims amount to speculation or cold propaganda, with no underpinning scientific evidence; others are well supported by sound scientific evidence. Sometimes the conclusions of scientific evidence are contradictory or inconclusive. In these instances we have examined the evidence, argued the points and reached conclusions based on the best scientific evidence available. In several instances we have identified significant gaps in knowledge and discussed how they might be dealt with. It is accepted that science can never prove that something cannot/will not ever happen or does not exist, and it is thus unreasonable to demand that it can or should.

1.4 WHO HAS BEEN INVOLVED IN THE REVIEW?

Many people have participated in the review in different ways. Members of the public who participated in the Public Debate Foundation Workshops[15] helped to identify the principal questions and concerns that are the focus of this scientific review. Similarly people who participated in the public meetings of the Science Review and responded to the dedicated website also helped to identify the salient scientific issues.

The review has been carried out by an independent Scientific Review Panel drawing on 24 experts in natural and social sciences with a broad range of relevant and complementary expertise. The Panel was chaired by Professor Sir David King[16] working with Professor Howard Dalton[17]. Various people on the Panel carried out the role of authorship on papers in the early phases of the review, this evolved into an editorship role as panel members interacted and exchanged views and finally the secretariat took on the role of mediator. At the last meeting we all progressed in such a way as to take common ownership of the entire report.

1.5 WHAT IS THE STRUCTURE OF THE REPORT?

The report comprises seven chapters. Chapter 2 describes the Methodology used in the review. Chapter 3 describes the role of science in the GMO regulatory system. Chapter 4 considers the reliability of GM plant breeding compared with conventional methods. Chapter 5 looks at food and animal feed issues related to safety. Chapter 6 looks at environmental impact and Chapter 7 at gene flow, detection and impact. Chapters 5-7 contain a selection of papers that address

[15] A report on the Corr Willbourn Foundation Workshops can be found on the GM Science Review website.
[16] Chief Scientific Adviser to the UK Government. The letter of invitation from Professor Sir David King to members of the Science Review Panel can be found on the website. http://www.gmsciencedebate.org.uk
[17] Chief Scientific Advisor to the Secretary of State for the Environment, Food and Rural Affairs.

specific issues under each of these headings. A list of abbreviations can be found at the back of the report. Additional Annexes to the Report can be found on the Science Review website[18].

1.6 WHAT IS THE RELATIONSHIP BETWEEN THIS REVIEW AND THE WORK OF STATUTORY UK ADVISORY COMMITTEES ON GM?

In the UK there are extensive regulations that apply to the safety and use of GM crops and their products. Detailed considerations of proposals to release GM crops into the environment or to use them for human food or animal feed are the responsibility of appropriate statutory Advisory Committees. These Advisory Committees consider each proposal on a case-by-case basis and make recommendations to the UK Government. It is then the responsibility of Government Ministers to decide whether or not to implement that recommendation. Regulations covering the cultivation and use of GM crops and their products are complex and are harmonised across the European Union (EU) in the form of EU Directives. These Directives require a detailed science-based risk assessment, and the regulations embodied in them are continually evolving to respond to new knowledge. Further information on the statutory regulatory framework covering the UK is relevant to this Report and is outlined in Chapter 3.

The guiding principle of this Science Review has been to consider the current state of scientific knowledge on specific issues. It is for the statutory bodies to make recommendations based on assessments of specific GMOs[19]. However, this has not precluded a consideration of evidence in particular cases, nor has our brief precluded the identification of new evidence that might bear on these considerations.

1.7 HOW WILL THE REPORT BE USED?

The report is presented to Government as a contribution to future policy and regulatory decisions about GM crops and food in the United Kingdom. The Report from the Prime Minister's Strategy Unit on the overall costs and benefits associated with growing GM crops in the UK was published on 11 July 2003 and the Public Debate strand ('GM Nation?') is due to publish a report in September 2003. The Science Review Panel will then meet again in Autumn 2003 to consider responses to the Science Review report and scientific issues raised by the Public Debate report, and, if timing allows, the results of the Farm Scale Evaluations. The Science Review Panel will produce a second report following these discussions in late Autumn 2003.

[18] http://www.gmsciencedebate.org.uk/panel/default.htm

[19] The terms of reference of the Science Review state that: 'It is not the role of the Panel to make recommendations on specific applications for consent to release or market GMOs. This is the statutory duty of the Advisory Committee on Releases to the Environment (ACRE) and the Advisory Committee for Novel Foods and Processes (ACNFP)'.

Box 1.1: The current status of GM crops and foods internationally

GM crops

The first GM plants (Petunia and tobacco) were produced in 1983 and the first GM field trials in the world were in 1986 and in the UK in 1987. Since that time there has been a rapid increase (% to % p.a.) in the area of GM crops grown internationally, although they still remain a small proportion of the total world agricultural production. Even so, GM crops are now an integral part of agriculture in some countries. Worldwide, commercial cultivation of GM crops increased from 1.7 million hectares in 1996 to 58.7 million hectares in 2002, with soyabean, cotton, maize and rapeseed occupying 99.9% of the area sown. Over a quarter (27%) of the global GM crop area in 2002 was grown in nine developing countries. Globally the principal GM crops in 2001 were soyabean (32% of the global area), maize (21%), cotton (12%) and oilseed rape (5%). The number of farmers growing GM crops was between 5.5-6 million worldwide. More than 75% were small cotton farmers mainly in China and in South Africa.

(Data from - ISAAA Briefs (2002) Preview. Global Status of Commercialized Transgenic Crops: No. 27)

GM enzymes for food production

A range of enzymes for food processing are produced by GM microbes. Chymosin, used mainly for the production of 'vegetarian cheese', is the best-known example. But other examples of enzymic preparations derived from genetically modified organisms which are commercially available for food use in the EU are listed below:

Activity	**Source**
alpha-Acetolactate decarboxylase	*Bacillus subtilis* containing *Bacillus brevis* gene
alpha-Amylase	*Bacillus subtilis* containing *Bacillus stearothermophilus* gene
alpha-Amylase	*Bacillus subtilis* containing *Bacillus megaterium* gene
alpha-Amylase	*Bacillus licheniformis* (self-cloned)
alpha-Amylase	*Bacillus licheniformis* containing *Bacillus stearothermophilus* gene
Catalase	*Aspergillus niger* containing *Aspergillus* gene
Chymosin A	*Escherichia coli K-12* containing calf gene
Chymosin B	*Aspergillus awamori* containing calf gene
Chymosin B	*Kluyveromyces lactis* containing calf gene
Cyclodextrin-glucosyl transferase	*Bacillus licheniformis* containing *Thermoanaerobacter* gene
beta-Glucanase	*Bacillus subtilis* (*B. amyloliquefaciens*) containing *Bacillus* gene
beta-Glucanase	*Trichoderma reesei* containing *Trichoderma* gene
Glucose isomerase	*Streptomyces lividens* containing *Actinoplanes* gene
Glucose isomerase	*Streptomyces rubiginosus* containing *Streptomyces* gene
Glucose oxidase	*A. niger* containing *Aspergillus* gene
Hemicellulase (xylanase)	*Bacillus subtilis* containing *Bacillus* gene
Lipase, triacylglycerol	*A.oryzae* containing *Rhizomucor* gene
Lipase, triacylglycerol	*A.oryzae* containing Thermomyces gene
Maltogenic amylase	*Bacillus subtilis* containing *Bacillus stearothermophilus* gene
Pectinesterase	*Aspergillus oryzae* containing *Aspergillus aculeatus* gene
Protease	*A. oryzae* containing *Rhizomucor* gene
Protease	*Bacillus amyloliquefaciens* containing *Bacillus gene*
Protease	*Bacillus licheniformis* containing *Bacillus* gene
Pullulanase	*Bacillus licheniformis* containing *Bacillus* gene
Pullulanase	*Kleibsiella planticola* containing *Kleibsiella* gene
Xylanase (hemicellulase)	*A. oryzae* containing *Aspergillus gene*
Xylanase (hemicellulase)	*A. oryzae* containing *Thermomyces* gene
Xylanase (hemicellulase)	*A. niger* var.*awamori* containing *Aspergillus* gene
Xylanase (hemicellulase)	*A. niger* containing *Aspergillus* gene
Xylanase (hemicellulase)	*Bacillus subtilis* containing *Bacillus* gene
Xylanase (hemicellulase)	*Bacillus licheniformis* containing *Bacillus* gene
Xylanase (hemicellulase)	*Trichoderma reesei* containing *Trichoderma* gene

Chapter 2

METHODOLOGY

This Review is conventional in that it evaluates the current state of scientific knowledge in the field. In other respects, it is distinctive. Scientific reviews are often 'in house affairs' aimed at a specialist scientific community. But, in addition to having scientific integrity, this Review has been designed to be conspicuous, and to be explicitly linked to public interests and concerns.

In the first phase of the Review, all scientists and the general public were invited to submit papers and science-based views on key issues. Letters of invitation were sent to large numbers of individuals to encourage the widest participation from scientists of all shades of opinion.

The second (peer review) phase enabled the Science Review Panel to consider the scientific rigour of the papers presented (and other information available) before summarising where consensus lay on the science and where there is real uncertainty or gaps in knowledge.

Figure 2.1 shows the routes of information exchange between the various components of the GM Review that are described in detail below. The Timetable of the Review is shown in Table 2.1.

2.1 PUBLICITY

The launch of the Science Review was publicised in the media to stimulate interest among the scientific community and more broadly. As well as tapping into familiar expertise, it also wanted to reach out widely to access fresh sources of knowledge that might offer new perspectives on GM issues. The communication strategy included a letter to the scientific community and high profile media interviews.

2.2 THE WEBSITE

Launched on 31 November 2002, the Science Review website provided the principal means by which the scientific community and the general public could contribute to and observe the Science Review. It also provided the principal medium by which the Panel communicated on the science and looked at the evidence. Neither the Web nor science has geographical boundaries. Although the Review was focused on UK issues, it invited contributions on an international scale. The website provided details of Panel meetings (Agendas, Secretariat papers, Minutes, instructions for members of the public who wished to observe), and Open Meetings (registration details, speakers abstracts, verbatim transcripts, reports specially commissioned by science writers).

Guidance on how to make contributions was also provided on the website. Although contributions did not need to be peer-reviewed, contributors were encouraged to focus on and address the science, which should be reasonably argued and be evidence-based, either directly

or by reference to identified and publicly available material. A gratifying feature of the Review was that most contributions conformed to these guidelines. Those that did not were not excluded but placed on a 'wider issues' page of the website. At the time of writing almost one hundred contributions have been received. The names and status of the scientists submitting contributions was requested so that readers could judge for themselves the accountability and experience of each contributor.

An 'interests and concerns' page was developed to make the Review especially accessible to the public. This hosted a review of public concerns (the Corr Wilbourn Report), questions arising from a series of foundation discussion workshops, popular summaries of Science Review Open meetings held around the UK written by science writers, and a glossary of terms.

2.3 THE SCIENCE REVIEW PANEL

The Science Review Panel, chaired by the Government Chief Scientific Advisor, Professor Sir David King, working with Professor Howard Dalton, Chief Scientific Advisor to the Secretary of State for Environment, Food and Rural Affairs, had two principal functions. First, to monitor the progress, quality and credibility of the Science Review itself and second, towards the end of the debate, to review and summarise the state of scientific knowledge, consensus and identify significant/relevant areas of uncertainty.

Two distinguishing features marked out this Panel immediately. Firstly it was quite large (25 members) and secondly, it has an exceptional breadth of expertise and experience including leading scientists and social scientists from a number of fields, and with a spectrum of opinions on GM. There was overlapping membership between the Science Review Panel and the Steering Group of the GM Public Debate, to help ensure that the scope of the Science Review evolved in the light of developments in the public debate. Details of Members and their affiliations and expertise and interests can be found on the website.
(http://www.gmsciencedebate.org.uk/panel/members/default.htm)

The Science Review Panel met on seven occasions over a six-month period, mostly at the Royal Society or the Royal Institution of Great Britain. Members of the public were able and encouraged to observe these meetings. Minutes can be viewed on the website.

Early in its work the Panel identified areas of interest and concern to the scientific community. It also took into account public attitudes through consideration of the outcome of the foundation discussion workshops published in the Corr Willborn Report, and developed a framework to review these issues. The Panel looked at contributions to the website, the Open Meetings, as well as reviewing the scientific literature. At several meetings members discussed at length, particular topics of concern raised by members of public that were posted on the Science Review website. These are recorded in the minutes and secretariat papers which can be found on the website.

2.4 OPEN MEETINGS

Between January and March 2003, a programme of four open meetings was organised by the British Association for the Advancement of Science (BA). The purpose of these meetings was to offer a wide spectrum of scientists the opportunity to put their views to the Science Review Panel and for the public to have an opportunity to enter into dialogue with experts. Diverse venues were chosen: The Science Museum (London), The Royal Society of Edinburgh, The Institute of Grassland and Environmental Research (Aberystwyth) and the Agriculture and Food Science Centre (Belfast). They were all well attended, with audience numbers ranging from 70 to 100. A fifth Open meeting was organised by the Royal Society itself in London.

Further details including programmes, reports and verbatim transcripts can be found on the Open Meetings page on the website.

2.5 STRAND CO-ORDINATION

Following the initial announcement of the GM Review by Rt. Hon Margaret Beckett MP, the three strands have developed their strategies and operations in close consultation with each other, recognising that good interaction is essential for success. The Science Review recognises that the GM debate is a very deliberative means of engagement with the public. The GM Science Review Panel was constituted with a deliberate element of cross-skills membership. Professor Philip Dale served on the Public Debate Steering Board and the Science Review Panel. There has been comprehensive two-way flow of information with the Strategy Unit, especially in those areas where there are direct linkages between their work and the Review. For example, the Strategy Unit held a shocks and surprises workshop to look at various scenarios and the review paper on allergenicity (Section 5.3) explicitly took into consideration a very specific scenario discussed at this workshop. Two members of the Science Panel also have roles on the Strategy Unit expert groups (Professor Jules Pretty and Dr Brian Johnson) and others have contributed to seminars held by the Strategy Unit as part of their work. The work published by Corr Willbourn on the outcome of the public debate foundation discussion workshops to assess grass roots interests and concerns has played a central role in setting the agenda of the Science Review process.

2.6 THE FRAMEWORK OF THE REVIEW

2.6.1 Framework

The Panel developed the framework in Box 2.1 to review the issues. The Framework is broader in extent than the checklist (Box 2.2) and specifically governs the structure of our sections in our Review; whereas the checklist was a discipline the Panel agreed it would go thought to respond to the questions raised in the framework. For each issue the Panel consider the following:

Box 2.1: Review Framework

1 **Range of views and quality of evidence.** What is the range of views based on peer-reviewed literature and other sources of information, and what is the quality of the evidence? Have all major perspectives been brought to the Panel? (See Checklist, point 1.)

2 **Is there general scientific agreement?** (See Checklist, point 2.)

3 **Is the issue unique to GM?**

4 **Are there gaps in our knowledge or scientific uncertainties and are these important?** What is or what might be the risk associated with the uncertainty? (See Checklist, point 4.)

5 **Looking to the future.** What potential developments are there is this area? Do they affect the Panel's conclusions?

6 **Where there is recognised scientific uncertainty, what is the potential way forward?** (See Checklist, points 5 & 6.) For example:

- Further research?
- Are there technological approaches or agronomic practices that could help to reduce uncertainty?
- Are there satisfactory regulatory approaches, e.g. assumptions in risk assessment, monitoring, tools to evaluate the risks?

It is important to recognise that closing gaps in knowledge may not always be possible in practice for all sorts of reasons, including technological or economic constraints.

2.6.2 Checklist

The overall aim of this Checklist was to provide a single consistent framework to assist the Science Panel to respond in an efficient and coherent fashion to issues identified from a variety of sources. These include questions formulated by the Panel itself, those arising from Open Meetings and the Public Debate, and those raised in submissions to the Science Review website.

The Checklist attempted to fulfill a number of objectives. It embodied the remit of the Science Panel to give (and be seen to give) full attention to 'uncertainties', 'divergences of view', 'unknowns', and 'gaps in knowledge'. It aimed to be clear and meaningful for a general audience, whilst helping to stimulate relevant and fruitful questions of specific bodies of scientific evidence. It assisted the Panel in addressing the full scope of public concerns over GM science whilst avoiding repetitive, time-consuming or unduly onerous burdens.

The Checklist comprises six basic questions. These are divided into two groups, concerning, respectively, 'what do we know' and 'what don't we know'. Each question is supported by a short explanatory paragraph.

Box 2.2: Review Checklist

What do we know? How robust is this knowledge?
1 What is the quality of the evidence? Where there are judgements over the relative likelihood of different outcomes, is this based on empirical field data, laboratory studies, scientific models or expert opinion? In considering different hypotheses, have false negatives been treated the same as false positives? What is the statistical power of any quantitative assessments? **2 Are there different interpretations of the evidence?** Does the Panel's response include attention to contending scientific understandings or minority expert opinions, both within the Panel itself and in the wider literature? What are the implications of any such divergent interpretations for the conclusions reached by the Panel? **3 What key assumptions do the Panel's conclusions rely on?** Which assumptions, if altered, might have a significant effect on the Panel's conclusions? For instance, what assumptions are adopted with respect to operating environments, individual behaviour, adherence to good practice, regulatory compliance or institutional trends? What would be the effect of altering these key assumptions?
What don't we know? How might we cope with this?
4 Might there be significant gaps in our knowledge? Are there scientifically founded questions concerning the completeness, sufficiency or applicability of the scientific models or data which inform the conclusions reached by the Panel? What can be said about any resulting 'unknowns' and their practical implications? **5 How might research help to address the gaps?** What kinds of scientific research or environmental (or other) monitoring might help to reduce particular uncertainties or address the unknowns identified by the Panel? **6 What risk management measures might help to address these gaps?** What operational practices or policy measures might help to address the key scientific uncertainties and unknowns identified by the Panel or to mitigate exposure to their consequences? What might be the limitations of these measures?

2.7 THE REVIEW OF PUBLIC CONCERNS (THE CORR WILLBOURN REPORT)

The work published by Corr Willbourn on the outcome of the public debate foundation discussion workshops to assess grass roots interests and concerns has played a central role in setting the agenda of the Science Review process. This work was an integral part of the GM public debate strand of the National dialogue. A copy of the report can be viewed on the Science Review website. The introduction to each Review chapter lists the 'questions' of particular relevance to science under Review. The questions are listed at Annex I.

Figure 2.1: Information exchange

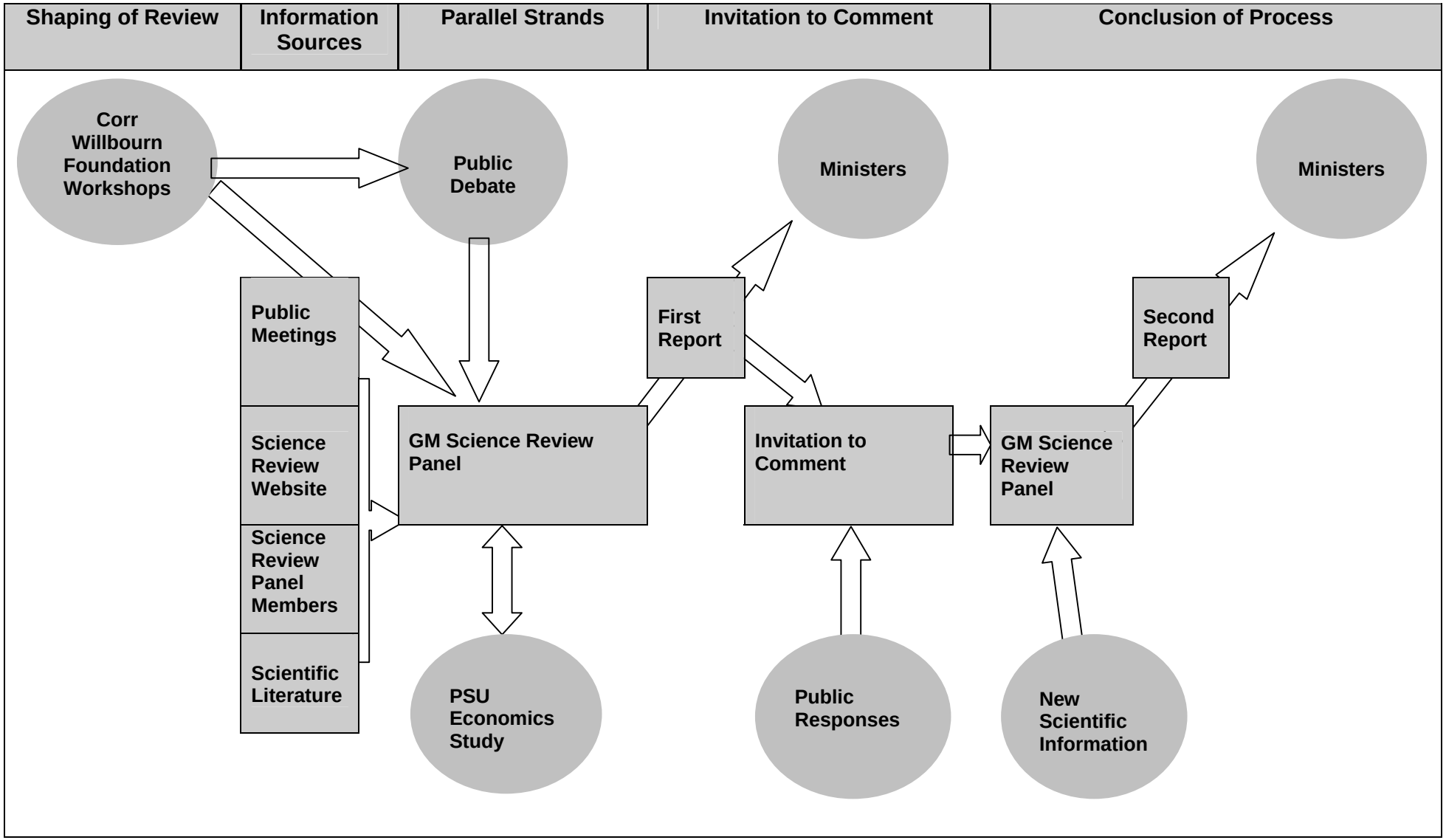


Table 2.1: Timetable

2002/2003	November	December	January	February	March	April	May	June	July
Dedicated Website	Launch								
Science Review Open meetings			23rd Science Museum, London 27th Royal Society, Edinburgh		11th Agriculture & Food Science Centre, Belfast 17th Institute of Grassland & Environmental Research				
Science Review Panel Meeting		10th		18th	19th	29th	13th	5th, 24th	
Drafting Group Meetings						1st Food & Feed Group 2nd Environmental Impacts Group 8th Geneflow Group	13th All three Groups		
Corr Wilborn report published					6th				
Public Debate Programme								3rd start	18th finish
Strategy Unit									Report
Science Review									First Report to Ministers

Chapter 3

SCIENCE IN THE REGULATORY PROCESS

This chapter describes the role of science in the regulatory process. Science has a central role in the regulation of genetically modified organisms because it provides the evidence base for decisions on safety to human health and the environment, and it is on the basis of safety (not benefits), that approvals are granted.

The regulatory process is dynamic, continuous (in that no approval is absolute, it is always under review to take account of advances in science and technology and prevailing knowledge), subject to critical challenge and continuously subject to improvement. It is through critical challenge that, for example, the farm scale evaluations and subsequent changes to European regulatory framework have taken place. The regulatory system will improve in future because we will improve understanding of the limitations of the scientific basis of the regulatory process and develop new tools to refine risk assessments.

Applications to cultivate GM crops, or to place foods or animal feed derived from them onto the market, are reviewed by scientists serving on advisory committees. In the UK these are: the Advisory Committee on Releases to the Environment (ACRE); the Advisory Committee on Novel Foods and Processes (ACNFP) and the Advisory Committee on Animal Feeding stuffs. Each has websites where detailed information is available.

The scientific quality of the initial scientific evidence submitted to these committees by applicants varies, and a process of iteration often follows between the committee and applicant with the applicant providing further information before the committee can formulate its advice (see Figure I) which gives an overview of how ACRE operate; other committees follow similar procedures). The application dossiers and the committees' advice are publicly available. Some of the evidence in dossiers is based on peer reviewed published papers. However, other material is based on 'in house' research and unpublished. The reasons for not publishing work are varied but it does not necessarily follow that the work is substandard. Science advisory committees in evaluating dossiers essentially 'peer review' unpublished data in the course of their evaluation.

Genetically modified organisms are required by legislation to be assessed on an individual, case-by-case basis. Consents are issued for a limited duration (for both field research or marketing applications) under specified conditions that might also include monitoring requirements. Consents can be withdrawn if regulatory examination of subsequent evidence finds the product unsafe or insufficiently safe.

Scientific advisory committees operate within the bounds of what is widely regarded as acceptable scientific standards of conduct and process (see background of Introduction for a more detailed explanation of what this means). Conditions are attached to consent and the consent holder is expected to adhere to good practice and regulatory compliance. The GM Inspectorate's role is to check for compliance.

Science is continuous and developing and there are often divergent viewpoints across the science community on issues. It is not necessarily the data which is disputed, but it can be its interpretation. Committees advise on the basis of current and widely accepted state of scientific knowledge and advice is reviewed in the light of latest scientific developments. Committees are not insulated from the scientific community or public concerns. The most recent large-scale example of public engagement in the UK has been the Chardon LL GM maize hearing.

As approval is based on safety to human health and the environment, a risk assessment carried out by the applicant forms the core of the applications. The scientific advisory committee assesses this. The risk assessment does not consider economic costs and benefits.

It is important to recognise the difference between hazard and risk. Confusion between the two can and does lead to problems in risk communication. A hazard is something that may cause harm. Risk is the product of two components: the likelihood that the hazard will take place and (in the event that it does) the consequence.

The relationship between hazard and risk is often illustrated by the function:

Risk = f (hazard, exposure)

Some generic limitations have been noted in the conventional risk assessment of complex technologies (Arrow, 1971; Porter, 1995; Wynne, 1996; Power, 1997; Morgan et al, 1990; Shrader-Frechette, 1990; Wynne, 1992; Amendola *et al.* 1992). The existence of these limits is a prominent theme in much of the international policy literature on risk assessment (DoE 1995; NRC, 1996; Treasury (UK), 1996; EPA, 1997; RCEP, 1998; HSE, 1999; House of Lords, 2000).

Scientific advisory committees operate in this complex climate and are aware of the challenges in providing advice. Committees such as ACRE have for example, grappled with some of the difficult issues related to what is environmental harm; provided guidance to raise the standards of submissions; and developed principles of best practice in the design phase of making GM crops; provided guidance on monitoring[1]. The philosophy of best practice in GM crop design is essentially an avoidance strategy to reduce unidentified risks. The approach minimises opportunities for potential harmful interactions to occur that may be difficult to anticipate, to evaluate or to monitor.

Science advisory committees, in assessing applications on a case-by-case basis, make use of a number of practical approaches to managing risks. Removing or 'bagging' flowers where there is uncertainty about the impact of cross-pollination is an example. Or, if there is no qualitative data on an event occurring, simply assuming it does occur and then estimating the consequences. This is particularly relevant in assessments on gene transfer to plants that can exchange genes in the field, or horizontal gene transfer where there is uncertainty about whether it occurs.

[1] http://www.defra.gov.uk/environment/acre/subgroups.htm

Chapter 6, Section 6.8 is relevant to those interested in considering further the main scientific approaches available for determining and predicting the environmental consequences of GM crops.

3.1 SUBSTANTIAL EQUIVALENCE

For many years now, the concept of 'substantial equivalence' has been a prominent feature of established international approaches to the regulation of GM technologies (CEC, 2003). It is not a safety assessment in itself but a way of structuring the comparison of a novel food with its conventional counterpart to identify any differences that then become the focus of the safety assessment. It recognises the fact that for most conventional foods, acceptable safety is established by their history of consumption rather than by formal risk assessment.

Substantial equivalence is used to identify differences that potentially could compromise this established level of safety in a conventional food. The approach is needed since standard animal toxicity tests designed to evaluate single defined chemical substances like food additives, pesticides and pharmaceuticals cannot be done on whole foods without careful consideration of nutritional implications. It is difficult to feed an animal a sufficient multiple of the anticipated human intake of the food being tested without compromising the nutritional balance of the test animal. The Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO) first addressed this problem in 1990 when the principle of comparing a new food with an existing food was identified. In 1991 the OECD formulated the concept of substantial equivalence. It has been revisited frequently since its inception and was reviewed in 2000 by FAO/WHO who found it to be a practical way to structure the safety assessment of foods.

In the past, substantial equivalence has been applied to determine an 'end point' in the safety assessment process (OECD, 1993). In this context it provides evidence that a GM crop or product is 'substantially equivalent' to a non-GM counterpart. The legislation on GM food and feed (see Appendix 2) has included this provision but it is little used and will not feature in new GM regulations that are about to be introduced. This application of substantial equivalence has been subject to significant criticism (e.g. Millstone *et al*. 1999; Royal Society of Canada, 2001; Levidow and Murphy, 2003).

Currently, substantial equivalence not used to determine a regulatory 'end point' but rather it is the framework for a comparative approach (SBC, 2001) that guides safety assessment. Significant differences between a new food and its traditional counterpart are identified as a basis for further investigation. The comparison identifies similarities and differences between the novel food and the existing counterpart with respect to composition, nutritional value and metabolism. The safety assessment then focuses on the health implications of any identified differences, which may or may not represent a hazard. For GM foods, the safety evaluation considers in detail:

- the genetic modification event including history of the host organism as well as the characterisation of the modified organism;
- safety assessment of the new gene product(s) and or metabolites;
- composition (fats, proteins, carbohydrates, vitamins, minerals) of the food;

- potential toxicity;
- potential allergenicity;
- any unintended secondary effects; and
- likely intakes and dietary impact.

Importantly, the end result is a decision on food safety and not a conclusion based solely on an analysis of similarity.

Readers who are particularly interested in food and feed safety may wish to refer to Chapter 5.

3.2 THE PRECAUTIONARY PRINCIPLE

The second key general concept bearing on the regulation of GM crops is 'precaution' (Cameron and O'Riordan, 1994; Sand, 2000; Fisher and Harding, 1999; Raffensberger and Tickner 1999; O'Riordan and Jordan, 2001). This is formally enunciated in various versions of the 'precautionary principle', which holds, in one influential version, that '*…Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation.*' (UNCED, 1992). Although providing a general evaluative guide, precaution has (like substantial equivalence) also been criticised on the grounds of scientific ambiguity (USDA, 2000; ILGRA, 2001). How serious is 'serious'? What exactly do we mean by 'irreversible'? How should we define 'full scientific certainty'? Concerns are raised that precaution constitutes an essentially pessimistic response to uncertainty and gaps in knowledge in regulatory risk assessment (Morris, 2000).

However, over recent years precaution too has begun to be interpreted in a somewhat different and more concrete fashion (ESTO, 1999; EEA, 2001). Rather than being treated as a firm 'decision rule', precaution is increasingly seen in terms of what it means for the regulatory appraisal process (Stirling, 2003). In this respect, precaution appears as an inherently scientific response to challenges of uncertainty, ambiguity and gaps in knowledge: by providing practical guidance to the types of information that might best inform decision-making and the most effective ways to gather this information (Renn 2003; van Zwanenberg and Stirling, 2003).

In particular, a precautionary approach to the appraisal of risk is generally held to embody the series of specific elements summarised in Box 3.1 (after ESTO, 1999; EEA, 2001). To the extent that the present Science Review process embodies many of these general characteristics, then it may be seen as part of a genuinely precautionary approach to the appraisal of GM food and crops.

Box 3.1: A summary of key characteristics of a precautionary approach to the appraisal of risk (after: ESTO, 1999; EEA, 2001; Renn, 2003)

- The inclusion of diverse scientific disciplines, to guard against an unduly narrow idea of the possible hazards, conditions or mechanisms of harm.
- The careful treatment of evidence, so that absence of evidence of harm is not presented as evidence of absence of harm. This is associated with a certain shift in the levels and burdens of proof, such as to give greater favour to the environment and human health.
- The open acknowledgement of uncertainty, ambiguity and gaps in knowledge, in order to avoid concealing the role of subjective judgement and the intrinsic limitations of risk assessment
- The transparent documentation of any assumptions and value judgements and an exploration of their scientific consequences by means of techniques like sensitivity and scenario analysis
- The involvement of stakeholders, lay people and participatory techniques to help ensure that the 'framing assumptions' explored in scientific analysis are consistent with wider social interests and values.
- The systematic and balanced assessment of the pros and cons associated with a series of different options, rather than simply focusing on the 'acceptability' of a single option in isolation or a comparison between this and existing tolerated poor or worst practice.
- Ensuring that the appraisal process allows expression of a balanced array of opinions, free from the exercise of coercive pressures and as independent as possible from particular financial or political vested interests.
- The serious consideration of issues such as the irreversibility of possible harm, the flexibility of possible responses, the diversity of policy options and the ease with which associated commitments may be withdrawn, to ensure that strategies are as robust as possible in the face of new knowledge and surprise.

Chapter 4

HOW RELIABLE IS GM PLANT BREEDING?

Does GM work? Is GM technology too imprecise? Are GM genes more unstable than resident genes? Is it necessary to produce many transgenic plants to obtain an acceptable one?

4.1 Summary

Some people have expressed concern that GM plant breeding is too unreliable and imprecise for crops to be used in agriculture at all or at least without more extensive testing. A principal argument used is that it is necessary to produce about 100 GM plants to obtain one that has desirable characters for use as a basis of a new GM crop variety. To address this concern it is necessary to place GM breeding in the context of non-GM breeding methods such as: gene transfer by pollination, mutation breeding, cell selection and induced polyploidy. Most of these now conventional plant breeding methods have a substantially greater discard rate. Mutation breeding for instance, involves the induction of unpredictable large-scale and undirected genetic changes. Many thousands (or millions) of undesirable plants are discarded in order to identify plants with suitable qualities for further breeding. Mutation arising spontaneously is the ultimate source of all variation allowing plant breeding and evolution.

The current and widely accepted view within the biological research and plant breeding community is that there are important parallels between non-GM and GM plant breeding although in certain respects GM breeding techniques differ significantly, and that the methods of evaluation of GM crops for food, feed and the environment currently carried out within the European regulatory framework, are generally robust if consistently applied and should be effective. All plant breeding methods have unique features. The special feature of GM plant breeding is that it allows a wider choice of genes for modifying crops in novel ways by enabling the use of genes from species outside the plant kingdom (animals, bacteria and viruses). This undoubtedly presents challenges for their regulation and management so that (along with non-GM crops) they will need to be addressed carefully and intelligently as GM breeding techniques evolve.

It is important that in the UK we have regulatory oversight that is proportional to the degree of risk, which recognises the distinctive attributes of GM and the different sources of uncertainty as well as the conventional breeding context and baselines.

4.2 Background

Modern molecular biology methods make it possible to isolate genes, and other DNA sequences, from different organisms or make synthetic DNA, and insert the DNA into crop plants. Usually many individual GM plants need to be produced to obtain a crop variety that has desirable qualities for use in agriculture and for consumption. This has led to expressions of concerns that GM technology may not be sufficiently precise and reliable. As this issue is relevant to food, feed and environmental impact, it is being considered in a separate chapter in this report. To address the topic, it is first important to discuss GM plant breeding in comparison with other methods of plant breeding (see Hayward *et.al.* 1993; Smartt and Simmonds, 1995; IAEA, 1995[1])

4.2.1 How different are GM and non-GM plant breeding methods?

Non-GM plant breeding includes a wide range of approaches. Some non-GM methods have been used throughout the history of plant breeding, and others apply the latest advances in molecular genetics and genetic mapping. Some examples are as follows.

Gene transfer by pollination involves the transfer of genes into crops by pollination with plants usually from the same species, but occasionally from different species or different genera. This is the basis of cross-breeding. This method makes it possible to recombine many thousands of genes from different plant parents. Plant embryo culture has extended the range of cross-breeding and made it possible to obtain hybrid plants that are unlikely to form in nature. Plants with desirable genetic combinations are selected and undesirable ones discarded following extensive testing of (tens of) thousands of genetically different plants.

Induced mutation in its simplest form involves exposing seeds or seedlings to ionising radiation (Cobalt60 gamma) or chemicals (mutagens) that cause unpredictable random changes (mutations) in the genes of the final crop plants. Some mutagens cause predominantly random single DNA base-pair substitutions, others cause random breaks in chromosomes, loss of chromosome fragments or rejoining of chromosomes in different combinations. A more subtle form of induced mutation is to induce destabilisation of naturally occurring mobile genes (transposons) that have the potential to silence other genes or cause them to be expressed in novel and unpredictable ways. The utility of mutation breeding relies on careful evaluation and selection of plants with desirable qualities in the progenies of the first generation mutants. This frequently involves the elimination of (tens of) thousands of plants with undesirable characters. Mutation breeding underpins the plant breeding pedigrees of many of the food crops we eat daily (especially cereals)[2].

Cell selection requires crop plants to be grown in culture vessels in a laboratory. The DNA in cell cultures becomes genetically unstable and this instability is used as a source of genetic variation for plant breeding. This method has been used, for example, in the selection of

[1] These three publications, and the references they cite, provide comprehensive reviews of plant breeding methods and the evolution and breeding of a range of crops.

[2] http://www-mvd.iaea.org/MVD/default.htm is reference to the list.

herbicide tolerance in crop plants (Marshall, *et al.* 1992). Herbicide is simply added to the culture media and plant cells that survive are rescued and used to regenerate whole plants with increased tolerance to the chemical. The method inevitably incorporates other mutations with unknown effect as part of the cell selection process. As with tissue culture methods there is evidence of random genetic changes in cultured cells (Karp, 1991) and the destabilisation of naturally occurring mobile genes. Where these extra mutations deleteriously affect the agronomic performance of the plant, they can, in some crops, be removed by undertaking conventional backcross programme to incorporate the trait in an otherwise acceptable genetic background.

Induced polyploidy involves treating plants with a chemical (such as colchicine); that doubles the number of chromosomes[3] in the crop and, therefore, doubles the amount of DNA in every cell in the crop plant. Induced polyploidy has been used in the breeding of some of the grasses, clovers and horticultural crops used commercially. It is also used in the breeding of other crops, especially for the production of interspecific and intergeneric hybrid crops e.g. hybrids between wheat and rye (triticale).

Molecular marker assisted breeding. A substantial and growing body of genetic information on crops is now making it possible for plant breeders to recombine and select genes where previously this has been impossible on a rational basis. Plant characters controlled by several genes, and those difficult to assess phenotypically (such as yield, or resistance to drought and salt) have traditionally been very difficult to modify by breeding. The use of molecular markers is significantly improving this efficiency and has the potential to allow breeders to assemble new groups of genes such as multiple disease resistance genes, to effect novel changes in crop plants.

Targeting induced local lesions in genomes (TILLING). Advances in genetic sequencing of crop plants mean that it is now possible to effectively select induced mutations in specific plant genes (Colbert et al, 2001). This advance in research and methodology is beginning to provide unique opportunities to modify the biosynthetic processes of plants in ways previously inaccessible to plant breeders. In the coming decade, with further advances in genetic mapping and gene sequencing, it is likely that plant breeders will be able to modify a range of crop characters in novel ways.

GM plant breeding. By comparison with the 'non-GM' plant breeding methods (described above), GM allows the incorporation into crops one or more specific gene sequences isolated from a range of classes of organisms. These can be: the same crop species, wild relatives of the crop species, other quite different plant species, microbes (viruses, fungi or bacteria) or even animals. The method can be used to incorporate one or several genes into a crop plant that has many thousands of genes (wheat probably has more than 100 thousand genes). As the inserted gene will have been characterised at the molecular level (its genetic information defined), its position in the genome and its function can be assessed with a greater degree of precision than for genetic changes made by most non-GM plant breeding methods. It is necessary to produce about 100 GM plants to obtain one that has the desired qualities following testing and evaluation. The remainder are discarded.

[3] A chromosome is a thread like structure in the cell nuclei that carries genes. Wheat has 42 chromosomes, barley has14 chromosomes and potato has 48 chromosomes.

All of the above methods have unique properties and find utility in specific breeding applications. GM breeding cannot be used to make polyploids, or recombine thousands of genes (pollination), or cause large-scale random unpredictable genetic changes (mutation breeding). Similarly mutation breeding cannot be used to introduce single genes into crops from radically different organisms (GM breeding).

4.3 Range of views and quality of evidence

This section considers some general concerns that have been expressed about GM technology and whether it is sufficiently developed to produce crops that are safe for food, feed and the environment in comparison with non-GM crops. The question in the Foundation Workshop report[4] of most relevance is: 'does GM work?'

Within the Science Review process, these questions were discussed mainly at the second meeting of the GM Science Review Panel and at the Open Meetings on 'GM Food Safety'[5]. Issues raised in contributions to the Review website included: comparisons with radiation induced mutagenesis; unpredictability, imprecision and scientific uncertainty in GM; the instability of transgenic DNA; and the high rejection rate of plants resulting from the GM process.

4.3.1 Range of views

It is necessary to produce about 100 GM plants to produce one that has a desirable combination of crop characters. Some view this as demonstrating that GM technology is too imprecise. Others consider that GM plant breeding is considerably more precise than many non-GM methods, and that the tests required in the assessment and regulatory process are sufficient to identify desirable GM plants and GM crop varieties.

Introduced transgenes are observed to vary in their effect on the plant and to be influenced by environmental conditions. Some view this as demonstrating that GM genes can be unstable and may behave differently from resident (endogenous or existing) genes, giving rise to important uncertainties. Others note that genes introduced by non-GM methods can also show variable effects and be influenced by the environment (Griffiths *et al.* 1993). They also consider that the extensive testing of GM plants ensures that the plants used to establish new GM varieties present no greater risk than non-GM crops (most of which have not been through comparative testing). Others are less confident and take the view that it cannot be ruled out for either GM or non-GM plants that such effects may not be manifested until they are in widespread use.

Views about the unintended effects of GM crops vary. Some consider that unintended effects may pose health risks. Others consider that the theoretical planning of the transgene constructs

[4] The Corr Willbourn Foundation Workshops report can be found at http://www.gmnation.org.uk/docs/corrwillbourn.pdf

[5] http://www.gmsciencedebate.org.uk/meetings/default.htm

and procedures, the selection of the transgenic plants, the trials of the new crop variety and finally tests required during the regulatory process are sufficient to detect undesirable properties.

Some reason that the long history of plant breeding means that phenotypic variation typically falls within a familiar range, yet even a single gene inserted via GM techniques can produce a plant phenotype of which there is little or no experience (Dale and Irwin, 1998).

There is variation in views about the nature of evidence required to conclude that GM crops are acceptably safe. Reflecting a basic principle of scientific inference, some argue that the absence of evidence of harm should not be treated as evidence of the absence of harm. This argues for greater reliance on scientific research and epidemiological monitoring. Others reason that the combination of testing by developers to satisfy regulatory requirements for clearance and extensive use around the world over long time periods and large exposed populations and absence of evidence of harm, does provide important experience of safety[6]. Many millions of tonnes of GM crops have been produced and consumed internationally over the past eight years without any substantiated evidence of harm when compared with non-GM crops. However, views vary on what kind of monitoring is necessary and how many years and millions of tonnes of GM crops should be grown and consumed to draw a conclusion of acceptable safety.

4.3.2 Quality of evidence

The number of transgene constructs inserted into the plant genome during genetic transformation usually ranges from one to three, but can be higher. Plant breeders generally select and use transgenic plants with a single inserted transgene construct. This simplifies subsequent transgene inheritance patterns, and any further breeding. Selection of single inserts also simplifies the molecular analysis needed to satisfy the regulatory risk assessment (Lindsey, 1998; Bavage *et al.* 2002).

The positioning of transgene constructs in the plant genome varies between different GM plants. Transgene insertion mutagenesis practised most comprehensively in *Arabidopsis*, but also now extensively in rice (Martienssen and Springer, 1998; Jeon, J-S *et al.* 2000), demonstrates that transgenes can cause insertion mutations in resident genes more or less throughout the plant genome. For risk assessment purposes it is, therefore, assumed that all endogenous genes are potentially exposed to the insertion of transgenes within and adjacent to them during the transformation process. The evaluation and testing required for GM organisms is based on this assumption. It is also assumed that naturally occurring mobile genes (transposons found widely in crops and other living things) in non-GM methods of plant breeding are capable of causing similar disruptions (Griffiths, 1993). The behaviour and consequences of mobile genes have been studied extensively in maize since the 1940s and many mutations from this cause have been described (Brutnell, 2002).

There is variation in the extent of border DNA sequences that are inserted into different transgenic plants during the transformation process (Lindsey 1998). Border DNA sequences are

[6] Very few species giving the foods we eat daily have been tested for safety at all, and certainly not as extensively as GM crops for foods and feeds. We rely principally on our experience of safe use.

those from the plasmid or vector used for the transformation, but lying outside the specific DNA designed for transformation. This is true for transformation by *Agrobacterium* and the gene-gun, but the identity of the border sequences is more predictable for *Agrobacterium* transformation. Regulatory risk assessment requires molecular analysis of the molecular integrity of the inserted transgene construct, including the extent of any DNA outside the immediate transgene construct. If detailed molecular data are not provided in a proposal to the regulatory authorities to carry out an experimental field release (for instance), an assumption should be made that the whole plasmid has been inserted and a risk assessment carried out on that basis.

Tissue culture methods are used in most transformation procedures and these can sometimes be associated with an enhanced rate of mutation or epi-mutation (induction of heritable variation other than through DNA sequence changes). This source of novel variation has been studied extensively in its own right (somaclonal variation) for plant breeding. The mutations can be of various types, including: point mutations, deletions, duplications and chromosomal mutations including loss or gain of whole chromosomes (Karp, 1991). Mutation is a natural phenomenon that occurs in all living things and is the original source of all differences between genes on which sexual genetic recombination and natural selection act during evolution of new species. Mutation frequency can be increased by various methods (chemicals and irradiation) and different forms of induction have been used widely as a plant breeding tool (as discussed). The frequency of mutation and epi-mutation is normally higher than is seen in seed-propagated plants, but much lower than in mutation breeding programmes. The phenotypic and molecular analysis required during selection of plants by plant breeders, and subsequently the regulatory risk assessment, is designed to detect significant changes of this kind. The extent and stringency of analysis of transgenic crops is substantially more exacting than for the products of spontaneous mutation (e.g. 'sports'[7]) or breeding with induced mutations.

There is sometimes variation in transgene expression in the early plant generations and phases of testing. During the first generation of transgenic plants (T0) there can be non-Mendelian inheritance[8] patterns because of the insertion of multiple copies of the transgene and sometimes because of chimeras where some shoots and flowers are transgenic and others not. In subsequent sexual generations, where plants are selected with single copy transgene inserts, genetic segregation patterns usually follow the expected Mendelian ratios. In a plant breeding programme, any plant that has undesirable characteristics (e.g. transgene expression levels are too high, too low or not in the appropriate tissues of the plant) is discarded just as in traditional breeding programmes.

Transgene expression can sometimes be silenced or altered in transgenic plants and in subsequent sexual generations. There is now an extensive literature on transgene silencing which shows that DNA homology between different transgenes, or between transgenes and resident genes, can result in gene silencing (Meyer 1995; Grierson *et al.* 1996; Matzke 2002). Indeed the phenomenon is sometimes used to modify plant phenotype (i.e down-regulate expression of endogenous genes in the original plant), or give resistance to infection by preventing expression of genes causing disease in transgenic varieties. Many aspects of the phenomenon of transgene

[7] 'Sports' are naturally occurring mutations that have been used as a source of genetic variation for plant breeding, especially in vegetatively propagated crops e.g. potato.

[8] Inheritance does not follow Gregor Mendel's laws of single gene inheritance.

silencing have been elucidated over the past decade. It is known for instance that infection of Brassica plants with a caulimovirus can silence transgenes regulated by DNA sequences originally taken from the same virus (Al-kaff, *et al.* 1998). The possible consequences of gene silencing depend on the particular transgenic modification and need to be assessed case-by-case. The silencing of a herbicide tolerance gene, for example, could cause the crop to become susceptible to the herbicide (Al-Kaff, *et al* 2000). A potential application of gene silencing is to remove an allergenic protein from a food crop. Crops with this application of silencing would need to be assessed very carefully because any variation in the efficiency of silencing (caused by variations in environment for instance) could result in an allergic reaction to a crop believed to be non-allergenic. The potential consequences of gene silencing need to be examined carefully during the regulatory risk assessment. Transgene silencing can be strongly influenced by environmental conditions such as light, temperature and nutrient availability and agronomic practices such as seedling transplantation (Brandle, 1995; Down 2001). It may not be possible to investigate all such scenarios in laboratory or field studies. The changes seen between growth chamber, greenhouse and field also indicate that extrapolation from one environment to another is not always possible.

There can be variation in the expression of transgenes in different parts (e.g. leaf, root, flower) of GM plants (tissue specificity). The expression of transgenes is controlled by tissue specific promoters or gene switches. Many of the early transgenes introduced into plants were regulated by constitutive promoters (e.g. the 35S promoter from cauliflower mosaic virus) which expressed in most tissues of the plant. However, there is now a move to more specific gene promoters. It is usual, however, to see variation between different independently transformed plants in the expression of transgenes using a tissue specific promoter. This is understood to result from 'position effects' i.e. the expression of a gene is influenced by its position in the genome or its genetic context. The nature of tissue specificity is important for food, feed and environmental safety where there may be a need to target transgene expression to the edible or non-edible parts of crop plants or to particular growth stages. The targeting of transgene expression is considered case-by-case and is particularly important for characters such as pest resistance where non-target organisms might be adversely affected. In practice, this variation is addressed by analysis of tissue specificity during the assessment of transgenic plants and is an important requirement in regulatory risk assessment.

As with non-GM plant breeding, there is genetic variation between GM plants within a breeding programme. In practice this variation is used to select plants that express the GM character in the most desirable manner (tissue specificity, transgene expression levels etc). The further 'fine tuning' of a new GM variety also frequently includes the use of conventional non-GM breeding methods over several years of additional breeding and evaluation. The production of a commercially acceptable potato variety, for instance, requires attention to about 40 different crop characteristics, only one of which might been introduced by GM breeding. In a conventional, non-GM breeding programme, many thousands (or millions) of candidate plant lines are assessed to produce one line that has superior characteristics. The discard rate is particularly high for mutation breeding where the nature and extent of genetic change is undirected, and unpredictable. In the regulatory risk assessment it is possible to analyse GM plants with a degree of molecular precision impossible for most products of conventional non-GM plant breeding.

4.4 Is there general scientific agreement?

The widely accepted view within the biological research and plant breeding community is that there are many parallels in the properties of plants produced by GM and non-GM plant breeding methods. Indeed, there is a substantially greater discard rate from most conventional breeding methods than from GM methods. A detailed molecular understanding of gene position effects and gene silencing effects in GM plants is developing with current research. But both phenomena are also features of conventional (non-GM) genetics and breeding where it is rarely possible to study the molecular properties of unstable resident genes in any detail. It is also widely accepted that there is the potential for quite novel molecular interactions, which may fall outside our current scope of knowledge. There is extensive field testing and agronomic evaluation for non-GM breeding. GM crops are exposed to similar testing, but in addition include further testing on safety for animal and human health and for environmental impact under the European Union regulatory system. It is important that the tools available are sufficient. A major strand of scientific opinion considers the testing currently carried out in the EU to be robust and sufficiently comprehensive to provide GM crops that are at least as safe as conventional crops. This analysis is supported by practical experience in the cultivation and consumption of many millions of tonnes of GM crops internationally over eight years[9]. Another strand in scientific opinion considers that GM crops need to be grown, consumed and analysed for a longer period in order to justify drawing such a conclusion.

4.5 Is the issue unique to GM?

Unstable genes have been known and exploited in conventional breeding since the early days of genetics studies last century.

Tissue culture-induced procedures are used in the majority of crop genetic modification procedures. Tissue culture induced genetic variation has been used as a source of variation for non-GM plant breeding and the range of genetic variation obtained from this source has been described extensively. Tissue culture (or micro propagation) is also a common way to increase the numbers of plants ‘uniformly’ through vegetative propagation.

Insertion mutation is caused by naturally occurring mobile genes (transposons). They have the potential to mutate genes throughout the plant genome. Mutation occurs from many other sources in the genome and is the origin of differences between all genes on which genetic recombination and natural selection can act. These are the essential requirements for evolution.

A much higher discard rate is common in conventional breeding than for GM plant breeding, reflecting undesirable, unpredicted or genetic events. The success of breeding relies heavily on the identification of desirable plants from a wide range of genetically different breeding lines. In

[9] The vast majority of crop varieties providing the foods we eat have never been tested formally or safety. In these instances safety is established by use. GM crops and foods pass through an extensive regulatory assessment as required under EU Directives.

many cases, transgenic lines are used as a source of new genes for further breeding by non-GM methods over several years.

A special feature of GM breeding is that it allows the transfer into crop plants of one or a few genes from what might be radically different organisms. Conventional breeding cannot, for example, form plants that can assemble complex human immunoglobulins as has been achieved in GM plants (see Ma *et al.* 1995). This inevitably raises uncertainty about whether there are any novel genetic interactions and whether these are potentially harmful (Lim *et al.* 2002). To determine definitively the relative scale of the uncertainties would require scientific investigation of a kind that has only recently begun (Pawlowski and Somers 1996; Wang *et al.* 1996, Labra *et al.* 2001; Sala *et al.* 2000) and for which there are no firm general results. As a result, this issue can only be approached on a case-by-case basis.

A further special feature of GM breeding is that the products of particular gene constructs may become present in radically different foodstuffs, effectively independently of any biological relationships (Firn and Jones, 1999; Schubert 2002) As is discussed later in this report (Chapter 5.4), this can hold important implications for risk management policy in areas such as the avoidance of exposures to any allergens that might pass through regulatory screening.

4.6 Are there gaps in our knowledge or scientific uncertainties and are these important?

Conventional plant breeding can produce gross undirected and unpredictable genetic changes and in that sense has considerable uncertainty. This is well documented and we know much about the types of change at a cellular level (see quality of evidence). Plants with undesirable characters or performance are discarded in the assessment stage of a breeding programme, so that only those plant genotypes that perform well over several sexual generations (progeny testing) and in different environments are accepted.

However, as has already been noted above, the GM process does introduce certain novel sources of uncertainty. The degree of uncertainty is related to our ability to detect and interpret changes at a molecular level. Our ability to do this relies on the tools that are available. GM plant breeding has not developed in isolation and it is possible to analyse the products with a degree of molecular precision that is not possible in non-GM methods of plant breeding.

For assessment of the future potential impacts of GM crops, it is especially important to gain a better understanding of the: (a) Genetic interactions associated with gene stacking; (b) Mechanisms of genome evolution and the induction of new variation within the genome (c) the biochemical implications of introducing familiar enzymes under the control of novel systems (e.g. EPSPS under the control of the CaMV 35S promoter) and the implications to the host of introducing novel enzymes.

4,7 Likely future developments

There is research on targeting transgene constructs to particular regions in the plant genome (already common in micro-organisms, lower plant forms such as the mosses and higher plant plastids by homologous recombination) as part of the transformation process. It has been claimed that this could reduce the variation between independent transgenic plants in transgene expression caused by position effects. The early indications from this research suggest that there is still variation in levels of expression when targeting is achieved in higher plants. It will probably still be necessary to produce several transgenic plants and test them for desirable performance (Ow, 2002).

There is likely to be an increase in the range of plant promoters to achieve more targeted transgene expression to particular plant tissues. This is a potentially valuable development for targeting transgene expression to edible or non-edible parts of plants. The use of less familiar gene promoters will mean that tissue specificity of transgene expression will need to be analysed carefully as part of any regulatory risk assessment (Topping and Lindsey, 1995).

There will be increased concentration on the regulation of endogenous gene expression using transgenic methods, including the utilisation of gene-silencing constructs. Underpinning research on factors affecting gene silencing will be important for risk assessment in applications of this kind (Grierson *et al*. 1996).

4.8 Where there is important scientific uncertainty, what is the potential way forward?

There is extensive research on molecular profiling methods to complement the current analysis of GM plants. This is a challenging area of research because plants from non-GM breeding programmes show genetic and phenotypic variation. Plants are also ‘plastic’ in that they respond and adapt to different environments by adjustments in expression of their genes. The challenge in this research will be to assess the significance of a change made by a genetic modification against existing substantial variations in background gene expression.

It would be valuable to gain a better understanding of the mechanisms of genome evolution to be able to see how genomes change under selection and during speciation. It would also be helpful to have a better understanding of epi-genetic phenomenon and their effect on gene expression. This topic is the subject of a number of current research programmes.

It is important that in the UK, we have regulatory oversight that is proportional to the degree of risk and the nature and scale of the uncertainties, and, which recognises the context and reference baseline provided by conventional breeding.

Chapter 5

GM DERIVED FOOD AND ANIMAL FEED SAFETY

5.1 INTRODUCTION

This chapter of the GM Science Review report considers the state of our scientific knowledge on issues of public and professional concern associated with the safety of food and animal feed of GM origin. This covers the consumption of GM crops, (whether processed or unprocessed) and the use of GM crops as animal feed resulting in the consumption of various animal food products, principally based on eggs, milk and meat. The term 'GM derived' means that products are included which are derived from genetically modified organisms, but in which it is not possible to detect any transgenic DNA or novel proteins.

Public concerns about GM were reflected in the report on the 'Review of Public Concerns', produced as a result of a series of 'Foundation Discussion Workshops' conducted by Corr Willbourn Research and Development under the GM Public Debate strand of the GM Dialogue. The 'public's questions', of particular relevance to the science related aspects of GM derived food and animal feed safety, can be found under the headings 'Possible risks to health' and 'Regulation and monitoring of safety' in that report. We have aimed to take account of these in this chapter.

More specifically, GM derived food and animal feed safety issues were raised under the Review at the various Open Meetings, as contributions to the Review website, and by GM Science Review Panel members at their meetings.

We consider the following four issues, where the text in italics aims to encapsulate the public issues and concerns from the 'Review of Public Concerns'.

5.2 Possible nutritional and toxicological differences in GM food
Could GM derived food be more toxic, more carcinogenic, or nutritionally less adequate when compared to other foods? And what is the potential for GM technology to produce foods with enhanced nutritional content or reduced toxicity compared with their non-GM counterparts?

5.3 Food allergies from GM crops
Is the risk of suffering food allergies greater in GM food?

5.4 The fate of transgenic DNA
Could transgenes (or parts of their DNA sequences) in food survive digestion and behave differently in comparison to traditional foodstuffs in their ability to relocate, recombine or modify the consumer's genome or that of associated gut microflora? If so, would this pose an increased risk to health compared to the consumption of non-GM derived food?

5.5 The effect of GM derived feed in the food chain

Could the consumption of GM derived feed and crops by farm animals pose more of a health hazard to consumers of the resulting food products, or to the animals, than the use of non-GM material?

These issues, as well as addressing concerns, also identify some of the potential benefits that could arise from the future use of GM technology. This includes improved nutritional quality, and reduced toxicity and allergenicity, of crops and food, and crops to produce pharmaceutical substances for medical and veterinary use.

The various references to aspects of the regulatory framework and to procedures for safety assessment and risk analysis are explained more fully in Chapter 3.

5.2 POSSIBLE NUTRITIONAL AND TOXICOLOGICAL DIFFERENCES IN GM FOOD

Could GM derived food be more toxic, more carcinogenic, or nutritionally less adequate when compared to other foods? And what is the potential for GM technology to produce foods with enhanced nutritional content or reduced toxicity compared with their non-GM counterparts?

5.2.1 Summary

Procedures for the safety and nutritional assessment of food and animal feed derived from genetically modified (GM) crops have been developed by intergovernmental bodies over the last 20 years, extending experience with traditional foodstuffs and different classes of chemical substance.

As with any new means of food production, there are potential human health risks that must be considered when crops and foods are developed by biotechnology. For example, this would cover allergy or toxicity from ingestion or from inhalation of pollen. In contrast, very few traditional foodstuffs which are considered to have a history of safe use have been subjected to systematic toxicological or nutritional safety assessment.

By identifying potential hazards and undertaking assessment of potential risks, it has been concluded by the FAO and the WHO that the food safety considerations for current GM crops and derived food and feed are fundamentally of the same nature as those that arise from conventional plant breeding (FAO/WHO, 1996). However, by virtue of the different processes involved, there will be some sources of uncertainty and potential gaps in knowledge that are more salient with respect to GM food production techniques. In summary, the risks may be toxicological/allergenic or nutritional in nature or may relate to the potential for gene transfer. In consequence, the available scientific evidence indicates that any potential effects are not different in nature from those created by conventional breeding practices and are already familiar to toxicologists and nutritionists (SOT, 2003). By assessing the hazards deriving from each component of the transformation of an existing ‘traditional’ variety to a new GM variety, it is possible to establish whether the new plant, food or feed is as safe as the conventional counterpart. The testing specifically addresses any potential for adverse nutritional or toxicological effects using established methods of analytical, toxicological and nutritional research (Codex, 2002a). When the testing is completed in accordance with current international guidance and best practice, a very detailed matrix of information should be available to permit the investigator to conclude whether or not the GM crop or derived food and feed is as safe and nutritious for its intended use as its conventional counterpart. However, as in all fields of safety assessment, the efficacy of the process inevitably depends on the rigour of the testing, reporting and compliance with regulatory guidance. In the UK and Europe, the process is tightly regulated and releases and marketing can only take place with explicit consent of the regulatory authorities. The stringency and consistency of application of the regulatory evaluation and oversight are essential for securing public health standards and confidence. In the United States there are 3 authorities, FDA, USDA and EPA involved in the regulatory approval process and opinions vary on its stringency (Gurian-Sherman, 2003; CLA, 2000).

For new foods, such as those derived from GM crops, the benchmark for comparison is that they should be at least as safe and nutritious as the traditional food or substance they replace or complement, and which have a history of safe use. Notwithstanding existing regulatory approaches, European consumers have voiced health concerns about the safety of GM crops, for example indicating that societal and ethical aspects must also be taken into account.

The extent of production and consumption of GM food over the last seven years and the lack of any convincing evidence of verifiable untoward toxic or nutritional effects resulting from its consumption, provides a measure of confidence in its safety when compared with the safety of other novel or non-GM foods. However, evidence for the absence of readily observable and relatively severe adverse effects in any food does not mean that milder, less widespread or longer-term effects can be completely ruled out. This raises the question of the sufficiency of existing monitoring for the potential health effects of food in general. The long-term assessment of the health effects of whole foods and feeds using post-marketing monitoring presents much greater difficulties when compared with that of a single compound or simple mixtures such as prescribed medicines. The main problem is establishing a causal link between consumption of a food and a particular negative or positive effect, which for all but major effects may be swamped by variability caused by changes in peoples diet and lifestyle. Countries are considering the implementation of some form of post-marketing surveillance of potential human late health effects of food in general, but at present there is nothing in place for GM foods in any country.

Looking to the future, the goal of producing safer more nutritious food is nothing new and indeed has long been practiced as part of traditional plant breeding, for example, selective breeding to remove erucic acid from Oilseed Rape (Canola) occurred over 30 years ago.

A number of ‘second generation’ GM crops are now under development which focus on providing foods which have improved characteristics, for example, which may be safer or have enhanced nutritional properties (ILSI Europe, 2001). Examples include:

- removal or decreased levels of antinutritional factors, toxins, allergens;
- introduction of or increased levels of health promoting factors (e.g. antioxidants); and
- modification of the levels of macro or micronutrients (such as fats and vitamins or minerals).

Such traits are likely to increase the complexity of the existing safety assessment process, as explained in Section 5.2.7.

Food safety and nutritional value and wholesomeness are related to a level of risk that society regards as reasonable in the context of, and in comparison with, other risks associated with a traditional diet. In short, food is not risk free. Safety depends on the way the food is prepared, processed and stored, and it is important that it is eaten according to its intended use. For example, potatoes must be cooked, and red beans must be boiled before consumption. The OECD has addressed this and it concluded that a food is safe if ‘there is a reasonable certainty that no harm will result from its consumption under anticipated conditions of use’ (OECD, 1993a). When reliable information is available making it possible to identify potentially dangerous effects to human health, or when there is scientific uncertainty making it

impossible to correctly assess the potential risks for consumers, it is appropriate to adopt a precautionary approach in risk assessment and management (EC, 2000).

Procedures have therefore been developed for the safety assessment of foods derived from GM crops taking into account that food safety is a relative concept. Approaches have been developed over the last 20 years by experts collaborating under intergovernmental bodies such as OECD, WHO and FAO. The framework underlying a comparative approach has been conceptualised as 'substantial equivalence'. Notwithstanding the limitations of this concept, which were mentioned in Chapter 3, the testing framework encompassing safety assessment and subsequent risk analysis, as set out by the OECD, WHO and FAO, is widely used in the European Union and in the UK, specifically by the Government's advisory bodies for food safety, the ACNFP and ACAF.

Inevitably, where food safety standards are concerned, it is desirable for consumer safety to have levels of international harmonisation recognising the need to maintain the best practices commensurate with ongoing scientific developments and national or international variations in diets. In this context, it must also be recognised that the evolution of food safety systems in different countries and parts of the world is impacted not just by science by also by society. Thus, while international regulatory frameworks show variations, it is generally agreed by the scientific and regulatory community that international consensus has been reached on the basic scientific principles presently used for the safety assessment of food derived from GM crops (Kuiper *et al*. 2001). There is however wider social and political contention over the scope and adequacy of the existing regulatory framework and the implementation of the scientific principles. Doubtless safety assessment procedures governing GM derived, as well as other novel and conventional, foods as embodied in institutions, policies, laws and guidelines, will continue to evolve.

5.2.2 Background

EU regulatory classification for GM food safety assessment

Most traditional food consumed today has a history of safe use, although there are exceptions for parts of the population for different staple foodstuffs, for example gluten allergy and milk intolerance. Moreover, imported new foods not hitherto eaten by a particular population such as kiwi fruit or even traditionally bred new varieties such as the Lenape potato (Coghlan, 1999) can sometimes cause toxic effects such as food allergy or intolerance. Similarly, traditional food and feed crops may also contain nutritionally undesirable constituents, as in the case of rapeseed plants and erucic acid (FAO/WHO, 2000) or corn and phytic acid. Notwithstanding the potential for adverse effects, very few traditional foodstuffs which are considered to have a history of safe use have been subjected to systematic toxicological or nutritional safety assessment.

Given the differing context, it is considered fully appropriate to assess the safety of any food or food ingredient designated as novel that has to enter the food or feed chain. To this end the EU Commission's Scientific Committee for Food published recommendations concerning six categories of food designated as novel and requiring detailed testing (SCF, 1997; EC Official Journal, 1997). See Box 5.1, where Class 3 relates specifically to GM plants and their products.

Box 5.1: Classification of novel foods as a basis for safety assessment (EC 1997)

Class 1: Pure chemicals or simple mixtures from non-GM sources
Foods and food components that are single, chemically defined substances or mixtures of these which are not obtained from plants, animals or microorganisms that have been genetically modified. Two subclasses can be identified: those where the source has a history of food use; and those where the source has no history of food use.

Class 2: Complex novel foods from non-GM sources
Complex foods or food components which are, or are derived from, sources which have not been genetically modified. Intact plants, animals and microorganisms used as foods as well as food components (e.g. complex carbohydrates, fats, proteins or those substances collectively described as dietary fibre) are included. Two subclasses can be identified: those where the source has a history of food use; and those where the source has no history of food use.

Class 3: GM plants and their products
GM plants can be consumed directly as unprocessed foods or after having been processed into foods and food ingredients including pure chemicals. This class of novel foods includes all such foods and food ingredients. Two subclasses can be identified: those where the host plant used for the genetic modification has a history of use as food or as a source of food under comparable conditions of preparation and intake; and those where the host plant used for the genetic modification has no history of use as food or as a source of food under comparable conditions of preparation and intake.

Class 4: GM animals and their products
GM animals can be consumed directly as unprocessed foods or after having been processed into foods and food ingredients including pure chemicals. Products directly produced by GM animals (e.g. eggs, milk) can be consumed either processed or unprocessed. This class of novel foods includes all such foods and food ingredients. Two subclasses can be identified: those where the host animal used for the genetic modification has a history of use as food or as a source of food under comparable conditions of preparation and intake; and those where the host animal used for the genetic modification has no history of use as food or as a source of food under comparable conditions of preparation and intake.

Class 5: GM microorganisms and their products
Living GM microorganisms may be used in food production or in the production of food ingredients. This class includes all novel foods which are, or are produced using GM microorganisms whether or not there are any living cells in the novel food as consumed. Two subclasses can be identified: those where the host microorganism used for the genetic modification has a history of use as food or as a source of food under comparable conditions of preparation and intake; and those where the host microorganism used for the genetic modification has no history of use as food or as a source of food under comparable conditions of preparation and intake.

Class 6: Foods produced using a novel process
This class comprises foods and food ingredients that have been subjected to a process not currently used in food production. Novel processes for food production may encompass, for example, new types of heat processing, non-thermal preservation methods, new processes to chill or freeze products, to dehydrate products, and the application of new processes catalyzed by enzymes. According to the scope of the Regulation (EC) No 258/97, the resulting product is only considered to be a novel food if the process results in changes in the chemical composition or structure of the food or food ingredient, which affect its nutritional value, metabolism or level of undesirable substances.

Sources of potential change in toxicity and nutritional content that could affect safety

To consider concerns over possible toxicity and nutritional changes to GM foods, it is necessary to dissect out the possible entry points for new hazards, as well as potential targets for the reduction of hazards, e.g. allergenic proteins, during the development of a GM crop, compared with traditional foods The following four sources can be identified, and should be checked systematically in the case of each new GM crop.

The 'parent' traditional crop or substance

This is relevant because as the starting point in the development of a new variety it is important to know its composition and variability in different geographical environments and under different growing conditions, and in particular the presence of known:

- natural endogenous toxins or food allergens;
- antinutrients; and
- biologically active phytochemicals, e.g. phytoesterols, caffeine etc.

The gene donor, new gene or transformation process

As the gene donor contributes the new DNA to the 'parent' crop during transformation it is important to know the gene donor's safety. This includes the comparative bioavailability of the active principle, its history and any prior information. For instance, sprayable Bt (*Bacillus thuringiensis*) has been used as an insecticide over the last 40 years but presents rather different issues of bioavailability than is the case with Bt proteins in GM crops. In the former situation the Bt proteins break down in sunlight, in the latter they are broken down during processing and/or in the gastro-intestinal tract of the consumer. It is also important to have a full description of the vector DNA, method of transgene delivery, characterization of inserted DNA sequences and the sequences bridging the plant genome and inserted gene. The last point not only permits the development of event specific detection methods but also ensures that no fusion proteins can be generated as a result of open reading frames spanning the plant genome and the new gene insert.

The primary gene product(s) or metabolites

It is the new gene-product (normally a protein), or different levels of plant metabolite(s), which characterise the new GM variety. These substances result in the new trait. Testing of the resulting substance(s)/metabolites is essential to determine that they could not lead to changes in toxicity or nutrition, unless these are intended from the perspective of reducing toxicity or enhancing nutritional qualities. Recognising that the new GM variety may contain one or more 'new' substances it is also important to test the whole crop in feeding studies (see below).

Introduced transgenes, under the control of specific promoters, encode proteins or can act to modulate the activity of, or switch, metabolic pathways on or off. Proteins are consumed daily in our diet and are broken down by digestion to peptides and amino acids which are assimilated for normal bodily growth. Proteins and any new metabolites expressed in the new GM variety are tested for stability to digestion, homology to known toxins or allergens, acute (single dose) and sub-acute (repeat dose) toxicity testing. In all spheres of toxicological testing, the efficacy of testing depends on protocol compliance and the quality of the programme design in relation to the substance(s) under investigation in conjunction with regulatory guidance (EC, 2003).

The new (transformed) crop

The new GM crop requires safety assessment to ensure that it is at least as safe and nutritious as the parent crop from which it is derived. Clearly if the new gene product or endogenous plant metabolites were not as intended they could potentially lead to toxic, allergic or antinutritional effects. By testing the composition of the new crop, food or feed in its entirety

in feeding studies, as well as the gene product/endogenous metabolites per se, there is a double safety check.

5.2.3 Range of views and quality of evidence

The issues and concerns

An underlying question with a new technology is whether it might pose unique safety issues or new classes of risk. In this context, concerns have been expressed about the safety of food derived from GM crops for man and food producing animals. Dr Pusztai has detailed some of these in his evidence to the GM Science Review and to the Clerk to the Health and Community Care Committee (HCCC) of the Scottish Parliament[1]. (In response to the HCCC, the Scottish Executive held that their report was fundamentally flawed.) A submission to the GM Science Review website[2] argues that subsequent work has failed to substantiate Dr Pusztai's findings. Today, we are not aware of any peer-reviewed scientific article which reports adverse effects on human health as a consequence of eating GM foods, (OECD, 2000). But equally, epidemiological studies are difficult to undertake for whole foods and no comprehensive ones have been conducted (see later in this Section and Section 5.2.6). In the USA recent reports indicate that approximately 60-70% of the processed food on supermarket shelves contains GM components (CDFA, 2003).

Nevertheless, there are different opinions over whether GM foods present a problem for human health and a number of the more important issues and concerns are addressed below based on a range of views and opinions taken from the literature, evidence presented to the Scientific Review Panel and the 'Review of Public Concerns'.

The potential for GM technology to improve nutritional value, food security and safety is considered in Section 5.2.7.

Concerns have been raised over the scientific validity of food testing strategies, for instance in relation to the sufficiency of animal testing and wider research (Chassy, 2002)[3,4]. Many approaches exist and a recent comprehensive review of food safety evaluation listing a number of studies performed is presented by Kuiper *et al.* (2001). Some of the issues involved in comparing whole food testing with single substance testing are described later in this section.

The Science Review Open Meeting on 'GM Food Safety'[5] was a major focus for the discussion of a number of these food safety issues and concerns under this Review. In addition, there was useful discussion material from other Open Meetings on 'GM Animal Feed: Safety Implications for the Food Chain'[6] and 'Gene Flow'[7] (although these issues are

[1] Pusztai, A. Submission of Evidence to the Clerk to the Health and Community Care Committee of the Scottish Parliament, November 2002. Report on inquiry into GM crops. HCCC, 1st report 2003.
[2] GM Science Review website. Burke D. http://www.gmsciencedebate.org.uk/topics/forum/0055.htm
[3] GM Science Review website. Smith A. http://www.gmsciencedebate.org.uk/topics/forum/0004.htm
[4] GM Science Review website. Halford N. http://www.gmsciencedebate.org.uk/topics/forum/0048.htm
[5] GM Science Review Open Meeting: 'GM Food Safety'. http://www.gmsciencedebate.org.uk/meetings/default.htm
[6] GM Science Review Open Meeting: 'GM Animal Feed: Safety Implication for the Food Chain'. http://www.gmsciencedebate.org.uk/meetings/default.htm
[7] GM Science Review Open Meeting: 'Gene Flow'. http://www.gmsciencedebate.org.uk/meetings/default.htm

considered elsewhere in this report). A submission to the GM Science Review website[8] considered the scientific concerns over the risk assessment and regulation of GM foods. Concerns raised by the Review website contributors are discussed below. In addition, some of the same questions were raised in the report on the 'Review of Public Concerns', while several new points are also addressed.

Two overarching issues are considered in this Section:

- could GM derived food or feed be more toxic, carcinogenic or less nutritional[9,10]; and
- could GM technology produce foods with enhanced nutritional content or lower toxicity for man?

While a wide diversity of evidence and concerns has been presented, most of the potential consequences to health derive from the following points:

- Any inherent toxicity of the transgenes and their products.
- Unintended (pleiotropic or mutagenic) effects resulting from insertion of the new gene construct into the recipient genome in the new GM plant. For example:
  - over expression of endogenous active substance;
  - gene silencing; or
  - altered metabolic pathways.

This summarises a range of concerns variously raised over the precision of the scientific basis of GM and our understanding of the process of expressing and control.

The scientific evidence in relation to questions raised

Is the new gene itself toxic?

Because the process of genetic modification has the potential to transfer genetic material between species, concerns have been expressed over the inherent toxicological properties of transgenic DNA. However, years of research indicate that dietary DNA has no direct toxicity itself. Humans typically consume a minimum of 0.1 to 1 gram of DNA (genes) in their diet each day (Doerfler, 2000). In this context it has been estimated that allowing for typical levels of transgenic DNA in plants, only 1:10,000 – 1:100,000 or less of the total plant DNA is the transgene (Lemaux & Frey 2002). DNA is rapidly hydrolysed and the new DNA is not a new type of material to our digestive system. The UK Royal Society concluded that the risks to human health of the ingestion of GM DNA are negligible, (Royal Society, 2002)[11]. The fate of DNA in humans and animals is discussed in Sections 5.4 and 5.5.

[8] GM Science Review website. Gasson M & Burke D. http://www.gmsciencedebate.org.uk/topics/forum/0045.htm

[9] GM Science Review website. Greenpeace. http://www.gmsciencedebate.org.uk/topics/forum/0024.htm
[10] GM Science Review website. ISIS. http://www.gmsciencedebate.org.uk/topics/forum/0030.htm
[11] GM Science Review website. UK Royal Society. http://www.gmsciencedebate.org.uk/topics/forum/0081.htm

Could the new gene be transferred to a human?

This is considered in Section 5.4.

Might the gene product or altered levels of endogenous metabolites per se (as in pathway engineering) present a toxic or allergenic risk to consumers, or handlers e.g. farmers/processors?

A case-by-case approach is adopted by testing the expressed proteins using standard *in vitro, in silico* and *in vivo* toxicological methods applicable to defined single substances. Secondly, the impact of the new product(s) in the context of the whole plant matrix is investigated in toxicology and feeding studies on the new GM crop/food. The uncertainties associated with methods for assessing food allergies are considered in Section 5.3. Current safety assessment of non-novel/GM foods does not involve this level of scrutiny.

Ultimately, for GM foods, only those products which are established to be at least as safe as those traditionally consumed, can be considered for approval by the regulatory authorities. Typically the development of a new GM crop takes of the order of 10 years and the new (transgenic) proteins are typically tested in animal models for acute toxicity.

In the case of enzymes or other proteins introduced into crops through GM, it is pertinent that there are no known examples of food proteins having teratogenic, mutagenic or carcinogenic effects in animal models (SAP, 2000b). Those which are toxic typically elicit their effects rapidly upon consumption (Sjoblad *et al*. 1992).

Quantitatively increased levels of endogenous metabolites can be evaluated by taking into account the daily food intake and comparing their new dietary levels with those established to be safe and without risk. If *de novo* substances are expressed with no structural analogy these must be tested as defined single substances.

Will expression of the intended gene product increase the toxicity (including carcinogenicity) or allergenicity of the new GM crop itself?

Concern has been expressed about the long-term effects of a new GM food such as carcinogenicity, reproductive toxicity or allergenicity and it is asked why GM foods are not tested like pharmaceutical products (allergenicity is addressed in Section 5.3). These are important questions which are not unique to GM foods. In conventional toxicology methodology an important consideration is the nature of the substance to be evaluated. The methodology can be applied to medicines, food additives and pesticides, all of which are usually very well defined chemically. Testing is carried out by feeding the substance under test to the test animal at a range of doses, some several orders of magnitude greater than the expected human exposure level, to determine any adverse effects, thus allowing safe levels to be set for human exposure. For example, in the case of the GM Roundup Ready® Soybean Event 40-3-2 a range of farm animal feeding and toxicity studies were performed within the substantial equivalence framework[12]. The extent of testing depends on the identity of the substance, whether it is known or not, its mechanism of action, structure and quantitative level. Typically, but not always, single gene products such as proteins (especially those which are readily digestible) and plant secondary metabolites are familiar substances with a relatively low order or toxicity which can be tested in the conventional manner.

[12] Safety Assessment of Roundup Ready® Soybean Event 40-3-2, September 2002. http://monsanto.com/monsanto/content/our_pledge/pss_roundupsoybean.pdf

It is also important to check the whole food for changes that might arise from any unintended effects, during the transformation. This is done by undertaking typically a sub-chronic 90 day rat feeding study using up to three inclusion levels of the GM crop or food/feed in comparison with the non-GM isogenic variety. This study serves as a good indicator that there are no unintended changes of toxicological significance that might render the GM variety less safe than the non-GM comparator (EC, 2003).

Foods are complex mixtures of compounds that can vary considerably in their composition and nutritive value. There are practical limits on the amounts that can be fed to animals without affecting the nutritive value of the overall diets and thus causing secondary health affects. While there has been much discussion on the feasibility and efficacy of such feeding studies because of the need to maintain nutritional balance in the diet[13], properly performed safety factors of up to 100 fold can be achieved depending on the novel food being tested. Farm animal feeding (nutritional) studies, e.g. broiler chicken or ruminant studies, can also contribute to the safety assessment. The need for additional toxicity studies should be considered case by case.

The absence of readily observable adverse effects does not mean that these can be completely ruled out for any food. The long-term assessment of the health effects of whole foods and feeds, which are complex mixtures, presents greater difficulties when compared with the post-marketing surveillance or monitoring of a single or a few compounds such as in prescribed medicines.

There is a wide diversity of studies that might be made using human subjects to confirm digestibility and palatability. These studies are not to investigate potential toxicity but are to confirm acceptance and tolerance. Guidelines which have been agreed and published for such human studies, discuss when such work is justified and how the work should be designed and conducted (ACNFP, 2002).

Standard OECD (OECD, 1993b) or EU Commission Directive on Dangerous Substances (EC, 1987) protocols should be used where practicable in testing, according to the principles of Good Laboratory Practice. Use of non-standard protocols should be justified.

Could the gene product or altered levels of endogenous metabolites (as in pathway engineering) present a nutritional risk to consumers?

Only GM foods or derived products which are as safe as their conventional counterparts, taking into account the dietary impact of any changes in nutritional content or value, are allowed to be registered for marketing. Detection methods primarily rely on targeted approaches to determine levels of known nutrients, antinutrients, allergens and toxic substances. The gene product(s) will be checked for safety and any significant antinutrient potential in relation to known compounds would be likely to be picked up by the wide battery of tests conducted. Novel substances will be tested in their own right as defined single substances (see Section 5.2.2).

In order to increase the potential to detect unintended effects, molecular profiling methods are under development which adopt a non-targeted approach. However, due to the wide inherent variation within any individual crop (both GM and non-GM) it has become clear that further development, validation and construction of linked databases will be required before they are

[13] GM Science Review website. Halford N. http://www.gmsciencedebate.org.uk/topics/forum/0048.htm

able to be used in formalised risk assessment procedures. If the practicality of these profiling methods can be proven, which is by no means certain, they could be particularly useful in increasing the certainty of the safety and nutritional assessment of foods from 'second generation' GM crops involving the reengineering of metabolic pathways (see Section 5.2.7).

Some gene product(s) will be utilized to improve human or animal nutrition. In this case careful checks and studies are then needed in order to validate such claims (see Section 5.2.7).

Could insertion of the new gene lead to unintended effects such as increased toxicity (including carcinogenicity) or allergenicity via pleiotropic, insertional mutagenic or promoter effects?

Agrobacterium mediated or microballistic techniques are used to introduce the new transgene into the desired crop DNA randomly. This may result in pleiotropic and insertional mutagenic effects. Such insertions might cause gene silencing, altered expression or the turning on or off of existing genes that were not previously expressed. For this reason, following extensive selection and laboratory testing prior to field release and evaluation, the new GM variety is checked for compositional equivalence (for major constituents) to its traditional counterpart; phenotypic and agronomic equivalence; and nutritional and toxicological equivalence. If no unexpected findings are seen in any of these comparative evaluations, and there are no confounding effects, the probability of there being a new toxin, allergen, carcinogen or anti-nutrient is widely regarded as being very low. Chapter 4 considered the reliability of GM plant breeding technology and compared this with other plant breeding methods.

Gene promoters and protein coding sequences derived from plant viruses are used in the construction of some plant transformation vectors. A recent report[14] suggested that because of a proposed 'recombination hotspot' the consumption of transgenic plants or food derived from them containing the CaMV 35S promoter may result in 'inappropriate over-expression of genes' leading to cancer in humans, or that recombination may lead to the reactivation of dormant viruses' or the creation of 'new viruses' (Ho *et al*. 1999). There is no evidence that if such recombination events occur, they occur at any different rate or produce any unique end products that would lead to human health consequences (see Section 7.5). Moreover, intact and unencapsidated plant viruses have been consumed safely for thousands of years by man and animals (Bouhida *et al*. 1993; Harper *et al*. 1999; Ndowora *et al*. 1999; Hull *et al*. 2000). Because the virus copy number per cell is very much higher than the transgene copy number per cell, the consumption of virus infected plant tissues may result in up to a 100,000-fold greater dosage of the CaMV 35S promoter DNA per gram of tissue than would be obtained by consuming transgenic plant tissues (Hull *et al*. 2000). According to what is currently known about processes of horizontal gene transfer in the gut (Section 5.4), there is no biologically plausible mechanism by which the consumption of food or feed containing the 35S promoter might lead to adverse health effects in animals or humans[15].

Will the new GM derived food or feed be less nutritious?

A range of analytical or compositional studies is undertaken to determine whether nutrients, vitamin and minerals in the new food occur at equivalent levels as in the traditional counterpart (Sidhu *et al*. 2000). Apart from chemical analysis, which gives a general guide to safety, in a similar way to the targeted screen of key elements from the blood of humans, feeding (also known as wholesomeness) studies are normally performed in a fast growing

[14] GM Science Review website. ISIS. http://www.gmsciencedebate.org.uk/topics/forum/0030.htm
[15] GM Science Review website. Morton R. http://www.gmsciencedebate.org.uk/topics/forum/0062.htm

species such as chickens, where day old chicks are fed on the GM and the isogenic non-GM crop comparator lines for 42 days to determine any difference in weight gain or other endpoints of nutritional adequacy. Ruminants, pigs and even fish may also be tested for nutritional equivalence. To date such feeding studies have shown no significant adverse changes in nutritional value (Kuiper *et al*. 2001)[16].

As discussed above, non-targeted profiling techniques may prove helpful as the knowledge of natural plant variability increases for different species.

5.2.4 Is there general scientific agreement?

Among food scientists and regulators there is a widespread view (FAO/WHO, 2000; OECD, 1993a; Cockburn, 2002) that GM food safety testing procedures employed systematically, sequentially and holistically under international food risk analysis guidelines ensure that food derived from the GM crops approved today is at least as safe and nutritious as the traditional counterpart[17]. Some of the uncertainties and gaps in knowledge bearing on this view are discussed below. Either way, it is generally agreed that this does not mean that GM food, as with traditional food, has zero risk, rather that in line with the OECD definition, 'there is a reasonable certainty that no harm will result from its consumption under anticipated conditions of use.' The use of substantial equivalence in safety assessment (see Chapter 3) has caused controversy, in part because it has been defined as an endpoint in novel food regulations. Currently, substantial equivalence is established as a useful comparative approach to identify significant differences between a new food and its traditional counterpart. These differences are not necessarily a hazard but they become the subject of further detailed safety assessment (EC, 2003).

Such testing, which has to meet current international standards, uses compositional comparison as the start point for safety assessment. Not only are any changes studied in their own right for safety impact but also considered in the context of the metabolic perturbation that may have resulted in such changes. Additionally, the parent crop is characterised as well as: the inserted recombinant DNA; the safety and allergenicity of any inserted proteins and metabolites; and the toxicological and nutritional status (using animal studies on the whole crop).

The sufficiency and robustness of testing protocols is sometimes the subject of scientific contention. But they are widely accepted as the best presently available, whilst recognising that they will be reviewed and improved, for example in light of technological developments.

Although modern genetic modification techniques may introduce a defined form of novelty, the scientific community has not identified new or hitherto unknown classes of hazard from the process or product of GM. There is general scientific agreement that any hazards that may occur are encapsulated in three possible types:

- toxicological/carcinogenic/allergenic;

[16] GM Science Review website. Monsanto. http://www.gmsciencedebate.org.uk/topics/forum/0061.htm
[17] GM Science Review website. UK Royal Society. http://www.gmsciencedebate.org.uk/topics/forum/0081.htm

- nutritional; or
- gene transfer.

5.2.5 Is the issue unique to GM?

Concerns over whether or not foods derived from GM crops pose unique safety issues or might have unintended effects was discussed in Section 5.2.3. It is necessary to put such concerns into context. Traditional crops may have their own hazards, which can include toxins, allergens, antinutrients and biologically/pharmacologically active substances. Unintended effects may arise from natural and other non-GM plant breeding techniques (see Chapter 4). It is as a response to these concerns that a comprehensive case-by-case testing programme is conducted on all new GM crops and derived foods.

The process of GM does not, in itself, create new classes of hazard different from the types identified above. And the same hazards are inherent in conventional breeding methods, (SOT, 2003). Different plant breeding practices (such as GM, pollination, cell selection, and radiation and chemical mutagenesis) involve different processes, different outcomes and by implication different uncertainties. Plant breeding practices and the reliability and sources of uncertainty in GM technology were discussed in Chapter 4. In the case of GM, the technology does have the potential for the widespread introduction of individual gene constructs whose gene product proteins will then appear across a wide range of food types. (See the 'shock' scenario discussed in Section 5.3.).

Antibiotic resistance markers (ARMs) have been used in GM technology and have been a source of safety concerns centred on the gene transfer risk. This is discussed in Section 5.4.3.

In studying the crop and derived food product for its comparative safety and performance, the same endpoints are chosen as those employed with non GM crops and novel foods, namely phenotypic appearance, agronomic performance, composition, nutritional content, nutritional performance in livestock feeding studies and safety based on toxicity studies. In the same way, a final decision can be drawn from the weight of test results and evidence as to whether the GM derived food is as safe as its conventional counterpart.

Overall, the available scientific evidence indicates that the potential toxicological or nutritional hazards and resultant risks are in nature no different or significant respectively from those created by existing breeding practices. The regulatory process, in dealing with applications on a case-by-case basis, will need to take account of increasing exposure to the products of specific transgenes. The abililty to identify foods containing these products will depend in part on the extent of GM labelling.

5.2.6 Are there gaps in our knowledge or scientific uncertainties and are these important?

The safety of all foods is subject to scientific uncertainty and gaps in knowledge and all plant breeding methods have unique features and are subject to some uncertainty. Modern GM technologies are no different in this respect and will have their own characteristics. The

assessment of uncertainty in relation to a GM crop or a GM derived food is best done on a case-by-case basis. Chapter 4 considered gaps in our knowledge and scientific uncertainties in relation to GM plant breeding in general**.**

Compositional analysis is used in a targeted way to measure key internationally agreed macro and micronutrients in plants, the precise details of which have been defined by OECD for a number of crops (OECD, 2001a; 2001b; 2002a; 2002b; 2002c)[18] but some of which remain in contention. Although this serves to sample the plant's metabolic status, there is always the possibility of 'what if' a new substance was produced due to the transformation process. The potential occurrence of unanticipated alterations in the composition of GM crops is then a key consideration in their safety evaluation.

The fact that GM food crops have now been grown on over 230 millions cumulative hectares worldwide over the past seven years (ISAAA, 2003) does provide evidence for the lack of harmful human health effects from the consumption of GM food products. In addition, the lack of successful litigation that would demonstrate a causal link between adverse effects and the consumption of GM crops and food products is a form of societal evidence for lack of harm. However, it is only evidence for the lack of more serious and readily observable health effects. Milder or less widespread or more delayed adverse effects can be completely ruled out with existing data and long-term epidemiological studies would be required to demonstrate their absence.

Various groups have expended considerable efforts looking into the options and feasibility of post-marketing monitoring and surveillance schemes for GM food. Problems limiting the interpretation of data from this approach have been highlighted by the Food and Agricultural Organisation and the World Health Organisation (FAO/WHO, 2000). A key difficulty is how well any methodology can be relied upon to establish a causal link between consumption of a GM food and a particular negative or positive effect. At present there are no post-marketing surveillance systems for GM foods in place in any country (Amanor-Boadu V&Y, 2002). Only the more severe, widespread and immediate health effects would be likely to be picked up by public health procedures. Countries are working towards the implementation of some form of post-marketing surveillance of potential human food-related late health effects. For GM food, this would provide an additional check on long-term safety, to complement the existing essential safety assessment framework. The FSA has commissioned a study to examine the feasibility of using supermarket and household survey data for post-market surveillance of novel foods including GM derived ones. The results are expected later in 2003.

A non-targeted approach using molecular profiling techniques such as DNA/RNA microarrays, proteomics and primary and secondary metabolite profiling may have utility in the future. Today, further exploration of the specificity, sensitivity and validation of such techniques is still necessary. Moreover, because of a naturally wide variation in plant composition due for example to different developmental stages (ripening) or environmental growth conditions, different profiles of 'normality' will need to be held on linked databases (Kuiper *et al*. 2003). A significant amount of research has been sponsored internationally to explore these possibilities.

[18] * International Life Sciences Institute (ILSI) Crop Composition Database www.cropcomposition.org

From the food safety perspective the final composition of a new GM crop must be compared against the conventional counterpart using the wide range of characteristics described in Chapter 3. This creates a weight of evidence. Ultimately, safety assessment of the new GM crop or derived food in rodent toxicity tests as well as livestock feeding studies, in conjunction with the weight of evidence from other tests, all have to be taken into account to eliminate any new or unexpected constituents which might have significant adverse effect for man. It is recommended that existing protocols are formalised for this purpose.

It is also generally recognised that in the case of food allergens (which are considered in Section 5.3) we do not fully understand the defining characteristics that cause a particular substance to result in IgE sensitisation and a tendancy to develop allergies. However, this is no different for traditional or GM foods. In both cases we use a weight of evidence approach, which provides a scientific basis that the new GM variety will be at least as safe as its conventional counterpart.

Nevertheless, because of real concerns that the GM derived food may contain unintended substances, and any health impact depends on their detection, research should continue to build testing paradigms that take a holistic approach and do not focus solely on a single characteristic. As in any scientific field, there is an ongoing need to develop safety assessment to the highest practicable standard, consistent with scientific and societal attitudes and knowledge.

5.2.7 Likely future developments

Detection of unintended effects

As mentioned earlier, GM food is not unique in raising the possibility of causing unintended effects. These can also occur as a result of the conventional breeding of new plant varieties.

Food is a complex matrix containing tens of thousands of different substances. This means that molecular profiling techniques such as RNA microarray, proteomics, metabolite profiling and other screening techniques may, in principle, offer an unprecedented view of very subtle alterations in composition during plant breeding, GM or non-GM. However, as already discussed, these techniques are currently the subject of wide research investment by the biotechnology community at large, including the FSA. Large amounts of data will be generated and as Kuiper *et al.* (2003) say, it is not clear how the data will be interpreted and whether these techniques will find general utility and application.

Safer, nutritionally enhanced, foods?

The goal to produce safer more nutritious food is nothing new and indeed has long been practiced as part of traditional plant breeding. The example cited earlier concerning rape seed and erucic acid occurred over 30 years ago. More recently, there has been an interest in ‘functional foods’; foods which have been specifically designed to provide a particular health benefit over and above their usual nutritional value. So far, most of the interest in these (and the regulatory scrutiny) has focussed on their creation by non-GM means and various non-GM functional foods are now available, for example to reduce cholesterol levels. Concerns have been expressed about the possible adverse effects of deliberate changes to the nutritional

balance of food of this type, for example through the intake of higher levels of micronutrients, but these concerns are not specific to GM products and relate to the broader regulation of functional foods.

A number of 'second generation' GM crops are now under development, these focus on providing foods with safer or enhanced nutritional properties (ILSI Europe, 2001). The application of biotechnology to the future of food and nutrition was highlighted in a submission to the GM Science Review website (J. American College of Nutrition, 2002)[19]. GM can be used to:

- remove or decrease levels of antinutritional factors, toxins and allergens;
- introduce or increase levels of health promoting factors (e.g. antioxidants); and
- modify the ratio of macronutrients (proteins, fats and oils, carbohydrates) or micronutrients (e.g. vitamins or minerals).

These are also the aims of conventional plant breeders in seeking to add value to commodity crops.

The safety and nutritional impact of such products will be a key consideration. In most cases genetic modification will involve targeting the basic biochemical processes in the plant; leading to alterations in its metabolism and chemical composition. This reengineering of metabolic pathways may alter other pathways and lead to the production and/or removal of not only the targeted substance(s) but also of unexpected ones. An increase in one component may be matched by a decrease in other compounds of nutritional or agronomic importance. It is not surprising that this has proved to be the case in experiments carried out to test various hypotheses and models (Shewmaker *et al.* 1999; Gura, 2000). Any safety aspects arising from unintended effects will need careful assessment for potential commercial products and the limitations of chemical analysis in predicting biological function was raised as an issue on the GM Science Review website[20]. The number and spectrum of metabolites formed by a plant can vary considerably according to environmental and other growth conditions, complicating the baseline comparison for the effects of genetic modification (Firn & Jones 1999).

As the development of such 'second generation' products continues it will be necessary to address: new challenges relating to the detection of compositional change(s); phenotypic change (including those at the cellular level); dietary impact for consumers; sensitive consumer groups; unintended effects both predicted and unpredicted; data for any health claims; and impact on the uptake of other nutrients, etc. Many of these points are also applicable in the general field of novel and functional food research, whether GM or non-GM.

Testing of second generation nutritionally enhanced products will therefore not only need to build on the paradigm and methodologies of first generation GM crops and novel foods and regulations, but will also require new considerations and regulations in their own right. Their characterisation is likely to make increasing use of molecular profiling techniques (Kuiper *et al.* 1999), which are still the subject of much active research and development. The FSA is funding a three-year research programme until September 2004 which is exploring new and

[19] GM Science Review website. Klurfeld DM. http://www.gmsciencedebate.org.uk/topics/forum/0013.htm
[20] GM Science Review website. GeneWatch UK. http://www.gmsciencedebate.org.uk/topics/forum/0006.htm

emerging techniques and their potential application for developing the current safety assessment procedures, so that they can keep in step with future developments in GM technology. The programme is examining the use of protein and metabolite profiling techniques in characterising a variety of plant species. Recognising the above caveats and needs for the testing and safety assessment of second generation products, the research community is discussing these topics within an International Life Sciences Institute (ILSI) Working Group and focussing on three main areas, which are outlined below.

Removing detrimental (antinutrient) substances

The impact of dietary components that have untoward health effects varies from country to country, often according to their concentration in food, level of consumption and sensitivity of the population. Examples of components causing illness include rice allergens, gliadin proteins, wheat gluten, (leading to coeliac disease), lectins, peanut allergens, and cyanogenic glucosides in crops like cassava.

Various targeted GM approaches are being employed to remove or significantly decrease the presence of toxicants involving antisense RNA and gene 'knock-out' techniques. This is hoped to have the potential to not only improve health, but actually save lives as in the case of food allergens which in the worst case can cause anaphylaxis. However, while the concept is simple, the work is complex, especially in the case of structural protein allergens.

Enhancing health-promoting substances

It is well known that in the western world, cardiovascular disease kills approximately one out of every two people and cancer one in four. The onset and progression of both diseases can be influenced by diet.

Until now a major beneficial dietary factor for certain types of cardiac disease has been omega 3 fatty acid. Typically, the only source has been from oily fish. Recent research has shown a plant source in algae and the application of GM technology has now led to the trialling of crops rich in omega 3 fatty acid which has a potent cardio-protective effects (ILSI News, 2002).

Similarly, increasing the level of oleic oil (a poly-unsaturated fatty acid) in rapeseed oil and soyabeans reduces the level of saturated fat intake with clear cardiovascular benefits (DuPont Agricultural Products, 1996). This is the basis for a number of non-GM food products.

Many vegetables and fruits contain important antioxidants which help to protect against certain cancers. The beneficial substances are known as phytochemicals and include flavanoids, antioxidants, phytoestrogens and glucosinolates which are being studied with a view to enrichment and potential health enhancement.

Vitamins and micronutrients

Nearly one sixth of the global population of six billion people do not have adequate diets. Micronutrient (vitamin and mineral) deficiencies are common. Solutions are limited because of often-limited local food production and a lack of income to buy foods from diverse sources.

In consequence, GM technology has been used to enhance the nutrient quality of staple crops by specifically modifying the secondary metabolic pathways. A recent example is 'Golden Rice' which has been modified to increase the content of pro-vitamin A (beta-carotene) by

introducing two genes originating from daffodils and one from a bacterium into rice. Other crops such as pro-vitamin A enriched 'Golden Mustard' are also undergoing development; mustard seed oil being used as a daily food and cooking commodity over much of the Indian sub-continent. Moreover, higher levels of beta-carotene can be obtained in mustard than in rice (Ye *et al*. 2000; Shrewmaker *et al.* 1999).

5.2.8 Where there is important scientific uncertainty, what is the potential way forward?

Research

Thorough consideration of uncertainties has been undertaken by the European Network on Safety Assessment of GM Food Crops (ENTRANSFOOD) which collaborated under the EU Fifth Framework for Research over the last three years. The report is currently in draft and will be published in 2003 following final consultation (ENTRANSFOOD, 2003). International research is ongoing in the following main areas.

- Food allergy to improve our understanding of cellular and molecular basis of sensitisation.

- Safety assessment techniques which have evolved over the last 50 years will continue to be refined which will help to reduce uncertainty over the presence or not of unintended effects, currently studied in a variety of tests including animal toxicology.

- Molecular profiling techniques will continue to be researched and validated to establish their utility for improved analysis and detection of unintended compositional effects.

- Epidemiological studies need to be established to show any untoward impact on human populations. Based on this, meaningful methods of post marketing surveillance can be evaluated.

- Standard protocols will be agreed/adopted increasingly by the international community.

- Methods of removing marker genes and non-essential DNA will be found which do not disadvantage researchers in less affluent countries.

- Bioinformatics for improved safety assessment.

Technological approaches

Increased clarity is desirable on the comparative approach for safety assessment, based on substantial equivalence.

Regulatory approach

It is often said that there are few regulatory requirements for GM crops and the foods derived from them. This is not correct: wide-ranging regulations have evolved for GM crops over the last two decades and development will continue. Over this period, governments and intergovernmental organisations have designed strategies and protocols, which are scientifically robust and proportionate to other spheres of safety evaluation and the associated hazards and risks (FAO/WHO, 1991; FAO/WHO, 1996; FAO/WHO, 2000; FAO/WHO, 2001; Codex, 2002b ; NAS, 1987; NRC, 1989; OECD, 1993a; SOT, 2003). Indeed, in many respects there is far greater safety evaluation of GM crops and derived foods, which require extensive testing in comparison with conventional crops, which often require no mandatory testing at all. As in all walks of life uncertainties exist but the benchmark for GM food is that it should be as safe as conventional food, which already has a history of safe use.

5.3 FOOD ALLERGIES FROM GM CROPS

Is the risk of suffering food allergies greater in GM food?

5.3.1 Summary

It is estimated that 1-2% of the adult population may suffer food allergies, rising to 5-8% of infants. Changes in potential allergenicity during the breeding of conventional crops are not assessed in a regulatory framework and are not formally evaluated.

GM technology enables a particular gene construct for a new protein to be introduced, and the potential allergenic effect of that protein is a focal point for safety assessment. In addition, the regulatory process, with its case-by-case approach, must take account of possibly increasing exposure to a GM protein, especially if it is expressed in a diversity of different GM plants, and thus introduced into a diverse range of foodstuffs. In the hypothetical case, where a GM allergen was not recognised in regulatory screening, and its effects only emerged in the longer term, avoidance of the allergenic protein by the consumer could be difficult, because they would not be able to recognise its presence in the foodstuffs. The likelihood of this scenario is very low for a number of reasons. However, avoidance in a GM or non-GM case would depend on the relative effectiveness of labelling, traceability and recall systems and it would be for the regulatory system to ensure that any GM allergen, once known with a potentially significant effect on any consumer, should be labelled in a fail-safe way or withdrawn from the marketplace.

It is easier to evaluate the risk of introducing allergenic proteins and altering the allergen composition of the target crops after use of GM than with mutation technologies or breeding with distantly related germplasm.

The first line of defence against the untoward introduction of an allergen in a GM crops is a set of safety tests that have been found useful in addressing, in a practical sense, a number of different criteria that have been developed as indicators of allergenicity.

The issue of potential problems arising from GM food allergy hinges on the reliability and confidence in the safety tests that have been developed. These are under continuous evaluation and improvements are published in the scientific and regulatory literature.

It is difficult to predict the allergenic characteristics of a given protein. The interaction with the gut immune system that is involved in generating an allergic response is not well understood. Absolute predictability never exists in this or other regulatory arenas.

GM technology provides an opportunity for the targeted removal of food allergens from existing foods.

5.3.2 Background

The allergies to pollen derived from conventionally bred crops, such as those resulting from the introduction and widespread cultivation of oilseed rape, are well known, although the

nature of the allergenic compound(s) is not yet known. The introduction of a new conventionally bred crop or food may elicit allergies in a number of individuals. One of the most serious and widespread allergies that now occurs is that to peanuts and tree nuts. Exposure to these can have serious consequence for the allergic individual, including death after anaphylactic shock. The public appears to consider this unwelcome side effect that affects a small minority of the population as an unavoidable and acceptable consequence of the introduction of such crops, even though allergic people find it difficult to avoid exposure.

Food allergies are most common in: fish, shellfish, milk, eggs, legumes (peanut and soy), tree nuts, cereals and fruits. These account for some 90% of reactions to food. It is estimated that 1-2% of the adult population may suffer food allergies with up to 5% of children affected and 5-8% of infants.

Almost all food allergens are proteins, but not all food proteins are allergens, despite the large numbers of different proteins in the diet (Townsend 2000). There is currently no single predictive test to define which proteins are, or are likely to become, allergens to humans. It is therefore a combination of tests, based around a decision tree approach, which have allowed scientists to address questions of potential allergenicity for GM crops (SOT, 2003; FAO/WHO 2001; SSC 2003).

Allergies are different from aversion reactions. Up to 20-25% of people believe themselves to have adverse reactions to specific foods. The nature of the reaction is not understood in most cases and the food(s) which provoke these reactions change over the lifetime of the person. Food intolerance can also manifest itself in some people and they may react to simple sugars like lactose. The detailed mechanisms by which these intolerances occur are not well understood. This Review deals primarily with food allergens, i.e. those compounds that elicit a reaction in which binding of the compound to immunoglobulin E (IgE) antibodies that are specific for the food allergen in question leads to the release of histamine and the serious consequences that derive from that.

5.3.3 Range of views and quality of evidence

The risk associated with the introduction of new food allergens by GM technology has been highlighted repeatedly as a public concern and in the 'Review of Public Concerns' elicited questions such as:

Is GM food harmful? Could harm take the form of allergic reactions?

There is a wide range of public views, from those who contend that the internationally accepted frameworks for regulation (FAO/WHO, 2001) assure a high level of safety to those who state that since there are a number of potential uncertainties in the regulatory framework it is impossible to be absolutely sure. The latter position is probably best exemplified by positing one of the 'shock' scenarios that has been discussed in the economic strand of the GM Dialogue[21]. In this scenario, a novel non-food-plant protein that would not have been shown by the current framework of safety testing to be an allergen, would have been

[21] * GM Economics Study, The Prime Minister's Strategy Unit. Note of a 3 April 2003 workshop, yet to be published on the website http://www.number10.gov.uk/output/Page3673.asp

introduced by GM technology in a very large range of crop plants. Subsequently, five years after the large scale introduction of these crops the protein, would be detected to be allergenic in a small fraction of the human population, which, due to the novel protein's wide distribution, would have difficulties in avoiding exposure to it. A new allergen introduced into a staple crop by non-GM breeding would also become widely used in processed food. There is a range of public views on the extent of the difficulty that people would have in avoiding this kind of exposure. If such a scenario were to occur, all safeguards would have failed.

The scientific views in this area of the debate range from a high level of confidence in the existing testing regimen, to those that see the regimen as useful but would like to see it further validated and extended, to those who say that it is inadequate. Hence, the main science-related issues about allergenicity relate to the level of confidence in the practical testing regimen.

There has been considerable research on the assessment of allergenicity in GM foods. For example, this is part of a current EU Fifth Framework Programme study on testing strategies for GM foods that will report early in 2004[22]. Allergenicity in GM food has been considered by Kuiper *et al.* (2001) and at an Open Meeting on 'GM Food Safety' under the GM Science Review[23]. The two documented and probably the most cited cases of potential concern over allergenicity are discussed Kuiper and Meredith. The first of these is the inclusion of a protein from brazil nut into soyabean as part of research to improve nutritional quality. The decision tree approach to allergenicity testing recommended in regulatory guidance (FDA 1992) was followed and as a result of this testing, development was stopped by the company prior to any commercialisation (Townsend 2000). The other case is the Starlink episode. In StarLink™ corn the truncated cry9C gene of *Bacillus thuringiensis* has been introduced to provide resistance to the corn borer. StarLink™ corn was first approved for animal feed only. This was because further studies needed to be completed on protein digestibility before the product could be submitted for human food tolerance approval. Due to inadvertant mixing of food and feed, contamination of the human food corn with the animal feed corn was detected. The allergenic potential of the cry9C protein was further very thoroughly assessed in an EPA SAP hearing. The detailed assessment of scientific issues (SAP, 2000a) and the negative tests on suspected allergic individuals have been documented and no-one has been found to have developed an allergy to StarLink™ corn. The episode highlights the challenges in the assessment of the human allergenic potential of a given protein.

5.3.4 Is there general scientific agreement?

There appears to be general scientific agreement on the approaches to safety assessment based on the analysis of a decision tree (Metcalfe *et al*. 1966). The main areas of contention appear to be the value of specific tests and if and how they can be improved (Haslberger, 2003).

Genuine food allergy is almost always associated with proteins or glycoproteins, which lead most often but not always (see below) to an IgE immune response. IgE is the main type of immunoglobulin that gives rise to allergic reactions. The assessment of allergenicity is thus based primarily on the ability of a protein to generate an IgE response.

[22] GM Science Review website. Smith A. http://www.gmsciencedebate.org.uk/topics/forum/0004.htm.
[23] GM Science Review Open Meeting. 'GM Food Safety'. http://www.gmsciencedebate.org.uk/meetings/default.htm.

There are also rarer, cell-mediated immune reactions to food allergens, which usually give a more delayed response (6-8 hrs after ingestion) and may give rise to serious reactions to food such as the one in coeliac disease where patients react against gluten. The cell-mediated immune responses are not well understood and it is likely that sensitisation and elicitation of a response are different from those for allergic responses mediated by IgE.

It is important to recognise that the prediction of food allergies is complicated by the fact that proteins can be altered during food processing in the factory and at home (e.g. by cooking) and further by passage of the protein through the human alimentary canal. This can result in either reduced or increased allergenicity.

Problems with allergenicity in a GM crop can be due to the allergenic potential of the product of the transgene itself, but also to unintended effects of the introduction of the transgene on the expression of levels of naturally present allergens in the target crop.

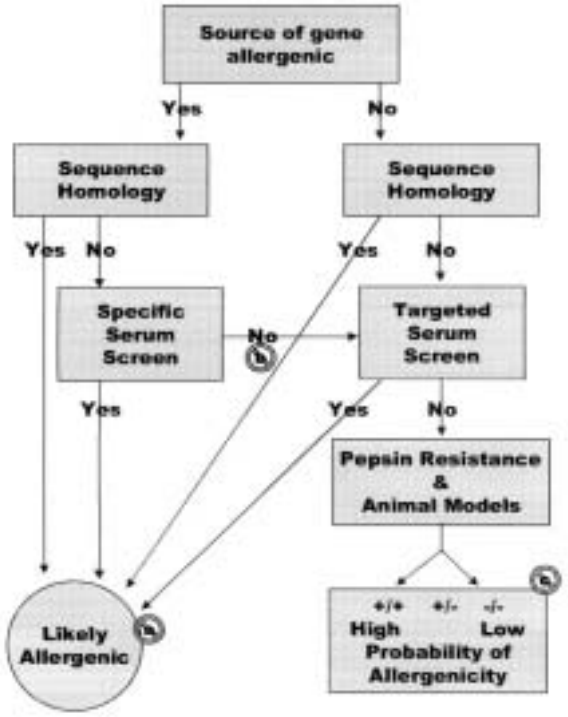
In 2001 the FAO/WHO (2001) developed and proposed a new decision tree approach for the assessment of the allergic potential of proteins (Figure 5.1). This refined previous decision trees (Metcalfe, 1996; FAO/WHO 2000). Whilst important information can be gained using this decision tree, there are divergent scientific views on the utility of some of the tests proposed (SOT, 2003; Selgrade *et al.* 2003; Haslberger, 2003). Areas of disagreement are: (a) the use of 6 vs. 8 amino acids as a trigger for identifying a potential allergen, due to the increased numbers of false positives; (b) functional similarity of proteins does not necessarily signal similarity of immunological behaviour; (c) direct assessment of sensitising proteins is not addressed. There has been considerable recent research in developing and testing of appropriate animal models (SOT, 2003; Kimber *et al.* 2003). The decision tree approach helps to structure the assessment of the allergenic potential of a donor gene product and leads to a weight of evidence estimate concerning the likelihood that a particular protein might have an allergenic potential. It does not lead to an absolute declaration of absence of allergenic potential, reflecting a precautionary approach.

The first analysis should be an assessment of amino acid sequence homology of the donor gene with genes known to produce allergens. Databases of amino acid sequences of allergenic proteins have been developed. The protein sequence of the donor gene is compared to all the proteins in the database. If sequence homology is greater than a specified level (28% identity in 80 amino acids or 6 consecutive amino acids) then the donor gene is considered to encode a potential allergen and the regulatory authorities would not approve the development of the GM crop. In practise, this method is really only able to detect similarity between known allergens and the donor gene encoded protein for linear epitopes, i.e. those dependent on the protein sequence rather than the shape of the molecules. However, this is considered an acceptable correlate since the shape of many of the proteins that we ingest in our food will be altered by boiling and/or stomach acid (*vide supra*). The denaturation of proteins that is inherent in these processes, allows only linear epitopes to remain intact when the protein enters the gut. It can also unmask linear epitopes that were not present in the non-denatured protein. In contrast, these processes will almost certainly destroy conformational epitopes.

A second assessment *in silico* is related to structural similarity. Even if the threshold level of sequence homology between the GM protein and proteins in the allergen databases, is not found, an assessment can be made of the family of proteins to which the GM protein belongs. Structurally related protein families such as lipocalins; non-specific lipid transfer proteins, napins (found in muscle and nervous tissue) and parvalbumins (found in seeds) may have a

higher probability of being an allergic protein than other proteins not part of these families (FAO/WHO, 2001). Others include in this group: seed proteins, enzyme inhibitors, profilins or defensins and pathogenesis-related proteins (Haslberger, 2003). But it is contested whether functional similarity without structural similarity is likely to result in cross-reactivity and point to an increased likelihood of allergenicity. Considerations about structural similarity may override considerations about sequence homology if the latter is found to be below the threshold level.

Figure 5.1: Assessment of the allergenic potential of foods derived from biotechnology (FAO/WHO 2001)



a: Any positive results from the *in silico* comparison and from serum screening protocols indicate that the expressed protein is likely allergenic.

b: The degree of confidence in negative results in a specific serum screen is enhanced by the examination of larger numbers of sera. When large numbers of sera are available, they should be used. The use of 50-60 sera will increase the power of the serum screen to exclude false negatives.

c: When positive results are obtained in both pepsin resistance and animal model protocols, the expressed protein has a high probability to be an allergen. When negative results are obtained in both protocols, the expressed protein is unlikely to be an allergen. When different results are obtained in the pepsin resistance and animal model protocols, the probability of allergenicity is intermediate, although rational explanation may be possible in some situations.

If the above assessments point to a low likelihood of allergenic potential for the GM protein, then three safety tests should be done including serum screens, an assessment of digestive stability of the protein and its ability to elicit allergy and IgE responses in test animals. However, there is not a universal correlation between stability in gastric fluid and allergenic potential.

The next element in the assessment of the allergenic potential of a GM protein depends on the origin of the donor gene. If the protein comes from a crop with known allergenicity to humans, a specific serum screen should be done. This involves testing the ability of IgE in serum to bind to the allergen, using sera from persons that are known to be allergic for the donor organism or gene product. It involves the evaluation of the response in 25 sera. The presence of one positive serum (> 10 kIU/L IgE) defines the product as allergenic.

If the donor gene comes from a non-allergenic source a targeted serum screen should be done using sera from people who are allergic to organisms/proteins similar to ones from which the donor gene/gene product was derived. It has been proposed that 50 sera should be used in such a screen and again that one positive result should define the GM gene product as

allergenic. Increasing the number of sera, to for example 60 or more, would increase the power to detect false negatives. For most allergens this number would be considered sufficient to detect cross-reactivity to an allergen in the human population. In these targeted serum screens again the presence of IgE that reacts with the allergenic protein is evaluated.

The test regimen for the important IgE response appears strong in its ability to detect pre-existing sensitivity in the population. It is very dependent on the availability of sera for the specific and targeted serum panels.

Concurrently with serum screens, the expressed protein should be subjected to an analysis of pepsin resistance and break down under acidic conditions. Those proteins that are digested in the human stomach and intestine and are sensitive to degradation by pepsin have been considered less likely to be allergenic, although the digestive process may also unmask allergic epitopes. The pepsin susceptibility is a relevant parameter though it is only a correlate of allergenicity since the test protocols do not mimic the complete process of gastric digestion (FAO/WHO, 2001) and many proteins that reach the small intestine intact are not allergenic.

The expressed and purified protein can then in a fourth assessment step be given to animals in order to assess its toxicity and allergenicity. Several animal models such as the Brown Norway rat have been proposed but none has been accepted as a validated routine animal testing model. They involve exposing the animals to high levels of expressed GM proteins, not just to the crop.

The assessment of potential unintended effects involves an analysis of the allergenicity of the proteins encoded at the insertion site or perturbations of the expression of natural endogenous plant allergens. Unintended effects may be separated into two groups. The first are those associated with the insertion of the gene such as insertion site effects and the second are those associated with the perturbation of the genome and consequent metabolic changes.

Unintended effects that derive from where the donor gene is inserted are not difficult to evaluate. Although the function of the proteins defined in the sequences that flank the inserted donor gene may not always be known, sequencing the insertion and the flanking areas will indicate whether fusion proteins may be formed. The allergenicity of these can in principle be assessed using the same decision tree approach as used for the donor gene. The same applies to protein products of any neighbouring genes.

For those crops in which the allergenic proteins and other components are well described it would be simple and desirable to ascertain that the insertion of the transgene has not altered the levels and/or the characteristics of the know allergenic compounds. If the host plant contains allergenic compounds, the possibility of such alterations should be evaluated.

In summary, we should probably refer back to the ‘shock’ scenario that was posited earlier, which assumes that all the normal safeguards to prevent the introduction of an allergen have failed. It also assumes that a diverse range of GM crops all based on the same gene construct are all introduced at roughly the same time, which is impractical from both the regulatory and developmental perspectives. Nevertheless, it would appear to be potentially problematic to put a single transgene encoding a novel non-food plant protein into a large number of staple crops and introduce these all at the same time. The regulatory process, in dealing with every application on a case-by-case basis, should take account of the possibility of increasing exposure to a single GM protein as in this ‘shock’ scenario. Furthermore, it may not be

desirable to introduce the same gene construct into a wide range of different crops. For example, insect resistance is conferred on crops via a family of (Cry) proteins, of which there are three classes based on their genetic similarity. Some are specific to certain classes of insects and are used in different crops because they have different insect pests. So identical Cry genes are not used for all crops.

Any risk would depend on how much of the protein is needed to achieve its desired effect and where the protein would end up in the plant. Since we consume seeds, oils, and bits of plants, including their fruits and roots, it would seem unlikely that the GM protein would be a component of the food products derived from all the crops, particularly if universal promoters for the expression of transgenes are replaced with tissue-specific ones.

Nevertheless, the threshold levels for sensitisation are not known for many foods, although the higher levels required to elicit an allergic responses are probably in the microgram range (Bindslev-Jensen *et al*. 2002), although the figure will vary considerably from one individual to another. Hence, it may not be as safe as could be achieved to rely on biological partitioning alone.

The scenario further requires that all the testing regimes have failed to pick up the allergenic potential of the protein: e.g. sequence homology, digestive stability and animal testing. The protein would have been evaluated in a targeted human serum screen from people allergic to monocots or dicots depending on whether the novel protein from a non-food plant was derived from a monocot or dicot plant. In this scenario, a larger targeted screening programme might have to be considered than the ones outlined above.

As far as cross-reactivity is concerned, it is very easy to do a simple power calculation that would suggest the size of the targeted serum screen necessary to detect the effect (serum IgE binding to the protein) if the reaction was present in a given percentage of the population, noting that the targeting of the serum screen would enhance the likelihood of detection of cross-reactivity.

At this moment it appears difficult to assess the likelihood of a false negative result in a screen of allergenic potential. However, since the four criteria that have been developed (sequence and structural homology; human serum screen; digestibility and toxic and allergic effects in animals) are assessing different parameters of the transgenic protein, this would *a priori* reduce the likelihood of false negative results in all four tests.

Finally, the scenario posits that the allergy is only detected years after the introduction of the crops. This is unlikely as it seems to be considered that the first manifestations of a new allergy will occur in pre-existing adult allergic individuals and could occur as a result of cross-reactivity (Haslberger, 2003).

5.3.5 Is the issue unique to GM?

Potential effects of modifications of crops and derived foodstuffs generated by conventional plant breeding programmes on food allergy have actually not been very thoroughly investigated. The relative expression levels of various food allergens may well have changed in conventional breeding programmes, but this has not been specifically assessed. Nevertheless, there appears to be a consensus that there are no problems associated with

conventional breeding technology, which also includes less conventional methods such as mutation breeding and embryo rescue[24].

The introduction of novel, non-GM, foods with as yet unknown allergens into human populations has been documented to be associated with the appearance of new food allergies. For example, after the introduction of kiwi fruit in the UK diet, it became clear that a fraction of the exposed population developed an allergy to it. This type of event is difficult to predict. Furthermore, it appears difficult to devise a regimen for testing this in post marketing surveillance. In any case the societal response appears to be that those who are allergic to kiwi fruits should simply try to avoid eating it. The removal of kiwi fruit from the UK diet would probably not be considered a reasonable response to the problem, because it is considered avoidable. It raises the question, though, as to what would have happened if a novel, now wide-spread, food elicited the same response.

Milling and processing of food products can also cause human allergies. There are many examples of allergies related to working in an environment high in processed food products, e.g. baker's lung etc. The Health and Safety Executive under the Health and Safety at Work Act regulate the exposure of people to allergens in such environments. Most of this exposure should be avoidable through good engineering control in the processing plant. The processing of GM food crops is in this respect no different from that of conventionally bred crops. Allergy problems with workers in processing plants may give an early warning about the allergenic potential of GM crops.

Because of the intense scrutiny to which GM crops are subjected with respect to the issue of allergenicity and the fact that usually only one or at most a few transgenes are inserted, it will be much easier to assess the allergenicity of the products of these genes. The probability that potential allergenicity will be detected is far greater than when a non-GM food is introduced or modified by conventional breeding. In the case of GM crops it will also be potentially easier to do post-marketing monitoring of allergenicity, as indicated above.

In relation to the 'shock' scenario previously discussed, GM technology is unique in the sense that a single gene construct has the potential to be placed into a diverse range of different food crops (although all crops have around 99% of their genes in common). If some of these food crops (e.g. soya) are those used extensively in the food processing industry, then the gene construct could become a common dietary constituent. However, food from a novel non-GM commodity crop with a potential risk of containing an allergen might also become quite widely consumed.

Avoidance is the main clinical response to allergy and this depends in practice on the comparative effectiveness of traceability and recall systems and the information available to the consumer (e.g. food labels) and to others. The identification and management of food safety issues is well established in UK[25] and throughout EU. Whether the avoidance of GM derived allergens would be more or less easy to achieve in this 'shock scenario', compared to the non-GM scenario was the subject of debate by the Panel. They were unable to reach a unanimous view, although, for the reasons given earlier, it was generally agreed that the scenario was highly unlikely to happen in practice.

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[24] GM Science Review website. Drobnik J. http://www.gmsciencedebate.org.uk/topics/forum/0001.htm

[25] Food Safety Act 1990. Code of Practice on Enforcement of the Food Safety Act 1990 in relation to the Food Hazard Warning System, Number 16 (revised).

It was felt by the majority of the Panel that the identification of any allergicity issue, GM or otherwise, would trigger appropriate risk management processes such as specific advice or labelling through to product phasing out or recall and that, although in the GM scenario various food crops might be involved, avoidance would be just as effective. Processes to handle recalls are already in place and are activated on a regular basis[26,27] The analysis of reports of alleged allergenicity to Cry9c insecticidal protein by the US Centres for Disease Control[28], and which found no evidence to indicate allergenic potential, is an example of how specific follow up analysis of reports of an allergic response to the product of an introduced gene may be handled in practice.

A minority view put greater significance on the potential for the GM allergen to be present in a diverse range of different crops and foodstuffs. It was thus felt that the consequences of the GM 'scenario' presented particular and important issues in the management of risk and uncertainty. And that the situation would be less easy to manage than in the non-GM case, placing greater demands on labelling (such as the identification of individual gene constructs) and requiring more extensive traceability and recall measures for effective avoidance. This does not imply an increase in the likelihood or severity of risks of novel allergens.

5.3.6 Are there gaps in our knowledge or scientific uncertainties and are these important?

The accepted safety assessment procedures are really only able to detect similarity between known allergens and the donor gene encoded protein for linear epitopes. There are important gaps in our knowledge in this area, as there are in relation to allergenicity and non-GM crops and food, and there have been examples of highly similar sequences of allergen isoforms that have been shown to lack allergenicity (Haslberger, 2003).

Predictive methods for conformational epitopes, i.e. those derived from the shape of the molecules do not exist.

There are currently no methods for the assessment of allergenic potential of small molecules or glycans but neither are there methods at present to modify specific glycans (without affecting others) by GM technology.

Our ability to predict cell-mediated immune responses that give rise to delayed type hypersensitivity is largely empirical and not open to easy testing in animals. These responses are difficult to predict or to assess on a human population basis. Our theoretical knowledge in this area is rudimentary only.

The correlation between digestibility and allergenicity has been questioned (Fu, 2002) and more standardised validated tests need to be developed before the contradictory positions between those who state that there is a correlation between these two characteristics and those who say there is not, can be resolved.

[26] http://www.food.gov.uk/enforcement/alerts
[27] http://europa.eu.int/comm/food/fs/sfp/ras_index_en.html
[28] http://www.cdc.gov/nceh/ehhe/Cry9cReport/complete.htm

5.3.7 Likely future developments

GM technology can be used to insert or silence specific genes in plants. The latter process provides an opportunity to use GM technology for targeted removal of food allergens from existing foods. Current technology allows gene expression to be inhibited[29]. This has potential for removing known allergens from foods and there are already examples of this being done successfully for the amylase/trypsin inhibitors of rice (Tada *et al*. 1996) and the Lol p5 allergen of ryegrass (Bhalla *et al*. 2001). Efforts to remove the allergen from peanuts would be beneficial to a substantial fraction of the population whose sensitivity to the protein can expose them to life threatening situations and work to this end is underway (Bannon *et al*. 2001). Although this would be beneficial, it is not simple to achieve. Peanut contains potentially more than 20 allergenic proteins. The removal of one or two of them are unlikely to make the peanut safe to eat for all peanut allergy sufferers.

It is certain that knowledge of the genomes of the main agricultural crops will increase. The rice genome has already been sequenced and others will undoubtedly follow. This will help enormously in assessing and predicting likely intended and unintended effects. The insertion sites of transgenes can then be properly evaluated and in relation to known genes.

Transcription profiling can assess unintended effect from disturbances of the genome. This will allow an assessment of the effect of the insertion on the expression of the surrounding genes. For a more comprehensive screen of the effect of the insertion of a transgene one could also develop proteomic screens, in which one would look for changes in the expression profile of various proteins after transgene insertion. The problem with both approaches is that the baseline data that would tell us whether a change in expression is significant are not available. The expression profile of proteins will vary with the crop variety and a number of other factors such as: time of year; where the crop is grown and under what management conditions; and whether it is infected with a pathogen or not.

5.3.8 Where there is important scientific uncertainty, what is the potential way forward?

Research

Our relative lack of knowledge about allergenicity suggests that we should exercise caution when assessing all new and improved foods. The factors that are important in sensitisation and eliciting an allergic response are not well understood and more research is necessary into the causes of food allergy and the mechanisms by which persons are sensitised and by which the responses are elicited. Hence it is difficult to evaluate the potential hazards in this area completely. The GM foods presently available appear not to have elicited allergic reactions, which is unsurprising as the proteins that have been added have no known history of allergenic potential.

[29] GM Science Review website. Halford NG. http://www.gmsciencedebate.org.uk/topics/forum/0044.htm

For non GM, GM foods and novel foods in general there is a need for:

- a better understanding of the factors that sensitise a person to a substance and elicit an allergic response;

- a continued improvement in the testing systems including the size and range of serum screens; and

- expansion of the allergenic protein sequence databases and the development and validation of animal models and cell-based assays.

The FSA has commissioned research in the area of improving our understanding of food allergy. The outcome of this research, which aims to reduce the likelihood that the allergenic potential of a conventional and a GM crop remains undetected, has not yet been reported.

Regulatory approach

Absolute certainty about lack of allergenicity cannot be achieved (EC, 2003) in this or any other risk assessment. The likelihood that all regulatory and safety testing procedures fail, is probably small but cannot be quantified at present as no data are available that allow us to do so. This, however, is not a unique situation in risk assessments. Absolute safety does not exist.

5.4 THE FATE OF TRANSGENIC DNA

Could transgenes (or parts of their DNA sequences) in food survive digestion and behave differently in comparison to traditional foodstuffs in their ability to relocate, recombine or modify the consumer's genome or that of associated gut microflora? If so, would this pose an increased risk to health compared to the consumption of non-GM derived food?

5.4.1 Summary

Transgenic DNA is no different from other DNA consumed as part of the normal diet and it will have a similar fate.

Food processing and ingredient extraction may remove or inactivate transgenic DNA, thereby reducing or eliminating the gene transfer risk.

DNA is degraded in the gastro-intestinal tract but the process can be incomplete.

Trans-kingdom transfer of transgenic DNA from GM plant material to bacteria is unlikely to occur due to a series of well-established barriers and this is supported by experimental evidence.

Transgenic DNA that includes homology to bacterial genomes provides a molecular mechanism for DNA recombination that has been observed in marker rescue experiments.

5.4.2 Background

The potential for transgenic DNA to be transferred from GM material following its consumption is a recognised hazard that is addressed during the safety evaluation process. It is well established that bacteria possess sophisticated processes for the acquisition and rearrangement of genetic material. These processes are important to bacterial evolution and good evidence for this in nature is provided by the development of multiple drug resistance. This has been analysed in detail and it represents a paradigm for the importance of gene flow and DNA rearrangement in bacteria. The transfer of DNA between bacteria can be achieved by several distinct mechanisms that include conjugation (mediated by direct cell to cell contact between bacteria), transduction (DNA is carried between bacteria by a bacterial plassmid) and transformation (released naked DNA is taken up by bacteria).

Whilst the existence of these processes makes gene flow amongst bacteria a significant natural phenomenon, the same is not true for the transfer of transgenic DNA from GM plant material to bacteria where a variety of natural barriers exist. In considering the safety concerns associated with the consumption of GM plant material, the possibility of plant to bacterium transfer of transgenic DNA within the human gastro-intestinal tract is generally considered to be the main concern. The only feasible mechanism for such a transfer event would be transformation of DNA released from GM plant material. In addition, the possibility that transgenic DNA (and ingested DNA from various sources) might interact with the human consumer's genomic DNA has been evaluated. Here, these possibilities and their consequences are discussed.

5.4.3 Range of views and quality of evidence

What is the effect of food processing on transgenic DNA?

The delivery of GM plant material in foods is varied in that the foods might be eaten fresh and unprocessed, as in a fruit or salad vegetable, or subject to different processing regimes. Examples of processing include the extraction and canning of tomato paste and the derivation of widely used food ingredients such as flour or oil from commodity crops. The latter represents the largest market penetration of GM food with respect to soya and maize.

Food processing and extraction of ingredients will impact on DNA, including transgenic DNA. In extracted oils it may be impossible to detect any remnant of transgenic DNA and in many other cases the DNA will be degraded. This is important with respect to gene transfer, as the presence of biologically active DNA is a prerequisite for this to be a risk issue. Size reduction of DNA fragments such that intact genes are no longer present is relevant.

Accurate data on the effects of food processing and extraction are important when considering their effect on the gene transfer risk. There are several published studies on the susceptibility of DNA to processing. Sugar purification and production of refined oils remove most, and probably all, DNA (Klein *et al*. 1998). Acid conditions accelerate thermal inactivation as has been demonstrated for the *Bacillus thuringiensis* toxin gene used in many insect tolerant GM crops (Hupfer *et al*. 1998). Published studies on DNA inactivation in foods include heat-treated pork (Ebbehoj & Thomsen 1991), processed tomatoes (Ford *et al*. 1996), heat-treated fermented sausage (Straub *et al*. 1999) and heat-treated maize flour (Hupfer *et al*. 2000). In addition, the desire to develop DNA-based detection protocols for GM food, usually based on PCR, has led to detailed investigation of remnant DNA present in a variety of target food materials.

What is the fate of transgenic DNA from GM plant material in the gastro-intestinal tract?

Transgenic DNA that is present in GM plant material will be subject to the same degradation processes as any other plant DNA. The healthy gastro-intestinal tract degrades DNA very effectively, thereby destroying intact biologically active genes. Deoxyribonuclease I produced by the salivary glands, pancreas and small intestine is a potent degradative enzyme and the low pH of the stomach acts to remove adenine and guanine residues, thereby eliminating biological activity (Beever & Kemp 2000).

Some experimental studies on the fate of DNA in the gastro-intestinal tract have been undertaken, adding data to the theoretical analysis. These experiments have involved both humans and animals. Usually, the detection of DNA is achieved by using PCR to amplify small amounts of genetic material. The biological activity of DNA has been measured by using established laboratory procedures with bacterial strains already known to be transformable.

Mercer *et al*. (1999) investigated the effect of human saliva on DNA survival *in vitro* using competitive PCR and tested biological activity by measuring transformation into the naturally

competent oral bacterium *Streptococcus gordonii* (competence is a natural process in which certain bacteria are able to take up DNA during transformation). Although DNA was degraded, sufficient biologically active DNA survived exposure to saliva to generate transformants. The frequency with which transformants were detected was reduced, reflecting the DNA degradation. Further work reported by Mercer *et al*. (2001) involved analysis of DNA degradation in the mouth of a human volunteer. This revealed a more rapid (4-fold) *in vivo* degradation process but nonetheless the potential for transformation was retained as demonstrated by *in vitro* transformation of competent *Streptococcus gordonii* cells.

Duggan *et al*. (2000) investigated DNA degradation by ovine (sheep) saliva and rumen fluid using *in vitro* experiments. They measured the biological activity of DNA using *E. coli* transformation. PCR amplification of DNA was possible for 30 minutes after exposure to rumen fluid but biological activity assessed by transforming ability was lost within one minute. In contrast, the ability to transform *E. coli* was retained even after 24 hours exposure to ovine saliva. These studies suggest that DNA may remain available for transformation in the oral cavity but is rapidly inactivated further down the gastro-intestinal tract. This work was followed up by conducting experiments in which sheep were fed GM maize and silage prepared from GM maize (Duggan *et al*. 2003). PCR amplification of a relatively large DNA fragment encoding the entire *cryIA(b)* transgene from rumen fluid was achieved 5 hours after feeding maize grains. The same target DNA was not detected after feeding silage although a smaller fragment of 211bp was amplified after 3 hours. In this paper additional *in vitro* experiments using ovine saliva were reported showing that plasmid DNA retained biological activity for 8 minutes.

The primary reason why fragments of DNA are available for uptake in the small intestine and beyond is that most of the DNA is encapsulated within a cellular matrix and so protected. This matrix is slowly degraded during intestinal transit and so intact DNA is constantly being leached out. Martin-Orue *et al.* (2002) found that DNA in food was much slower to degrade than naked DNA.

Chambers *et al*. (2002) used chicken feeding experiments to explore the *in vivo* fate of the plasmid pUC18 ampicillin resistance gene *bla* that encodes β-lactamase. Both bacteria carrying pUC18 and transgenic maize carrying the *bla* gene were studied. PCR-RFLP (restriction fragment length polymorphism) was used to differentiate the *bla* transgene from naturally occurring *bla* genes that may have been present already in the bacteria inhabiting the gastro-intestinal tract. This was possible because the pUC18 gene lacks a *Pst*I restriction site that is present in the wild type gene. The antibiotic resistance marker in GM maize was found in the crops of all five birds studied and the stomach contents of two birds, but it was not found in the lower intestine. The survival of the introduced antibiotic resistance gene was mirrored by the survival of a natural plant gene (*nad5*) emphasising the fact that transgenic DNA has the same fate as other consumed plant DNA. In contrast to the plant results, feeding bacteria that carried pUC18, led to the detection of the *bla* gene throughout the intestinal tract.

Netherwood *et al.* (2002) used a group of seven human ileostomists to monitor the survival of transgenes in GM plant material during passage through the human gastro-intestinal tract. Meals containing GM soya were used and the presence of the introduced herbicide tolerance gene for 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) was monitored using PCR amplification. In all seven subjects it was possible to detect survival of the transgene in the small intestine with a maximum recovery of 3.7%. A second trial involving human volunteers

with an intact gastro-intestinal tract was undertaken. In this case, no transgene survival was found when their faeces was monitored.

The scientific literature on DNA fate includes a series of papers that demonstrate significant persistence of DNA following its consumption. It is important to emphasise that these studies are not focused on transgenes and they are relevant to the fate of all consumed DNA. This data suggests that intact DNA may survive in the gastro-intestinal tract, cross the gut epithelium, enter the blood stream and interact with mammalian cells. Schubbert *et al*. (1994 & 1997) fed mice with bacteriophage M13mp18 DNA chosen as a test molecule that lacked homology to mouse DNA. The fate of this foreign DNA in the animals was followed using a variety of methods. Fragments of M13mp18 DNA were detected in the contents of the small intestine, cecum, large intestine, faeces and blood. It was calculated that 2–4% of orally administered DNA was detected in the gastro-intestinal tract and 0.1–0.01% was retrieved from blood. M13mp18 DNA fragments were traced by PCR to peripheral leukocytes and located by fluorescent *in situ* hybridisation (FISH) in about 1 of 1000 white blood cells between 2 and 8 hours after feeding and in spleen or liver cells up to 24 hours after feeding. M13mp18 DNA could be traced by FISH to the columnar epithelial cells, in the leukocytes, in Peyer's patches of the cecum wall, in liver cells, and in B cells, T cells, and macrophages from spleen. These findings suggest transport of DNA through the intestinal wall and Peyer's patches to peripheral blood leukocytes and into several organs. Upon extended feeding, M13mp18 DNA could be cloned from total spleen DNA into a lambda vector. Schubbert *et al*. (1998) extended this study and obtained similar results using a plasmid that expressed the gene for green fluorescent protein. They also demonstrated placental transmission to fetuses and newborn animals. This work involved the administration of purified naked DNA and more recently Hohlweg and Doerfler (2001) described a more natural scenario. The fate of the natural plant-specific gene for ribulose-1,5-biphosphate carboxylase (Rubisco) was followed in mice after feeding soybean leaves. This gene or its smaller fragments were recovered from the intestine 2 to 49 hours after feeding and from the cecum after 121 hours. These data show that plant-associated DNA survives better than naked DNA. RT-PCR was used to investigate the possible expression of the consumed plant DNA with negative results.

Other animal studies have generated data suggesting that DNA in the diet can be detected in the blood and leukocytes (Klotz & Einspanier 1998; Einspanier *et al*. 2001). This work includes experiments with feed from GM plants and in this case small fragments of natural plant chloroplast DNA were detected in the blood leukocytes of cows although there was no detection of the transgenic DNA. This result may have been influenced by the fact that the chloroplast genome is present in multiple copies per plant cell thereby increasing the copy number of chloroplast genes. In these studies and those conducted by the Doerfler laboratory, it is clear that DNA detection in areas of the body beyond the gastro-intestinal tract lumen is a natural phenomenon that does not impact on human health. Consumed transgenic DNA would have the same properties as any other DNA in the diet and equally would not impact on human health. Given that consumed DNA can be detected beyond the gastro-intestinal tract lumen, safety evaluation of transgenic DNA should consider on a case-by-case basis the potential for enhanced interaction with the human genome.

In conclusion, there is a body of experimental evidence demonstrating that the amounts of DNA consumed as a normal component of the diet are subject to degradation in the gastro-intestinal tract. This process is not 100% efficient and surviving fragments of DNA can be detected from various sites throughout the human and animal gastro-intestinal tract. There is evidence that degradation is progressively more complete during passage through the gut and

the retention of biological activity has been demonstrated in the proximal regions, notably in the oral cavity. There is evidence that DNA can move from the gastro-intestinal tract lumen to other areas of the body and this is a normal occurrence. There is no evidence that transgenic DNA behaves differently from other DNA in the diet both with respect to its survival and its fate following consumption in GM plant material.

What is the fate of transgenic DNA from GM plant material if it is taken up by bacteria in the gastro-intestinal tract?

The status of bacterial gene transfer by natural genetic transformation processes was reviewed by Lorenz and Wackernagel (1994). It is very well established that some bacterial species possess highly evolved processes that allow them to take up DNA from the environment. Under certain circumstances this can lead to the maintenance and expression of a new genetic trait. However, there are severe restrictions which limit the extent of successful bacterial transformation. The development of 'competence' in natural transformation is generally a tightly regulated process that depends on specific environmental circumstances. Bacteria produce enzyme systems (the restriction endonucleases) that differentiate and degrade incoming foreign DNA. In order to be maintained, DNA that is not degraded must be capable of DNA replication. This depends either on the presence of a genetically linked plasmid replicon that is functional in the transformed bacterial species or on an integration event. Efficient integration could occur by host controlled generalised recombination but this is dependent on the existence of DNA homology between the incoming DNA and the recipient bacterial genome (Lewin, 2000). Bacteria possess other efficient site-directed integration mechanisms but these are highly specific. At a very low frequency, maintenance as a result of an 'illegitimate' recombination event is possible. Natural transformation processes have been characterised in molecular detail for a wide variety of taxonomically distinct bacterial species. Two types of transformation machinery have been described which have components related to those found in type II and type IV secretion systems (Chen & Dubnau 2003). In these transformation processes DNA is taken into the cell as a single strand and this has implications for subsequent formation of a circular self-replicating molecule (e.g. a plasmid replicon present in a GM plant). In order to create the necessary circular molecule, more than a single copy of the DNA is needed and this is unlikely if it is presented as a linear tract of transgenic DNA sandwiched by plant DNA sequences. Thus the molecular mechanism of transformation can provide a barrier to the acquisition of a bacterial plasmid that may be present within transgenic DNA in a GM plant. In contrast, some laboratory protocols such as electroporation or calcium chloride treatment can effect very efficient plasmid transformation. Plasmid transformation of *E.coli* in calcium-containing freshwater has also been reported (Baur *et al*. 1996). In addition, it is very relevant that the microflora of the gastro-intestinal tract is not fully characterised. It includes uncharacterised bacterial species that cannot be cultured making the existence of novel mechanisms for DNA acquisition a possibility. Lastly, DNA acquired by a gastro-intestinal tract bacterium is unlikely to be of significance unless it is expressed or facilitates altered expression of other resident genes. Gene expression in bacteria depends on specific genetic signals that are not universal between species. Thus an incoming gene would either need to have a compatible promoter and ribosome binding site or it would need to be integrated into the genome in such a way that read through from a resident gene was possible.

Thus, there are significant restrictions to the expression of consumed transgenic DNA in gastro-intestinal tract bacteria. It can be predicted that DNA integration into the bacterial

genome is the greatest risk factor when considering plant to bacterium DNA transfer. This would be facilitated by DNA homology between the transgene and the recipient bacterial genome. This conclusion, based on a consideration of molecular mechanism, is supported by the experimental data on plant to bacterium DNA transfer and this is outlined below.

Experimental studies of trans-kingdom DNA transfer from GM plant material to bacteria

A limited number of experimental studies have investigated DNA transfer from GM plant material to microorganisms. Very few of these studies were directed at transfer events involving the gastro-intestinal tract and its microflora. However, data from other environments are very relevant in assessing the molecular principles involved.

Schluter *et al*. (1995) exploited the plant pathogenic species *Erwinia chrysanthemi* as a recipient when investigating the transformation of plant DNA. Erwinia causes soft rot by lysing plant tissues with extracellular pectinolytic enzymes and this provided an intimate association between plant material and the potential bacterial recipient. In these experiments, a transgenic potato carrying the bacterial plasmid pBR322 was used. *Erwinia* can support the replication of the pBR322 plasmid and it will express the plasmid antibiotic resistance genes thereby facilitating selection for transformation. Evidence for plant to bacterium transfer was not found in this study. However, a series of *in vitro* experiments were also undertaken and these provided quantitative data on the probability of plant to bacterium transfer. This was estimated as a maximum of 5.8×10^{-14} for an experiment using 0.9g of potato tuber and 6.4×10^{8} bacteria, suggesting it to be a very unlikely event.

DeVries and Wackernagel (1998) used naturally competent *Acinetobacter* and a marker rescue strategy to investigate plant to bacterium gene transfer. Marker rescue is a process in which the recipient bacterium has DNA homologous to that being transferred but is differentiated by the presence of a mutation. Successful transformation is detected by correction of the mutation as a result of homologous recombination. The plant selection marker derived from the *nptII* kanamycin-resistance gene was studied and the recipient bacteria carried an inactive homologue of the same *nptII* gene controlled by a bacterial promoter. In these experiments the incoming DNA was provided with an opportunity to be maintained by recombination with the bacterial genome. Homologous recombination between the plant-derived *nptII* gene and the mutant resident gene would repair the defect in the latter gene leading to the recovery of kanamycin-resistant transformants. Transformant detection did not depend on circular molecule formation, autonomous replication or integration by a rare illegitimate recombination event. In this experimental system, transformants were detected at a frequency of 0.9×10^{4} per *nptII* gene. If the *nptII* gene homology was removed from the *Acinetobacter* recipient, transformation fell below the 1.3×10^{-13} limit of detection. These experiments are important in demonstrating that homology between a GM plant transgene and a transformable bacterium provides an efficient mechanism for gene transfer by marker rescue. As few as 2.5×10^{3} transgenic potato cells could generate a transformant and marker rescue of the kanamycin-resistance was effective in the presence of a more than 6×10^{6}-fold excess of plant DNA. It is important to emphasise that this process depends on the provision of DNA homology and that it involves marker rescue rather than the recovery of unique DNA from the transgenic plant.

Gebhard and Smalla (1998) reported similar data on marker rescue by *Acinetobacter* in experiments using DNA from GM sugar beet. De Vries *et al*. (2001) reported similar data for transgenic potatoes using *Acinetobacter* and *Pseudomonas stutzeri*. In the absence of DNA homology to facilitate marker rescue, gene transfer was not detected and the event frequency fell by a factor greater than 10^8 or 10^9 for the two bacteria, respectively. Recent work by Kay *et al*. (2002) extended observations of marker rescue to include GM plants in which the transgene DNA was located within the chloroplast genome.

Relatively little direct experimental data on gene transfer from GM plant material to bacteria within the human or animal gastro-intestinal tract has been reported. During their investigation of DNA survival in saliva, Mercer *et al*. (2001) demonstrated that the naturally transformable oral bacterium *Streptococcus gordonii* would efficiently integrate foreign DNA into its chromosome provided that a region of DNA homology was present. During their analysis of the fate of GM soya transgenic DNA in ileostomists, Netherwood *et al*. (2002) isolated a mixed bacterial culture that gave a weak positive result for the presence of a 180bp fragment derived from the herbicide tolerance gene (5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene). This result persisted through six sub-culture rounds which would dilute any non-replicating DNA beyond the PCR detection limit. This result has been cited as evidence of horizontal transfer of consumed transgenic DNA to a gut microbe and comment from the GM Science Review Panel was specifically requested in a contribution by the Soil Association to its website[30]. The data obtained are unexpected and warrant further investigation but fall short of evidence for horizontal gene transfer. Importantly, a pure bacterial culture giving a positive PCR reaction could not be isolated and thus molecular evidence for integration of transgenic DNA into a bacterial genome was not be obtained, making interpretation of the observation difficult. Horizontal gene transfer of the *pat* gene from GM oil seed rape to *E.coli* and yeast present in the gut of young bees has been reported in the media[31], but the data have not been published or subject to peer review.

Antibiotic resistance marker genes

The introduction of a trait gene into a GM plant depends on the ability to select the transformed cells that have acquired transgenic DNA. This is achieved by the use of a marker gene that can be selected and during the development of GM technology antibiotic resistance marker genes have frequently been used. These are derived from antibiotic resistant bacteria and the *nptII* gene, which confers resistance to kanamycin and neomycin, is used frequently. The *nptII* gene was originally derived from the *Escherichia coli* transposon Tn5 but it was engineered for expression in plants using a plant-specific promoter. Safety concerns associated with the use of this marker for the construction of GM plants centre on the gene transfer risk. Use of the *nptII* gene is justified on the basis that both kanamycin and neomycin are of limited importance in the treatment of bacterial infections in humans, mainly as a consequence of their relative toxicity and the availability of safer alternative antibiotics. In addition, it is recognised that antibiotic resistance is already widespread in bacteria and rare gene transfer from a GM food source is unlikely to be of practical consequence (Nap *et al*. 1992). A comprehensive argument about the safety of *nptII* was developed by Calgene (Calgene Inc. 1990) and this is generally accepted by regulatory authorities.

[30] GM Science Review website. Soil Association. http://www.gmsciencedebate.org.uk/topics/forum/0093.htm
[31] Kaatz, University of Jena, Germany. Reported by German TV (ZDF), Sunday May 21, 2000.

In addition to *nptII*, other antibiotic resistance genes have been introduced into GM plants. The most common reason is that the trait gene was first engineered into a bacterial vector containing the antibiotic resistance genes during *E. coli* cloning before delivery to the GM plant. Such genes are not directly selectable in plants and their use is not an essential part of the GM plant construction process. Genes in this category include *bla* conferring ampicillin resistance, *aad* conferring streptomycin and spectinomycin resistance and *nptIII* conferring resistance to amikacin in addition to kanamycin and neomycin. The *aad* gene is also used as a selection marker in chloroplast transformation.

The use of antibiotic resistance genes is readily avoidable in the case of genes that are not used for direct selection. Also, there are a variety of approaches to GM plant selection that avoid or eliminate the antibiotic resistance selection marker. In the UK, ACNPF has produced advice that strongly encourages the development of alternative selection methods[32]. Alternatives to antibiotic resistance genes include selection for growth on mannose which relies on a gene for phosphomannose isomerase (Anon, 2000). Mechanisms, such as the *cre/lox* system (Dale & Ow 1991) have been developed to facilitate the removal of selection markers after GM plant construction. Other approaches involve the use of co-transformation of trait and selection genes followed by segregation of the latter. This has been effective in both *Agrobacterium* transformation (Komari *et al.* 1996) and biolistic transformation (ACNFP, 1995). In the case of chloroplast transformation, the genome has similar properties to bacteria. This should facilitate the development of marker elimination strategies based on homologous or site directed recombination.

5.4.4 Is there general scientific agreement?

There is general agreement on fate of transgenic DNA in GM plant material following its consumption. It is subject to degradation as is all DNA, but the process is not complete. Degradation is progressively more complete as it passes through the gastro-intestinal tract. Biologically active DNA is detectable in the mouth but not in the faeces.

The potential for interaction of consumed DNA within the host has been studied and there is evidence that it is detectable in the blood, leukocytes and other sites. There is general agreement that such processes are generic for all DNA and there is no suggestion that transgenic DNA behaves differently.

There is a consensus that there are a series of well-characterised biological barriers that restrict the transfer, integration and expression of transgenic DNA from GM plant material to bacteria present in the gastro-intestinal tract. Experiments designed to investigate the transfer of transgenic DNA from GM plants to bacteria have been undertaken and generated consistently negative results with one exception. If DNA homology with transgenic DNA is provided artificially in a potential recipient bacterium then evidence of marker rescue is readily obtained. Bacterial DNA in GM plants provides regions of potential DNA homology that might increase the risk of a gene transfer event taking place.

With respect to safety evaluation, it is generally agreed that the specific property of the transgenic DNA, including the trait to be expressed, is of greatest importance when

[32] ACNFP fact sheet, FSA/0550/0302.

considering gene transfer. In this regard, the use of antibiotic resistance genes in plants is controversial with differing views on the potential impact. There is a scientifically well-supported argument that any rare gene transfer event from GM plant material would have no impact as resistance is already widespread as a consequence of antibiotic and feed additive usage. Increasingly, it is clear that the presence of antibiotic resistance genes in GM plants intended for food use can be avoided and in future for new events this issue should no longer be a problem. However there can also be safety issues with alternative systems and the use of safe ARMs is an enabling technology for research workers in smaller laboratories, including those in developing countries.

The potential for transgenic DNA to be transferred from GM crops to plants and other organisms, other than by its consumption, is considered in Chapter 7.

5.4.5 Is the issue unique to GM?

There is no evidence that transgenic DNA *per se* behaves differently from any other DNA with respect to its fate following consumption in food.

The presence of bacterial DNA in GM plants is unique to GM technology and may increase the gene transfer risk. Given that this DNA is derived from the bacterial gene pool, it is questionable whether there is any overall increased risk of gene flow. It is also worth noting that wild-type *Agrobacterium* introduces bacterial DNA into plants as part of the infection process and that ancient integration and inheritance of *Agrobacterium* DNA has been found in certain tobacco species.

5.4.6 Are there gaps in our knowledge or scientific uncertainties and are these important?

The extent of direct investigation of trans-kingdom gene transfer from GM plant material to gastro-intestinal bacteria is limited. Whilst much can be concluded from general molecular biology principles and extrapolation from other experimental systems, there is a case for greater investigation within the gastro-intestinal tract *in vivo* or via appropriate models. The fact that many gastro-intestinal tract bacteria cannot be cultured *in vitro* is a relevant limiting factor.

Limited experiments in humans have generated PCR-based evidence for the persistence of transgenic DNA in mixed bacterial cultures derived from the gastro-intestinal tract. The authenticity and significance of this observation warrants further investigation.

5.4.7 Likely future developments

For the future, it will be important to evaluate the properties of new transgenes that might be used in GM plants. The emphasis of safety evaluation should be on any potential impact that might result following a rare and unexpected gene transfer event.

The use of plant organelles (chloroplasts) as sites for the introduction of transgenic DNA is of growing importance. It facilitates the use of homologous recombination to direct transgenic DNA to a predetermined site in the plant genome and it has advantages in minimising horizontal gene flow via pollen. It should be recognised that plant organelles have evolved from microorganisms and hence share similar gene expression machinery. This could increase the risk of gene expression following plant to bacterium transfer of transgenic DNA designed for organelle integration. In addition, many plant organelles are present per cell. This increases the relative copy number for transgenic DNA located in the chloroplast and it is inevitable that this will increase the risk of a horizontal gene transfer event.

5.4.8 Where there is important scientific uncertainty, what is the potential way forward?

There is limited scientific uncertainty in this area. Confidence might be enhanced by further direct investigation of gene transfer in the human gastro-intestinal tract, either directly where experimentally possible or by taking advantage of available model systems.

5.4.9 Concluding remarks

DNA is degraded during its passage through the gastro-intestinal tract but this may be incomplete. The detection of DNA movement out of the gastro-intestinal tract to the bloodstream and other parts of the body illustrates normal processes that are not of specific relevance to transgenic DNA in GM plants. There is no reason to expect transgenic DNA to behave differently to other DNA that is present in the normal diet.

Both known molecular mechanisms and experimental evidence suggest that trans-kingdom DNA transfer from GM plant material to bacteria in the gastro-intestinal tract would be a very rare event. Homology between transgenic DNA and the bacterial genome would provide the opportunity for marker rescue to take place and this has been observed experimentally.

The GM plant to bacteria gene transfer risk might be minimised by the restriction of bacterial DNA sequences in GM plants and this is an argument that supports a best practice in which unnecessary DNA sequences are eliminated (i.e. those sequences not associated directly with the expression of the desired trait in the GM plant). This represents something of a circular argument in that greatest risk is associated with bacterial sequences that are already present in the bacterial gene pool where gene flow is a much more significant natural process.

If a gene transfer event did occur its persistence would depend on it providing selective advantage to the transformed bacteria and any human impact would depend on the precise nature of expressed genetic material. This emphasises the fact that the case-by-case safety assessment of transgenic DNA is of great importance. Issues such as potential physiological effect and potential to enhance bacterial virulence are obvious considerations.

5.5 THE EFFECT OF GM DERIVED FEED IN THE FOOD CHAIN

Could the consumption of GM derived feed and crops by farm animals pose more of a health hazard to consumers of the resulting food products, or to the animals, than the use of non-GM material?

5.5.1 Summary

Both traditional plant breeding and GM techniques are being employed to produce animal feeds with enhanced value. The aim is to meet an increasing world demand for animal protein and to substitute high protein plant materials, since feeds of animal origin have been banned, specifically meat and bone meal. This Section addresses two broad concerns about the use of GM derived animal feed. Firstly, can the transgenic components of this feed be found in the resulting animal food products, enter the human food chain and affect our health? Secondly, does GM derived feed pose any more of a health concern for the livestock consuming it compared with non-GM feed?

The processing of animal feed will in some cases completely fragment the DNA, but this is often not the case and in general if GM crops are grown to feed animals these animals will be eating intact DNA, including any transgenic DNA. The vast majority of DNA and proteins are completely broken down within the animal's digestive system but it is normal for some surviving fragments of DNA to appear throughout the gastrointestinal tract. Some of these fragments can be taken up by animals and detected in the blood and internal organs. Known molecular mechanisms and experimental evidence suggest that the integration and expression of consumed DNA in gastrointestinal tract bacteria (horizontal gene transfer) would be a very rare event. Section 5.4 considered the fate of DNA in the gastrointestinal tract, and the possibility of horizontal gene transfer, in more detail. In summary, there is no evidence that transgenic DNA and novel proteins behave differently from other DNA and proteins in the diet both with respect to their survival and ultimate fate following consumption in GM plant material.

Studies on thousands of animals in recent years have found no adverse effects on animal health or productivity as a result of the use of GM feed compared to the non-GM equivalent, and no detectable difference in the animal products or adverse effects from their consumption. Many hundreds of millions of people have been eating food derived from GM fed animals as a significant proportion of their diet for up to seven years with no substantiated adverse effects reported. This provides confidence in the technology, but it does not mean that adverse effects can be ruled out: they may for example be too mild to detect, have a very low incidence or a long gestation period.

We have identified three future trends of significance for GM animal feed:

- the development of more GM crop plants with enhanced value as animal feed, e.g. improved digestibility, reduced pollution, enhanced nutrition, and increased protein;

- the appearance of an increasing number of crop plants that each contain a number of transgenes (gene stacking); and

- the development of GM crops, traditionally used for feed purposes, to produce biologically active proteins and peptides for medical and veterinary use or other products for industrial use.

In areas of scientific uncertainty we have identified a number of ways forward. In particular, the need for the relevant UK and EU regulatory bodies and their scientific committees to ensure that there are effective methods to assess the safety of new developments in the technology.

5.5.2 Background

The global population is expected to increase from six billion today to approximately 7.5 billion by 2020[33] and around 9 billion by 2050. With increasing population in developing countries comes urbanization. This in turn drives an increased demand for meat partly due to improved economic standards. IFPRI have shown (Delgado *et al*. 1999) that this change in population and demography may require a doubling of animal protein production with a corresponding doubling of demand for feed grain (Persley, 2000). In addition, ingredients of animal origin such as meat and bone meal used to provide much of the protein, and DNA, in the diet for farm animals. With the banning of these ingredients, high-protein plant materials are now of greater importance than before. However, their amino acid composition tends to be imbalanced for some animals, or developmental stages of animals, and genetically engineering crop plants to increase the proportion of some amino acids, particularly lysine in cereals and methionine in legumes, is an attractive proposition.

In consequence, changes in the type and quality of nutrients in specific crops and the impact of these to optimise food conversion efficiency of animal feed to milk, meat and eggs is now a high priority. Both traditional plant breeding and GM techniques are being employed to produce grains with enhanced value for animal feeds. The next commercial wave of nutritionally enhanced crops will focus on improved feeding value related to protein quality (better balance of amino acids), digestibility (fibre and starch) and metabolisable energy (oil). Nutritionally enhanced feed stuffs will also address anti-nutrients such as phytate, protease inhibitors and tannins that affect digestibility and feed value (Cockburn & Phipps 2003). Increasing the utilisation of nutrients also has the benefit of reducing soil and water pollution with manure (and in particular phosphate).

5.5.3 Range of views and quality of evidence

The Issues

Two broad concerns about the use of GM derived animal feed have been voiced by the public and have been the focus for considering this issue under the Review. Firstly, can the transgenes, transgenic DNA and novel proteins in this feed be found in the resulting food products and enter the human food chain? And if they can, what is the significance of this in terms of human health effects? The main food products of interest are eggs, milk and meat,

[33] GM Science Review website. UN. http://www.un.org/esa/population/publications/wpp2002/WPP2002-HIGHLIGHTSrev1.PDF

although there are others such as farmed fish and honey. Secondly, does GM derived feed pose any more of a health concern for the livestock consuming it compared with non-GM feed? One of our Open Meetings also raised a subsidiary concern over the health of livestock if they strayed and consumed GM crops not intended for use as feed.

These issues have much in common, in terms of the science and the health and safety concerns, as others in this report which address GM derived food safety in relation to nutrition, allergenicity, toxicity and horizontal gene flow. In this section we have aimed to focus on aspects specific to GM derived animal feed and animal products but there is inevitably some overlap.

The main exposure route considered in this section is via the gastrointestinal tract following ingestion of foods, but exposure could also occur via the lungs or eyes (e.g. contact with pollen or dust during processing), or through skin contact (during handling). Occupational exposure to allergens was considered in Section 5.3. It was also noted by the Royal Society in its report (Royal Society, 2002), which emphasised the importance of including all exposure routes in any risk analysis of the allergenic potential of GM (and other) plants.

The main focus for discussion of these feed issues under the Review was the Open Meeting on 'GM Animal Feed: Safety Implications for the Food Chain'[34]. In addition, some of the discussion from the Open Meetings on 'GM Food Safety'[35] and 'Gene Flow'[36] are relevant. Concerns raised in contributions to the Review website covered: the limitations of animal feeding studies and the possibility of animal DNA being incorporated into GM crops entering the food chain. Some questions in the report on the 'Review of Public Concerns' raised concerns over the perceived risks to health associated with GM food, but there was no specific mention of animal feed.

The effects of feed processing

Animal feeds are produced in a variety of ways. For example, oil is extracted from rapeseed to create meal, crops are made into silage and grains are heat-treated. In many cases, raw plant material is simply fed to animals without any processing. In addition, a range of by-products and residues from the brewing industry, and processing for human food are used as animal feed. (The effect of food processing on transgenic DNA was considered in Section 5.4)

In order to enter the food chain, transgenic DNA in processed feed would need to remain sufficiently intact. Fragments of DNA smaller than 200 base pairs are generally considered to be too small to transmit genetic information. Research has revealed varying degrees of DNA fragmentation as a result of feed processing. For example, the DNA remains largely intact in raw plant material and some silage, whereas subjecting wheat grains to 95°C for at least five minutes completely fragments the DNA. In general, in feed that has undergone heat processing, chemical expulsion or extrusion DNA is degraded to the point that it can no longer act as a source of functional genes (MAFF, 1998 & 2000; Forbes *et al.* 1998). We

[34] GM Science Review Open Meeting: 'GM Animal Feed: Safety Implications for the Food Chain'. http://www.gmsciencedebate.org.uk/meetings/default.htm
[35] GM Science Review Open Meeting. 'GM Food Safety'. http://www.gmsciencedebate.org.uk/meetings/default.htm
[36] GM Science Review Open Meeting: 'Gene Flow'. http://www.gmsciencedebate.org.uk/meetings/default.htm

conclude that some animal feed processing may fragment transgenic DNA to the point where it looses all functional integrity, but in many animal feeds the transgenic DNA may not be fragmented at all.

Livestock can stray and consume neighbouring crops, which might include GM crops not intended for use as feed, at least in their unprocessed form. We do not feel that this is likely to lead to novel proteins entering the human food chain, because of the various very high barriers to the transfer of proteins from an animal's diet to human food and to the human consumer. Livestock will of course also stray and eat a range of hazardous hedgerow and garden plants and crops, for example cows eating high erucic acid rape.

The survival of transgenes, transgenic DNA fragments and novel proteins in animals

Humans and livestock consume large quantities of DNA as a normal component of their diets. Typically, a dairy cow might consume as much as 24kg of dry matter per day. If on a 60% GM maize ration, it is estimated that it is consuming just under 60 grams of DNA per day, only 54 micrograms of which would be transgenic. In order to determine if any transgenic DNA or novel proteins consumed by farm animals have the potential to affect animal or human health we need to consider the fate of these molecules, and their non-transgenic counterparts, within the animal.

This fate of transgenic DNA from GM plants in the gastro-intestinal tract was considered in Section 5.4.3. In summary, it was concluded that DNA is progressively degraded as it passes through the gut, but that this process is not 100% efficient and some surviving fragments can be found in decreasing amounts throughout its length and in some other areas of the body. Transgenic DNA appears to be no different to other DNA in this respect. There are significant barriers to the integration and expression of consumed transgenic DNA in gastrointestinal tract bacteria, suggesting that this trans-kingdom DNA transfer would be a very rare event. Homology between transgenic DNA and the bacterial genome would provide the opportunity for marker rescue to take place and this has been observed experimentally.

Within bacteria, low concentrations of antibiotics, and certain other substances, are known to initiate or stimulate antibiotic resistance gene transfer and expression (Salyers & Shoemaker 1996). For example in the case of conjugative transposons, tetracycline has been found to enhance transfer frequencies by up to 100-fold (Salyers & Shoemaker 1995). Whilst these are examples involving highly evolved bacterial genetic elements, there is a concern that the use of low-dose antibiotic supplements in animal feed could provide a selection pressure and increase the risk of trans-kingdom gene transfer from GM plant material to microbes in the animal gut. More research is required, targeted at animals receiving therapeutic antibiotics. Antibiotics used as growth promoters are being phased out and those that are still licensed are unlikely to apply a selection pressure directly upon the marker genes currently used in GM plants.

There appears to be a very low probability, for a normal gut, that proteins expressed from a transgene in GM feed (or non-transgenic proteins) would enter into an animal and then into the human food chain. The proteins, or substantially sized fragments thereof, would need to survive digestion and enter the animal's circulation where they would be subject to immune attack and degradation. *In vitro* digestion studies show that most plant proteins are relatively

unstable when exposed to simulated gastric fluid (Astwood *et al*. 1996). Research on the *in vitro* degradation of a transgenic protein (*Pat*) showed nearly complete digestion within five minutes in the presence of pepsin (Wehrmann *et al*. 1996). The vast majority of proteins are rapidly degraded *in vitro* and *in vivo,* although a few (e.g. some seed lectins) are resistant and will survive gut transit relatively intact. A small proportion may be taken up intact and appear in the blood stream, although this is likely to trigger an immune response. Thus, while it theoretically possible that an intact protein could be transferred to eggs or to milk, this is an extra-ordinarily remote possibility. The role of proteins and peptides in allergenicity and the limitations of degradation analysis were discussed in Section 5.3.

A number of studies have been unable to find transgenic DNA or its gene products, or any other detectable difference, in milk, meat and eggs produced from animals receiving GM feed (Faust, 2000; Phipps & Beever 2002; Phipps *et al*. 2001)[37,38]. Since some DNA fragments from feed have been detected in the blood and internal organs of animals, and transgenic DNA is expected to behave in the same way as any other DNA, more sensitive detection methods would be expected to find transgenic DNA fragments in the blood and internal organs. In summary, there is no evidence that transgenic DNA and novel proteins behave differently from other DNA and proteins in the diet both with respect to their survival and ultimate fate following consumption in GM plant material.

Effects on animal and human health

Animal feeding studies, including toxicity testing, were considered in Section 5.2 in relation to the overall safety assessment of GM food, rather than GM feed. There have been many scientific studies, particularly in recent years, involving thousands of pigs and poultry and hundreds of beef and dairy cattle where no evidence has been found for adverse effects on animal health, in terms of performance, as a result of the use of GM feed containing herbicide tolerant or Bt constructs. Food and feed safety studies have considered animal feed safety and nutrition, animal productivity and quality, comparability of animal products and reproduction (Hammond *et al*. 1996; Clark & Ipharraguerre 2000)[31,39].

It is the sensitivity and statistical power of these studies that is important in achieving the desired endpoint rather than their size. Food animals are a very specialised population, rarely surviving for more than a small part of their natural lifespan. As such, they may be a sensitive indicator of the adverse effects of feed, since any impact on growth or breeding performance would be immediately picked up. On the other hand, any chronic effects of consumption of feed and any interactions with age-related disease would be difficult to identify. There is a lack of long-term studies in this area.

Compositional analysis has in the vast majority of cases failed to show any significant unintended difference between the GM feed ingredient and its conventional comparator. In one of the comparative feeding studies, the recorded difference in animal response was attributed to the measured difference in the concentration of mycotoxins present in the two feeds. There have now been several European studies (Brake & Vlachos 1998; Munkvold & Hellmich 1999; Valenta *et al*. 2001) in which field infestations (particularly with fusaria) and

[37] GM Science Review website. Monsanto. http://www.gmsciencedebate.org.uk/topics/forum/0077.htm
[38] GM Science Review website. Monsanto. http://www.gmsciencedebate.org.uk/topics/forum/0061.htm
[39] GM Science Review website. Halford NG. http://www.gmsciencedebate.org.uk/topics/forum/0048.htm

concentrations of mycotoxins present have been shown to be significantly reduced in *Btk* plants compared to conventional lines.

In terms of human health, many hundreds of millions of people have been eating food derived from GM fed animals as a significant proportion of their diet for up to seven years with no substantiated adverse effects reported. This is also the case for GM food not derived from GM-fed animals, and safety assessment criteria such as nutrition, toxicity and allergenicity were considered for all GM food in Sections 5.2 and 5.3.

This record gives us some confidence in the safety of GM food of animal origin. But as with animals and GM-feed, the absence of reported adverse effects does not mean that they can be completely ruled out. It just means that any impact is below the sensitivity of any epidemiological data, and not so acute as to be able to be directly linked to cause. In other words, epidemiology cannot prove a negative, especially without a defined endpoint to a study. However, the same problems arise in relation to the safety on non-GM food. There was some discussion of the detection of rare adverse events at the Open Meeting on 'GM Food Safety'[40].

5.5.4 Is there general scientific agreement?

The extent of DNA fragmentation reported by different research groups as a result of the same type of feed processing does appear to differ, but this may be because of detailed differences in the processing conditions. For example, processed transgenic oilseed rape meal was still found to contain significant amounts of high molecular weight transgenic DNA (Alexander *et al*. 2002) , but others (Chiter *et al*. 2000) have reported its complete degradation after processing. However, since in many types of animal feed the DNA will not be fragmented at all, it will generally be the case that if GM crops are grown to feed animals they will be eating largely intact transgenic DNA.

There is general scientific agreement that transgenic DNA and novel proteins behave in the same way as other DNA and proteins in the diet, both with respect to their survival and ultimate fate following consumption in GM plant material.

There is general scientific agreement on the lack of evidence of adverse effects on animal health as a result of the use of GM feed. There are no substantiated reports of adverse effects in terms of human health from the consumption of products from animals fed on GM derived feed. However as previously discussed, the absence of evidence (at least in the short term) should not be treated as evidence of the absence of harm.

5.5.5 Is the issue unique to GM?

No, in that animals eat large quantities of DNA and protein from a range of external sources and at one level, if transgenic DNA is broken down into non-functional DNA fragments then its origin is irrelevant as it all contains the same four nucleotides as in non-GM food. An animal's diet will also include some DNA from any contaminating microbes and viruses in

[40] GM Science Review Open Meeting: 'GM Food Safety'. http://www.gmsciencedebate.org.uk/meetings/default.htm

their food and DNA from their own gastrointestinal microbial flora and from their own bodies. Most of the transgenes currently used in plants were already present in the environment. For example, farm animals will ingest some soil, which will contain *Bacillus thuringiensis* (Bt), some strains of which produce insecticidal toxins**.**

So, given this long history of varied DNA consumption by farm animals and humans, is the ingestion of transgenic DNA that different in terms of risk to human and animal health? Any untoward consequences would probably be due to ingestion and transmission of intact autonomous genetic elements. The integration and expression of consumed DNA would be a very rare event, although over evolutionary timescales there is evidence of gene transfer events (Kidwell, 1993; Capy *et al*. 1994; Luo *et al*. 1998; Schouten *et al*. 1998). For example, a number of genes of apparent microbial origin, and not associated with mitochondrial function, have been identified in the human genome. These have a high homology with the genes found in mycoplasmas, which are intracellular parasites. Similarly, a novel DNA sequence might be incorporated into gastrointestinal microbial flora and persist and deliver a new product into its surroundings. But this has occurred throughout mammalian evolution (Stanhope *et al*. 2001). Horizontal gene transfer was addressed more fully in Section 5.4.

5.5.6 Are there gaps in our knowledge or scientific uncertainties and are these important?

Safety assessment of novel GM and non-GM feed is of course not absolutely foolproof. But existing methods substantially reduce the probability of any unintended and deleterious effect escaping detection. These methods will need to continue to evolve to keep pace with developments in the technology. Whilst existing evidence on animal and human health indicates a lack of adverse effects, there is uncertainty about the extent of any hidden adverse effects which might be too mild to detect, have a very low incidence or a long gestation period.

An important development in terms of safety assessment is the production of crops with significantly altered nutritional qualities, either in their gross composition or modified bioavailability. At present compositional similarity is taken as indicating that any historical knowledge of assumed safe use can be applied and any safety assessment geared to the novel components. But where there are clear compositional differences, history of assumed safe use no longer applies and other measures are needed. Simple ‘wholesomeness’ trials are for most species relatively insensitive. The natural variation present may be sufficient to mask any (chronic) effects, particularly in short term studies.

There are limitations to the PCR technology which make low-level detection and general quantification of plant DNA in animals difficult, particularly plant nuclear DNA, and where improvements would help to remove scientific uncertainty. It is difficult to quantify the amount of DNA present in the gastrointestinal tract because the biological fluids involved are very inhibitory to the PCR reactions. Variable extraction efficiencies mean that only rough, relative measures can be taken. However, some of these difficulties can be overcome by use of appropriate protocols. It is possible to detect chloroplastic DNA fragments in animals because of their relative abundance in animal feed but it is not possible to detect copies of nuclear DNA fragments in the same sample. It is nearly impossible with current technology to trace the fate of DNA in human subjects. If plant DNA is of interest, human subjects would have to eat a large amount of plant material for several weeks. Further, the human genome has

much in common with that of other organisms and if homologous sequences in plant and animal food are of interest, the subject's own DNA can interfere, making precise detection impossible.

Research does not appear to have been carried out on animals under different levels of stress. It has been suggested that the gut of diseased animals or those stressed just prior to slaughter is more permeable. This is relevant to the ability of DNA to pass into the circulatory system and also of much broader relevance, for example to pathogen shedding.

Little is known about the biological activity of DNA recovered from silage. Free chromosomal DNA was rapidly degraded when added to silage effluent (Duggan *et al*. 2000), but DNA contained within the plant tissue is differently protected and tests on its biological activity still need to be carried out.

See also, crops to produce biologically active proteins and peptides and gene stacking in the next section.

5.5.7 Likely future developments

An increasing number of crop plants will be developed with enhanced value as animal feed, e.g. improved digestibility, reduced pollution, enhanced nutrition, increased protein.

Some combined herbicide tolerant and insect resistant GM crops are now in use, produced by the crossbreeding of GM plants with each of the individual traits. In the future, we are likely to see increased development of crop plants containing a number of transgenes using this 'gene stacking'. It would make economic sense in the production of nutritionally enhanced animal feed, but it raises the question of whether this approach is more risk-prone. Would there be interactions between the various transgenes that were inherently different from the interactions with, and between, the thousands of other genes in the crop, both at the genetic and metabolic levels? One would expect less chance of interactions where the transgenes conferred quite different attributes from one another (e.g. herbicide resistance and increased lysine content). This seems to be an area where there is a lack of scientific knowledge.

A new class of GM crops under development are designed to produce biologically active proteins and peptides for medical and veterinary use (or other products for industrial use), for example edible vaccines. These crops would have animal health and welfare benefits, including reduction of disease in intensive agricultural systems. They could also become an important new crop for specialist growers. It was reported by Dr Fleming[41] that functional antigens had been produced experimentally in transgenic plants. These were not readily broken down in the gut and even small amounts of ingested antigen appeared to be effective in creating an immunological response. A potential risk is that the antigens present in the plant material may have an unanticipated detrimental effect on the animals eating them. A further risk is that by-products of industrial crops might enter the food chain, particularly when comparable by-products from conventional lines are a normal feed ingredient. However, these new GM products would be subject to a safety assessment process and, in relation to antigens or other pharmaceutical proteins, the same risk would apply to non-GM sources.

[41] GM Science Review Open Meeting. 'GM Animal Feed: Safety Implications for the Food Chain'. http://www.gmsciencedebate.org.uk/meetings/default.htm

5.5.8 Where there is important scientific uncertainty, what is the potential way forward?

Research

The following research issues have been identified:

- studies of animals under different levels of stress (e.g. effect on gut permeability);
- the biological activity of DNA recovered from silage and the fate of GM silage in animals - initial studies on the latter have not been as clear cut as other animal feeding experiments;
- the interaction of multiple transgenes in a single crop plant and the implications for animal feed.

Regulatory approach

Studies indicate that the use of existing GM feeds (particularly those containing the herbicide tolerance and Bt traits) do not compromise the welfare of the animal or result in compositional changes to animal products. However, the development of new constructs in GM crops will still need rigorous testing. Future testing may be more complex and more sensitive as refinements in testing procedures are made, but there seems to be no evidence that it needs to be inherently different.

The safety assessment of crops with significantly altered nutritional qualities will need careful consideration where there may be no historical knowledge of assumed safe use. Testing for safety where there are multiple transgenes in one GM crop is not necessarily any more difficult in principle but it may be more complicated. At present there is a lack of information about any undesirable immunological consequences that might be associated with edible vaccines. Testing for secondary effects is currently being developed with the aim of working out regulatory schemes for certification in the USA. The relevant UK and EU regulatory bodies and their scientific committees will need to ensure that there are effective methods to assess the safety of these new developments.

Chapter 6

ENVIRONMENTAL IMPACTS OF GM CROPS

6.1 INTRODUCTION

This chapter of the GM Science Review report considers the state of our current scientific knowledge on the issues of public and professional concern associated with how GM plants behave in the environment and the impacts they may have. The focus is on the possible direct and indirect environmental impacts arising from the GM crops themselves and not other crop varieties or related plants that might have acquired the transgene as a result of gene flow. Gene flow mediated impacts are covered in Chapter 7 on Gene Flow.

Public concerns about GM were reflected in the report of the 'Review of Public Concerns', produced as a result of a series of 'foundation discussion workshops' conducted by Corr Willbourn Research and Development under the GM Public Debate strand of the GM Dialogue.

More specifically, issues related to the Environmental Impacts of GM crops were raised under the Review at the various Open meetings, as contributions to the Review website, and by GM Science Review Panel members at their meetings.

Seven key areas were identified and are considered in this chapter.

6.2 Invasiveness/ Persistence
Could GM plants be invasive or persistent, and what might be the impacts?

6.3 Toxicity to Wildlife
Could GM plants be toxic to wildlife, and what might be the impacts?

6.4 Development of Resistance
Could crops engineered with novel resistance genes lead to the emergence of new forms of pests, diseases and weeds that are resistant to chemical sprays? Will new forms of insects and diseases evolve which are able to bypass GM resistance genes?

6.5 Changes in weed control strategies
Will herbicide tolerant crops offer new weed control strategies and, if so, what are the likely impacts, positive and negative? What are the real benefits of HT crops, and what will their effect on biodiversity be?

6.6 Horizon Scanning
Apart from HT crops what are the traits that might give rise to significant environment impacts, positive or negative?

6.7 Changes to agricultural practices

Might GM crops significantly change agricultural practice in the UK? If so, what might be the consequences?

6.8 Limitations of Science

Is the science available to predict the environmental impacts of GM plants?

6.2 INVASIVENESS / PERSISTENCE OF GM PLANTS

Could GM plants become invasive or persistent and what might be the impacts?

6.2.1. Summary

There is a conjectural risk that genetically modified crop plants might be more invasive of natural habitats than their conventional counterparts. Not withstanding the case-by-case approach taken by the regulatory authorities in evaluating invasiveness, there are two principal models that have been influential in considering the potential for GM crops to become more invasive of natural habitats than their conventional counterparts. One is the Alien Species Model. The hypothesis is that roughly 0.1% of introduced GM plants would become pests, because that was the rate of invasive alien plants species (c. 15 problem plants out of an estimated 15,000 species introduced). The other is the Crop Model, which argues that GM crops will behave in much the same way as conventional crop plants except for the GM trait that may influence fitness.

Evidence from the PROSAMO[1] experiments indicates that the Alien Species Model may provide a poor estimate of the probability of the GM crops used in the experiments becoming invasive. Well replicated field experiments on GM HT oilseed rape, sugar beet, and maize, and GM insect resistant potato showed that these GM plants were not more invasive or more persistent than their conventional counterparts. This suggests that the crop model is likely to be more predictive of the behaviour of the GM plants used in these experiments than the Alien Species Model.

Therefore, for some GM crops and constructs the probability of a problem arising is lower, and the environmental consequences are less severe than predicted by the Alien Species Model. However, in future, it is likely that the trend in transgenics will be to produce crops that are better adapted to biotic and abiotic conditions found on agricultural landscapes. These crops will need less human intervention to survive and thrive. By definition, because they are better adapted to harsher environments, they may be more able to persist and become invasive. The probability of invasion might be expected to be closer to the Alien Species Model. However, many domesticated crops are selected for traits that give them a disadvantage in the wild (e.g. big seeds, non-dehiscing pods, high nutrient requirements) which may limit their fitness outside cultivation.

For unfamiliar crops and constructs, invasiveness needs to be examined on a case-by-case basis, and the only reliable evidence is likely to come from field experiments.

6.2.2 Background

The alien species concept on invasiveness has a long history (Elton, 1958), and the consequences of plant invasions are well documented (Drake *et al.* 1989; Pysek *et al.* 1995; Simberloff *et al.* 1997). The Crop Model is a more recent concept, and was developed in the context of the PROSAMO experiment (Planned Release of Selected and Modified

[1] Planned Release of Selected and Modified Organisms (PROSAMO). These experiments studied genetically modified rape, maize and sugar beet that were herbicide tolerant and potatoes that expressed the insecticidal *Bt* gene.

Organisms) which compared the ecology of conventional and GM HT rape, maize and sugar potatoes that expressed the insecticidal *Bt* gene in a range of natural habitats (Crawley *et al.* 1993, 2001). The Crop Model assumes that some GM crops, especially those that exhibit traits that would not be expected to increase fitness in semi-natural habitats, behave like the non-GM crop with respect to invasiveness. Concerns regarding invasive species were the subject of one website contribution [2]. This subject was also addressed at the Royal Society meeting [3].

The relationship between the biological traits of a plant species and the likelihood that a species becomes invasive when introduced into a new habitat is complex, and all the evidence suggests that invasive potential cannot be predicted on the basis of traits alone (all plant species are capable of rapid increase in abundance under the right conditions; Crawley *et al.* 1996). The only reliable predictor of whether or not an introduced species will become invasive is whether it is known to have been invasive in other places (Veltman *et al.* 1996). There was a view that weediness was predictable on the basis of plant traits. For example, the attributes of the 'ideal weed' were listed (Baker, 1965), but it turns out that the traits of weediness identified by Baker have absolutely no power in predicting whether or not a species will be invasive when it is introduced into a new environment (Williamson, 1993).

6.2.3 Range of Views and Quality of Evidence

The PROSAMO programme studied genetically modified rape, maize and sugar beet that were herbicide tolerant and potatoes that expressed the insecticidal *Bt* gene. The survival was shown to be about 3%. After 10 years, there were no rape plants remaining. Maize never survived longer than a year and the longest-lived sugar beet was 2 years (Crawley *et al.* 2001).

For a plant to increase from a low frequency to become persistent or invasive in a non-target habitat, it must go through several stages. It must first escape from the location where it is cultivated, become established and survive to reproductive stage, producing viable seeds or vegetative propagules that form a second generation. For the population to increase in abundance they must leave, on average, one mature descendent. These stages are considered below.

Presence of GM plants outside arable fields

Is the mere presence of an individual GM plant a problem?

Yes, if it was a source of noxious products (pollen, seed, leaves, allelopathic or toxic chemicals) or attracted to it and subsequently harmed beneficial organisms (e.g. toxic foliage or nectar, tainted pollen). But there is no evidence for such effects in GM HT crops studied so far.

Many plants with noxious properties are grown without harm to people or the environment in agriculture, gardens and arboreta, and several familiar plants have poisonous seeds, leaves or fruits (laburnum, potato, rhubarb, etc.).

[2] GM Science Review Website. Cates 2003 http://www.gmsciencedebate.org.uk/topics/forum/0015.htm
[3] Royal Society meeting. Crawley 2003 http://www.gmsciencedebate.org.uk/meetings/pdf/110203-speakers.pdf

Introduction of seeds or vegetative propagules

What is the chance of GM plants escaping into non-target habitats?

Escape of plants from cultivation or from spillage in transit is almost certain to occur. Most crop plants are recorded outside arable fields in most parts of Britain (Crawley, 1987).

GM plants are likely to be of concern only if they increase in abundance once they have arrived at a location. However, constant recruitment does have the ability to produce large populations. Large ephemeral populations from seed transport and spillage could produce problems in agricultural landscape e.g. weed beet populations are proving problematic for growers and breeders in France and the Czech Republic (Bartsch *et al*. 1999; Soukup *et al*. 2002). The mere fact of increasing in abundance does not necessarily constitute a "problem" if there are no negative impacts associated with this increase.

Establishment of first generation individuals from these propagules

Will GM escapes become established?

It depends upon the habitat into which they are introduced. The Parable of the Sower (Matthew 13:4) is worth recalling here: “some seeds fell by the wayside, and the fowls came and devoured them up: Some fell upon stony places, where they had not much earth: and forthwith they sprung up, because they had no deepness of earth: And when the sun was up they were scorched; and because they had no root they withered away. And some fell among thorns; and the thorns sprung up, and choked them: But others fell into good ground, and brought forth fruit, some an hundred fold, some sixty fold, some thirty fold”. This catalogues the hazards facing a seed (predation, unsuitable microsites, plant competition). A major problem with predicting invasiveness is the difficulty of predicting which (if any) habitat an invader will colonise, so assessing the performance of a novel phenotype (which is always relative to a habitat/community) in the context of invasiveness is almost impossible with our present state of ecological knowledge.

Establishment is more likely in some habitats than others and in some successional stages. In the UK, seedlings of crop plants are common on disturbed open ground in towns, in arable land and on open roadsides, and rare or absent in closed grasslands and woodlands. Early successional habitats, with much open ground, and typically low levels of interspecific plant competition, are more likely to support crop seedlings than late successional closed vegetation. There may be GM plants in future (e.g. trees) with seeds sufficiently large that they are capable of establishment in late successional vegetation, but current GM crops show no such tendencies (Stace, 1997). In the future, GM herbicide tolerant or high yielding grasses could be capable of invading semi-natural grasslands (pasture, golf-courses, parks) and possibly other habitats, especially if they exhibited small increases in fitness because specific herbicides were used periodically on these areas.

Survival to reproductive age or size

Will GM seedlings grow to reproductive size?

This depends almost entirely on the habitat in which the seedlings are established. The greatest threat to the young plant is competition from established plants (shading, exploitation of soil water or nutrients). Plants surviving competition may be eaten by herbivores and either killed directly, weakened so that they succumb to plant competition, or kept at a size below the threshold for reproduction.

In the PROSAMO experiments, GM plants of oilseed rape, sugar beet, potato and maize all grew to reproductive size in one or more of the 12 natural habitats (woodlands, grassland, waste ground, heathland, wetland etc.) distributed over Britain (Crawley *et al*. 2001). It is possible therefore for some escaped GM plants to grow large enough to reproduce, at least in some habitats.

Reproduction (production of seed or vegetative propagules)

Will GM plants produce viable seeds or vegetative propagules outside arable cultivation?

In addition to the ecological effects mentioned above, reproduction may require the presence of other individuals to ensure cross pollination for plants that are not self compatible. In addition, pollination may require the services of more or less specialized pollinating animals (e.g. bees or moths). Absence or shortage of such mutualists might reduce the rate of seed production per plant.

In the PROSAMO experiments, GM HT plants of oilseed rape, sugar beet and insect resistant potato produced viable seed in one or more of the 12 natural habitats distributed over Britain (Crawley *et al.* 2001), but GM HT maize did not produce viable seed at any of the locations. Nevertheless, the evidence suggests that we should assume that GM crops would produce viable seed or vegetative propagules (e.g. potato tubers), at least in some habitats.

Dispersal and recruitment

Will GM plants form a second generation by dispersal and recruitment from escaped parent plants?

Just as introduced seed can produce recruits (see above) then so, in principle, could seed dispersed from established escapes.

In the PROSAMO experiments, GM HT plants of oilseed rape and sugar beet produced second-generation plants in one or more of the 12 natural habitats distributed over Britain (Crawley *et al.* 2001), but GM insect resistant potato and HT maize did not. The evidence suggests that we should assume that at least some GM crops will produce second generation plants following escape from agriculture, at least in some habitats. The key point is the number of such second generation (and subsequent generation) plants produced per parent plant (see below).

Formation of a self replacing population

Will GM plants increase in abundance following escape from arable culture?

All plants exhibit the potential to increase in abundance under appropriate conditions, “some an hundred fold, some sixty fold, some thirty fold”. The ability to increase when rare is a fundamental ecological trait, known as the “invasion criterion”. Technically, it requires that population change must be positive when plant density is low. We would not expect a large population of plants to go on increasing (e.g. because of competition for space), so there is no requirement for increase in large populations.

Will escaped GM plants leave more than one mature descendent on average (i.e. will populations tend to increase in abundance)?

In the PROSAMO experiments, none of the GM plants of oilseed rape, sugar beet, potato or maize increased in abundance in any of the 12 natural habitats distributed over Britain (Crawley *et al.* 2001). All the GM crops (and their conventional counterparts) failed the invasion criterion, and declined to extinction within 1 – 4 years (non-GM potato survived more than 10 years at one site). In all cases, failure to pass the invasion criterion was due to the combined effects of plant competition and herbivore attack. Thus, while it is possible in principle for GM crop plants to increase in abundance following escape from arable cultivation, the evidence suggests that this will not occur in any of the habitats so far investigated (woodlands, grassland, waste ground, heathland, wetland etc.), for the GM crops currently available.

A ten-year study addressing the question of whether arable crops are invasive of adjacent natural habitats showed that the population of the crop in the natural habitat was seed limited. The study focused on *Brassica napus* subspecies *olifera* (oil seed rape) on both verges of the 189 kilometres of the M25 London orbital motorway and provides a model system for the ecology of crop plants that grow outside arable fields (Crawley & Brown 1995). This study showed that there was no evidence that oil seed rape is invasive of adjacent semi-natural habitats, despite the fact that it is known to persist for long periods in disturbed habitats.

In principle, however, transgenes which confer a clear fitness advantage on a GM crop plant (for example insect-resistance or drought tolerance, rather than simply herbicide tolerance) might enhance their performance outside of arable fields. Such traits require case-by-case field testing for invasiveness and it would be unwise to generalise from GM HT plants to all other transgene constructs.

Increase in abundance to problem status

Will GM crops become problem plants?

It can be argued that if the mere presence of GM plants outside arable cultivation is not in itself a problem (see above), then GM crops would only become a problem if they were to increase in abundance.

Evidence to date, for current GM crop species and current GM constructs like herbicide tolerance (for oil seed rape, sugar beet and maize) or insect resistance in potato, indicates clearly that GM crops will not become problem plants following escape from cultivation. This evidence is strong, based as it is on long-term widespread replicated field experiments.

We need to be circumspect, of course, about future transformed plant species and novel GM constructs that might be expected to increase plant fitness under field conditions. It is possible, however, that fitness-affecting GM constructs will involve trade-offs of one sort or another. Traits that enhance fitness in one habitat may have exactly the reverse effect in another habitat. Only field testing is likely to provide definitive answers to these questions.

Critique of the Alien Species Model

The Alien Species Model to predict the invasive ability of GM plants is a simple analogy with the invasiveness of alien plant species. The hypothesis is that roughly 0.1% of introduced

GMs would become pests, because that was the rate of invasive alien plants species (c. 15 problem plants out of an estimated 15,000 species introduced; Crawley, 1987; Williamson, 1993). However, the risks of a GM crop being invasive cannot based on probabilities like this, but on the nature of the transgene(s) that has been inserted. Multi-trait transformations could be used to increase fitness of the crop in agricultural habitats, thereby increasing the probability of invasiveness of disturbed habitats.

The Alien Species Model is good in that it shows the extent of the problem should it happen, but overstates the risks (in particular, the *probability* that a GM crop will become invasive) associated with the current GM constructs and crops. Alien invaders have attributes which are quite different to the attributes of the crops which are currently GM. They are usually thicket forming perennials, which are horticultural rather than arable species.

6.2.4 Is there general scientific agreement?

The PROSAMO experiments comparing GM HT oil seed rape, maize and sugar beet and insect resistant potato crop plants with non-GM crops plants demonstrate convincingly that the GM plants studied were not more invasive or more persistent in semi-natural habitats, and provide convincing evidence that GM itself does not make these plants more invasive. Escaped plants of all crop species are found throughout those parts of Britain where the crops are grown; these are known as 'casual species', and none of them is regarded as being a problem in semi-natural habitats.

The scientific consensus is that, at present, there is no evidence that the GM crops currently available for commercial use in Europe, would be more invasive than their non-GM counterparts if released into the environment, or that gene flow from them will generate more invasive populations of wild relatives (see 7.3). There is, though, considerable uncertainty as to the invasiveness of GM crops with fitness enhancing traits such as resistance to abiotic stress.

6.2.5 Is the issue unique to GM?

The possibility of 'alien' species becoming invasive is a reality as is clearly shown by non-native plants being brought into the UK (Crawley *et al.* 1996). An example of this is *Rhododendron ponticum* which is invasive of shaded native woodland and has caused the massive loss of biodiversity, especially ferns and mosses. Other examples are *Buddleja davidii, Mimulus guttatus, Impatiens glandulifera* and *Fallopia japonica.* These species have become invasive in the UK because they have found a niche not previously occupied or have superior competitive ability compared with the native species. The fact that more than 1,200 alien species (see box 6.1) are present in Britain, draws attention to the fact that mere presence of alien species is not itself a problem. We estimate that about 15,000 alien species capable of growing under British climatic conditions have been introduced (intentionally or unintentionally) and only about 15 species have increased in abundance to the point at which they are considered to be a problem. However, it is difficult to get two people to agree about what constitutes a weed – a plant in the wrong place is the standard definition (Naylor & Lutman, 2002), but Mark Twain had a different perspective when he defined a weed as "a plant whose virtues have yet to be discovered").

The issue is unique to GM in that GM techniques enable traits to be put into crop plants that may not occur through evolution or conventional breeding. This fact is the reason that a regulatory system has been constructed around GM crops to require consideration of whether those crop/trait combinations might lead to undesirable environmental impacts, including invasiveness. Although this shows that a GM plant could theoretically become invasive, there is general agreement that equating current GM crops to exotic plants provides a very limited model for predicting the effects of gene flow and GM crops. This is due to a difference in biology and life history of these problematical 'alien invaders' and GM crops. The most common alien invaders tend to be thicket-forming woody perennials which have unfamiliar genotypes. The GM crops tend to be herbaceous annuals that are genetically close to familiar crops and have been studied and improved for use in agriculture over many years by selecting traits very different to weeds, demanding significant inputs and husbandry. However, the potential for invasiveness has to be considered crop by crop and trait by trait. If genetic modification was applied to potentially more invasive plant species, or the traits put into crop plants conferred significant advantages in terms of survival beyond the agricultural environment (salt tolerance is one example), the possibility of 'alien species' behaviour would have to be carefully investigated.

In the future GM plants may not be comparable with non-GM, because transgenic technology may have the ability to fundamentally change the physical and reproductive architecture and metabolism of crop plants to the point where they could effectively become new species. For these plants comparison with alien species may be more useful for assessing their invasive potential.

Box 6.1

Numbers of species and subspecies in the flora of Great Britain

Sexual species	1698
Agamospecies*	806
Total Native	**2504**
Naturalized Aliens	1274
Subtotal	**3778**
Casuals	c.3138
Total	**6916**

***** Numbers of agamospecies refer to those in the genera *Hieracium, Rubus* and *Taraxacum* only.
Source: Table from C Stace, 2002

6.2.6 Are there important gaps in out knowledge or scientific uncertainties and are these important?

We do not have an exact understanding of what changes in a plant's life history will affect its invasiveness.

More knowledge on the potential effects of releasing GM plants with traits such as virus resistance and drought and salt tolerance is required (see Chapter 7.3). In particular, we need to know how plants control traits such as growth rate, longevity, plant size, or survivorship in crops and plant species with potentially more invasive life histories (e.g. woody plants,

perennial grasses, thicket-forming herbs), and apply this knowledge to understanding effects in GM crops.
Further research should also focus on potential invasiveness in farmland habitats where, for example, herbicides and fertilisers are used, and periodic disturbance is a characteristic feature.

One of the difficulties of risk assessment is that invasiveness can take many generations of the plant to emerge, and may involve hybridisation with related species.

6.2.7 Likely future developments

As GM technologies are applied to a wider range of plants, the review of their potential to become invasive will need to be applied on a case-by-case basis (this case-by-case assessment of invasiveness is already carried out for each crop, and is also part of the regulatory approval process). Plants with large seeds such as trees, patch-forming pasture grasses, or crops with resistance to key stresses such as salt might have the potential to be more invasive than current crops.

6.2.8 Where there is important scientific uncertainty, what is the way forward?

Understanding the stages of plants' life history which makes them invasive. Understanding which traits, when subject to GM, are likely to affect plant performance in natural habitats, when exposed to the full rigours of competition and predation.

Technological approaches

As well as introducing agronomic or quality traits, GM methods can introduce traits which stop a plant reproducing, particularly by seed. Although currently not entirely reliable, these technologies could be used in future to prevent any possibility of invasiveness, for example in turf or pasture grasses.

6.3 TOXICITY TO WILDLIFE

Could GM plants be toxic to wildlife, and what might be the impacts?

6.3.1 Summary

There is little scientific dispute about the fact that a GM plant engineered to produce a toxin can sometimes be toxic to non-target wildlife, since toxins are rarely species-specific. Conventional breeding techniques can also lead to unintended effects on non-target species, although the nature and specificity of these effects will depend on the mode of action and levels of expression of the transgenic or endogenous toxin.

On the other hand, finding out whether commercially grown transgenic crops many have ecologically significant impacts is more complex. It does not necessarily follow that toxicity demonstrated in the laboratory will translate into an ecological impact in the field. Currently, little information is available on the ecological impacts of GM crops on non-target species obtained from experimental field research under realistic commercial release conditions.

Conventional crop management practices, including pesticide applications, already have significant adverse impacts on biodiversity and soil functioning and the impacts of GM crops need to be assessed in this context.

No significant adverse effects on non-target wildlife resulting from toxicity of GM *Bt* plants have so far been observed in the field (with the possible exception of Event 176 *Bt* corn). This suggests that *Bt* crops are generally beneficial to in-crop biodiversity in comparison to conventional crops that receive insecticide applications. However, benefits would probably be restricted or even negated if *Bt* crops required broad spectrum insecticide applications to control secondary pests that were not sufficiently controlled by the *Bt* toxin.

The differences in soil microbial communities observed beneath GM crops have been within the range of variation in microbial community structure and of the order of magnitude of the differences observed under different crops of even different cultivars of the same crop (Dunfiled *et al*. 2001). However, almost all our information is drawn from small-scale, short-term studies and there is a need for larger, more agronomically realistic studies to be undertaken to demonstrate absence of harm to non-target organisms.

Introducing potent and/or broad spectrum toxin(s) into crop plants may create novel ecosystem dynamics, by effectively removing the crop plant as a source of food for some herbivores, detritivores and higher trophic levels. Therefore, longer-term research that compares the population dynamics of key pests and their predators and parasitoids in transgenic pest-resistant and conventional sprayed crops would be of value, although not necessarily a prerequisite for risk assessments

There is a need to develop better protocols to test the impacts of GM crops on non-target species. Future advances in knowledge of the behaviour and fate of natural or transgenic plant toxins in the environment should enable the development of predictive models that could be populated by data from field or laboratory research. Such modelling may be the best way forward for predicting environmental risks from novel GM or non-GM plants containing toxins.

6.3.2 Background

The current UK regulatory system for deliberate release of genetically modified organisms assesses a range of possible risks that could result from experimental or commercial growing of transgenic crops. One class of risk that is assessed is whether a transgenic crop may have adverse impacts on non-target organisms, (i.e. wildlife associated with the crop that does not cause economically significant levels of damage). Risk assessment for non-target toxicity applies to all GMOs, regardless of whether they have actually been engineered to contain active toxins. Since the greatest risks are likely to result from crops designed to express compounds toxic to pests, and most scientific evidence on non-target impacts of GM crops is concerned with these traits, potential toxicity of pest-resistant GM plants will be a main focus of this section. However, to date there are no commercially available applications of pest-resistant GM crops that are likely to be grown in the UK in the near future. Much of the information contained within this review may not be directly relevant to the UK at this stage of GM crop development, but there are important lessons that we can learn from experience elsewhere about techniques for risk assessment that could be useful in the future.

Although the title of this paper asks whether GM crops could be 'toxic' to wildlife, there is in fact a range of adverse impacts that both GM and non-GM crops containing altered or novel plant defences could have on non-target biodiversity. The common definition of the term 'toxicity' – the quality or condition of being poisonous, harmful, or destructive – implies a direct result of a chemical compound coming into contact with an organism. Toxicity can be 'acute' (adverse effects resulting from a single or short-term exposure to a substance) or 'chronic' (the ability of a substance to cause harmful effects over an extended period, usually upon repeated or continuous exposure sometimes lasting for the entire life of the exposed organism). Toxicity may be lethal, resulting in the premature death of an organism, or it may have various sub-lethal effects, including reduction in fertility (male) or fecundity (female), longer development time and subnormal weight, all of which could have significant effects on population dynamics of affected species. Predators or parasitoids consuming herbivorous prey that have been feeding on toxin-containing plants may inadvertently ingest the toxin(s) and suffer 'tri-trophic' effects (the plant being the first trophic level, the herbivore the second and the predator the third).

These are all examples of direct toxicity mediated by biologically active compounds. However, experimental studies have shown that the impacts of direct toxicity are often difficult to separate out from 'indirect effects' caused by changes in availability or quality of target herbivores as prey items. For example, if pest populations are strongly suppressed or even eliminated by toxin-containing plants, the predators and parasitoids that feed on those species may also decline if they lack sufficient alternative food sources. Although not strictly defined as a toxic effect of a crop, the toxicity of the crop could be said to have indirectly harmed this species (in a similar way to the non-target impacts of herbicides on the insects and birds that feed on arable plants – discussed in 6.5).

In many cases, the nature of a GM plant will indicate the obvious starting point for risk assessment. For example, a crop plant expressing a pest-resistance transgene might have novel interactions with its pest species and also with any other non-pest herbivores, or predators of crop herbivores, which are susceptible to the toxin. These kinds of interactions are sometimes predictable from previous research and theory.

Sometimes, however, the nature of new ecological interactions may be less obvious. A contribution to the GM Science Review website raises the example that some varieties of insect-resistant maize (*Zea mays*) containing a gene from the bacterium *Bacillus thuringiensis* (*Bt*) have been found to contain elevated lignin levels which cause the stalks to be broken down more slowly in soil than conventional varieties[1] (Saxena & Stotzky, 2001a). This was apparently confirmed by a study on the decomposition of *Bt* corn by the woodlouse *Porcellio scaber* (Wandeler *et al.* 2002). On the other hand, another study examined the breakdown of *Bt* and conventional lines and found increased digestibility by woodlice and found more rapid decomposition in the *Bt* lines (Escher *et al* 2000). These examples of "pleiotropic effects" might not pose significant risks to the environment, but they illustrate the importance of considering the whole plant as well as the expected effects of the transgene.

At the time of writing, the only type of insect resistance to have gained widespread marketing consent elsewhere in the world exploits a group of bacterial proteins known collectively as 'delta-endotoxins', also known as '*Bt* toxins', derived from the bacterium *Bacillus thuringiensis*. Over 100 types of delta-endotoxin have been discovered, each of which is specific to certain species of Lepidoptera or Coleoptera. The *Cry*stals of pure protein endotoxin contained by *Bacillus thuringiensis* have been used for many years in agriculture as a bacterial spray (using the whole organism), mostly on organically grown crops on which synthetic pesticides cannot be used. However, biotechnology now makes it possible to produce a single *Bt* toxin inside plant cells increasing the physical tergetting and hence the efficacy of the treatment (most sprayed *Bt* misses the plant, is washed off by the rain or does nt get nearto the target insect), eliminating the need for crop spraying. One potentially important difference between the compound produced by *B. thuringiensi*s and the toxins expressed in '*Bt* crops' is that the bacterium produces a 'protoxin' which is only converted into its toxic form once it has been ingested by an insect, whereas *Bt* plants directly express the active (truncated) toxic compound. However, evidence so far does not suggest that truncated *Bt* toxins lead to altered specificity (Evans, 2002).

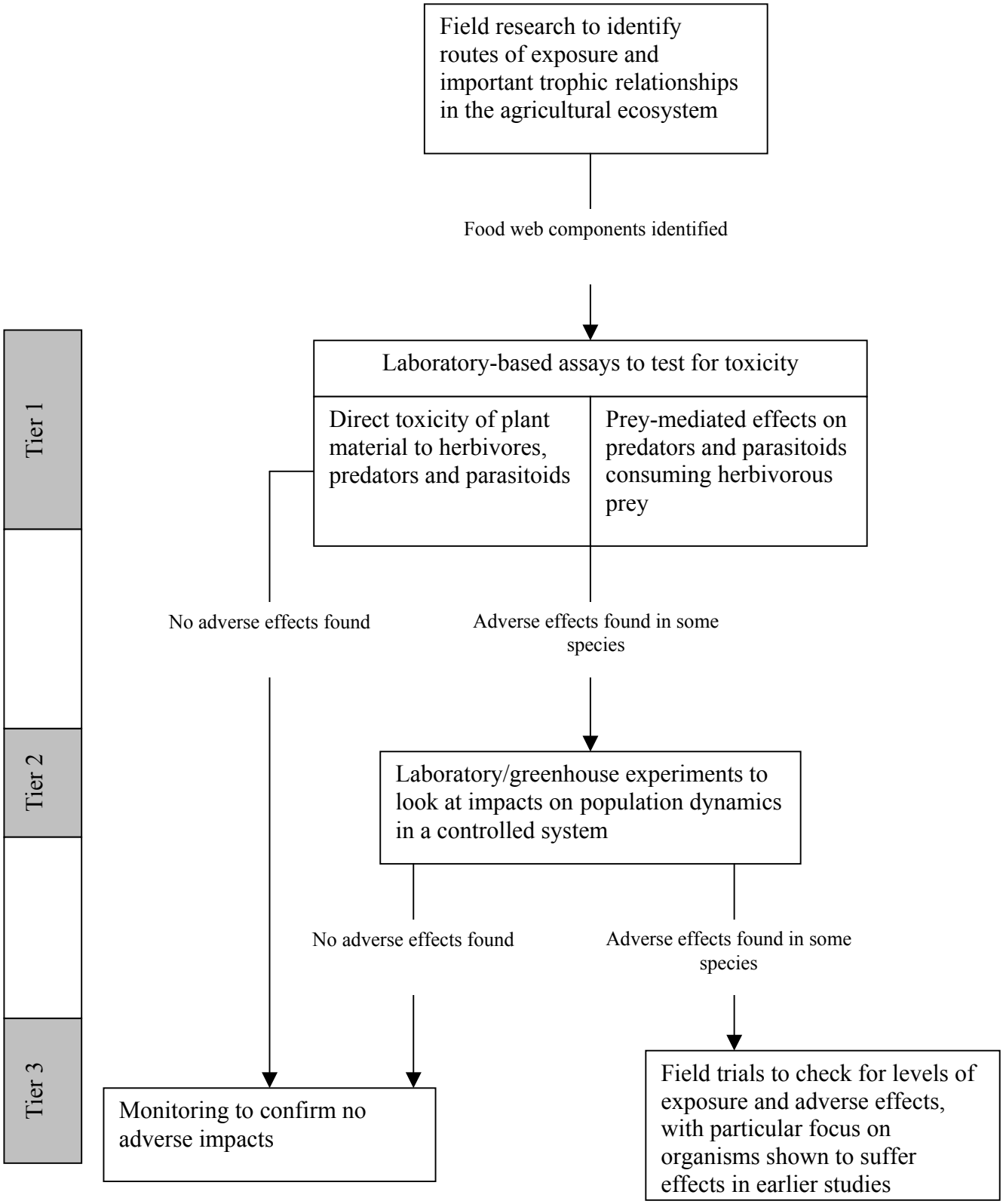
It seems unlikely that any *Bt* crops will be grown commercially in the UK within the next five to ten years. A survey of field trials conducted in the EU since 1990 revealed that *Bt* varieties of the following plants have been released: cotton, maize, rice, potato, tomato, cauliflower, broccoli, sunflower, coffee, strawberry and poplar. Of these, several are not currently suitable for commercial production in the UK (cotton, rice, sunflower, coffee) and one is a tree (poplar). *Bt* varieties of some UK crops might not be grown here for agronomic reasons. For example, although insect resistant *Bt* maize has consent for commercial cultivation in the EU, the pests it is designed to control (European corn borer or corn rootworm) are not currently a problem in the UK so there would be little incentive for farmers to grow these varieties unless they were agronomically attractive for other reasons. *Bt* tomato, cauliflower and broccoli might be possible candidates for UK growing but these still seem to be a long way from commercial development. In fact, only one field trial of a *Bt* crop (strawberries in 1995) has been carried out in this country so far. Research on the ecological implications of growing *Bt* crops is useful in terms of establishing protocols for experimental design, and perhaps for elucidating interactions between different species or guilds, but is not yet directly relevant to any forthcoming decisions on commercialisation in the UK.

In addition to *Bt*, a number of other insecticidal toxins have been experimentally introduced into crop plants, although none yet have commercial approval. They include cholesterol oxidase, vegetative insecticidal proteins, *Photorhabdus luminescens* toxins, proteinase

[1] GM Science Review Website Genewatch 2003 www.gmsciencedebate.org.uk/topics/forum/0072.htm

inhibitors, lectins and chitinases. In the UK, small-scale experimental releases have been carried out on strawberries transformed to express cowpea trypsin inhibitor and snowdrop lectin, conferring resistance to strawberry vine weevil, and potatoes expressing pea lectin, snowdrop lectin, proteinase inhibitors and pokeweed antiviral protein to confer resistance to phytophagous insect pests and potato cyst nematodes. Some of these traits may have broader pest toxicity than *Bt* and could in the future be combined with *Bt* genes to delay the development of pest resistance, a technique known as 'pyramiding' (Stewart, 1999). A number of nematicidal, fungicidal and antimicrobial compounds have also been transgenically expressed in plants (e.g. Glandorf *et al.* 1997), although many of these have been done on a purely experimental basis and none have so far been released commercially.

Diagram 6.1. Example of a three tiered risk-assessment procedure.



6.3.3 Range of views and quality of evidence

As risk is a product of hazard and exposure, both must be quantified in order to classify risks to the environment as high, medium, low or negligible. For example, the *Bt* toxin expressed in the pollen of some transgenic maize is known from laboratory studies to be toxic to some non-target species, including Monarch butterflies. These studies have identified possible hazards to non-target species, but more work needs to be done in the field to quantify exposure of arthropod larvae to the toxin, and assess the impact of toxins on population dynamics. Field studies may often be essential to quantify ecological exposure to hazards, and thus estimate risk.

But even if such risks are understood, they must still be assessed in relation to the biodiversity impacts of existing agricultural systems. In the case of *Bt* toxins in maize pollen, a valid comparison might be with current insecticide regimes used to control stem borers, the main target of *Bt* varieties of maize. Some comparative studies are already under way in the US and Europe, but are likely to be territory-specific because the relationship between biodiversity and agriculture varies between continents, countries and regions within countries, as well as with intensity of farming practices.

The discussion below has been structured in two sections to answer the question: *Could GM plants be toxic to wildlife* (hazard)*, and what might be the impacts* (exposure/risk)*?*

Could GM plants be toxic to wildlife?

There is little scientific dispute about the fact that GM plants engineered to produce toxins can sometimes be toxic to non-target wildlife. Indeed, many plants have evolved chemical defences against being eaten such that they are not food sources for many herbivorous animals. However, because there is an ever-expanding range of toxins being introduced transgenically into crops, and the range of species in agroecosystems that could potentially be harmed by these toxins can vary considerably between different regions, rigorous case-by-case assessments are required to test for toxicity. A considerable amount of research has been carried out in the laboratory to test for toxicity of GM plants to non-target species. There is not yet sufficient evidence for any one crop to demonstrate absence of toxicity to non-target species. However, to put this into context, many conventionally bred crop plants, (not just those bred for pest-resistance) can also have adverse effects on non-target species, and it would be next to impossible to develop a pest control system that would have no knock-on effects past the target pest(s). More relevant issues to consider when comparing the toxicity of natural or conventionally-bred pest resistance traits (or pesticide sprays - organic or modern) with transgenically inserted traits include: novelty, specificity and dose.

Studies looking at toxicity of GM crops (or even conventional crops) to vertebrate wildlife are generally not present in the published literature. The toxins involved so far are understood only to have toxic activity on insects. However, GM crops to be used for food or feed purposes must demonstrate lack of toxicity to mammals and/or birds through feeding studies, and these are reviewed in the chapter on human health.

The fact that a GM (or non-GM) plant may exhibit toxicity to a particular organism or group of organisms does not in itself indicate a risk unless a route of exposure can be identified, i.e. that organism must come into contact with the crop or the toxin at some point during its normal life cycle. Therefore the first stage of a risk assessment should be to assess possible

routes of exposure and the classes of organisms that would be exposed. Organisms could come into contact with plant-produced toxins via the aerial surfaces of the plant (leaves, flowers and stem), through its disseminated propagules (pollen, seeds and fruits), or in the soil surrounding the roots (rhizosphere). They may also contact the plant toxin directly by consuming plant material or released toxin, or indirectly by consuming other organisms that have the toxins in their gut. There may also be a temporal element to consider: plant toxins might persist in the environment for some time after the plant itself has been harvested. The section below examines evidence for toxicity of existing GM crops to some organisms that could be exposed to toxins through various routes.

Toxicity to herbivores and pollinators

Bt crops produce toxins that have a fairly narrow host range, depending on the specific *Cry* protein being expressed. The *Cry*1 and *Cry* 2 groups of toxins are specific to *Lepidoptera* (butterflies and moths) while *Cry*3 toxins are specific to *Coleoptera* (beetles). Non-target organisms likely to consume GM plant material may include species from these groups, as well as insects from other families, other invertebrates and vertebrates.

The mode of action of *Cry* toxins on target insects is well known – they bind to receptor cells in the midgut epithelium, resulting in the formation of pores which immobilise the gut, breaking up the epithelial cells and resulting in death of the organism. However, it is largely unknown what happens to *Bt* toxins in non-target herbivores and/or whether these herbivores may act as intermediaries through which the toxins may be passed on to predators and parasitoids (Groot & Dicke, 2002). It is possible that effects on predators and parasitoids may be observed due to a lower quality of their prey if they feed on/parasite species that are impacted by the *Bt* toxin(s)

In addition to the risk assessments carried out by applicants for commercial release of GM crops, there are a number of published studies that have examined the impacts of *Bt* crops on non-target organisms. Hilbeck *et al.* (2000) published a review of research on *Bt* plants and non-target organisms, which concluded that the experimental protocols used in many studies were inadequate to test for ecotoxicity, especially chronic lethal and sublethal effects. Experiments did not always adequately simulate routes of exposure that would occur in the field, and selection of test organisms was not always conducted on ecologically relevant species. However, as you cannot test every single species present in a field, non-target arthropod risk assessment has to concentrate on a limited number of indicator species. Although not always "ecologically relevant" to a given field/location, they are used because of their high sensitivity in general, making them good monitors for potential effects on other generally less sensitive species

Because of the case-by-case nature of the toxicological tests that have been carried out on *Bt* crops, it is impossible to draw any general conclusions from the research as to the toxicity of GM plants. Since *Bt* toxins can affect a range of Lepidoptera and Coleoptera, some research has focussed on the impacts on non-pest species in these families. Two studies examining the effect on Monarch butterfly larvae of consuming *Bt* maize pollen attracted a lot of attention a few years ago when they claimed to demonstrate a potential risk of growing this crop on a large scale. Losey *et al* (1999) fed Monarch larvae milkweed leaves that had been dusted with maize (corn) pollen to simulate a field situation, and found that survival of *Bt*-fed larvae was reduced by 44% in comparison to those fed on non-*Bt* pollen. Hansen and Obrycki (2000) attempted to simulate the field situation more closely by collecting milkweed leaves from the

field and placing larvae on them in the lab. They found 19% mortality in *Bt*-fed larvae compared to 0% in the non-*Bt* control. These studies suggested that there could be impacts on Monarch populations in the field, but they represented a "worst-case scenario" in which larvae were given no choice of food substrate. This led to further research at the field scale (discussed in the next section) to test for impacts on Monarch populations, and none were found. A toxicological study from this research programme demonstrated that only one *Bt* variety studied (event 176) caused significant adverse effects in Monarch larvae when fed on its pollen in the laboratory, while two other events had no significant effects (Hellmich *et al.* 2001). This emphasises the need for event-specific analysis of toxicity in transgenic crops, and the need for field data as well as lab data. Further studies investigating the exposure of Monarchs to *Bt* toxin in the field and developing a full risk assessment are discussed in the following section.

Other groups of non-target herbivorous arthropods, including Coleoptera (beetles), Hemiptera (bugs), Thysanoptera (thrips) and Tetranychidae (spider mites), will ingest the toxins when feeding on *Bt* plants. Lab studies on various predatory insects have showed no non-target effects of feeding on corn pollen containing *Bt* toxin. One study examined the effect of *Bt* pollen containing the Coleopteran-specific protein *Cry*3Bb on the pink spotted ladybird, *Coleomegilla maculata*, a polyphagous predator that is responsible for suppressing pest populations in the US Midwest. No significant effects were found on a number of fitness parameters including development time, pupal weight and reproductive capacity (Lundgren & Wiedenmann, 2002). Another study found no adverse impacts of *Bt* corn pollen on *Coleomegilla maculata*, insidious flower bug *Orius insidiosus* (Heteroptera) and common green lacewing *Chrysoperla carnea* (Neuroptera), although in any case the latter is not known to feed on pollen in the field (Pilcher *et al.* 1997).

Several studies have demonstrated that aphids do not take up *Bt* toxins, since these do not seem to be expressed in the phloem, and therefore neither aphids nor the predators feeding on them are likely to be affected negatively by *Bt* plants (e.g. Raps *et al.* 2001). There is no evidence to suggest that honeybees, *Apis mellifera*, are adversely affected by *Bt* pollen (e.g. Malone & Pham-Delègue, 2001). Beekeepers often use whole *Bt* sprays to prevent wax-moth infestations of combs, apparently with no effect on the bees inhabiting the combs.

No laboratory studies looking at direct non-target effects of a non-*Bt* GM plant on herbivores or pollinators were found.

Toxicity to soil organisms

A wide range of taxa could come into contact with transgenic plant-produced toxins in the soil, including bacteria, fungi, protozoa, nematodes, springtails, mites, enchytraeid worms, millipedes, centipedes, woodlice, molluscs, earthworms and a range of soil-dwelling insects (Evans, 2002). Possible routes of exposure include direct contact with transgenic plant roots, exudation of toxins into the rhizosphere from roots and incorporation of plant debris into the soil post harvest (Saxena *et al.* 1999; 2002; Saxena and Stotzky, 2000). For example, *Cry*1Ab is present in root exudates from several varieties of *Bt* corn, but not from *Bt* cotton, oilseed rape or tobacco; *Cry*3A was found to be present in exudates from *Bt* potato (Stotzky, unpublished, reported in Evans, 2002). In certain soils *Bt* toxins can persist and retain insecticidal activity for considerable periods of time (Tapp & Stotzky, 1998; Crecchio and Stotzky, 2001). Root exudates containing *Cry*1Ab were found to have no significant effects on earthworms, nematodes, protozoa, bacteria and fungi (Saxena & Stotzky, 2001b).

A review of impacts of fungal and bacterial-resistant transgenic plants on soil microorganisms showed that research is scarce and incomplete, and mainly focussed on mycorrhizal symbiosis (Glandorf *et al.* 1997). Most studies indicate that there are no obvious effects on the saprophytic soil microflora, but these conclusions cannot be generalised. One study demonstrated that mycorrhizal symbiosis can be adversely affected, indicating that non-target effects on beneficial fungi can occur (Vierheilig *et al.* 1995). Another study of transgenic bactericidal potatoes expressing T4 lysozyme showed increased killing of the non-target bacterium *Bacillus suBtilis* on potato root hairs, although the study was insufficient to demonstrate that negative impacts would be seen on bacterial communities in the field (Ahrenholtz *et al.* 2000). Griffiths *et al* (2000) examined the impacts of a transgenic potato, producing the lectins GNA and Con A, on non-target soil organisms and processes. Laboratory studies with soil bacterial communities and a ciliate protozoan could detect no direct effect of either lectin at a range of concentrations. However, a bacterial-feeding nematode was limited in its ability to detect prey when either lectin was present in the medium.

Toxicity to predators and parasitoids

Investigating the effects of toxins on higher trophic levels (predators and parasitoids) is more complicated, since the experimental protocol needs to realistically simulate the route of exposure to the toxin. It should also be able to distinguish between different kinds of effects, no effect, and compare with current non-GM practices. These include direct toxic effects of the compound, prey-mediated effects (for example, if the prey organism alters the toxin in some way that makes it harmful to the predator), a reduction in size or nutritional value of prey due to exposure to plant toxins, and behavioural effects.

A series of tritrophic studies on the effects on green lacewing *Chrysoperla carnea* of eating *Bt*-fed prey (Hilbeck *et al.* 1998a, 1998b, 1999) demonstrated potential harmful effects on an important natural enemy. Mean total immature mortality for lacewing larvae fed on *Bt*-fed European corn borer (*Ostrinia nubialis*) and Egyptian cotton leaf-worm (*Spodoptera littoralis*) was always significantly higher than the control, and this was true whether or not the prey species was adversely affected by the toxin. Analysis revealed that in addition to prey-herbivore by *Bt* interactions, prey/herbivore by plant interactions also exist. Again however, these laboratory studies provided no food choice.

Another study examined the effect of *Bt* cotton and *Bt*-cotton fed lepidopteran prey on adult survivorship of four important lepidopteran predators of cotton pests: *Orius tristicolor* (minute pirate bug), *Geocoris punctipes* (big-eyed bug), *Nabis* sp. (damsel bugs) and *Zelus renardii* (assassin bug). Adult survivorship is particularly important as these predators often migrate into cotton fields as adults. Longevity was significantly different between control and *Bt*-fed *O. tristicolor* (-28% in *Bt*) and *G. punctipes* (-27%) but not in *Nabis* sp. and *Z. renardii*. A review of previous studies on these species shows that no significant effects had been found where the predators had been fed on *Bt* leaves. This indicates a possible prey-mediated effect (Ponsard *et al.* 2002).

Schuler *et al.* (1999) studied the impacts of *Bt* oilseed rape on diamondback moth (*Plutella xylostella*) larvae and the parasitic wasp *Cotesia plutellae*. Parasitoid larvae developing inside *Bt*-fed susceptible moth larvae inevitably died within their hosts. But wasps developing inside *Bt*-fed resistant moths suffered no measurable adverse effects from the presence of the *Bt* toxin inside their hosts. In a second study, *Bt* oilseed rape was observed to have no adverse

impacts on the population dynamics of the hymenopteran parasitoid *Diaeretiella rapae* or its ability to control aphids *Myzus persicae* feeding on the crop (Schuler *et al.* 2001).

Studies of the impacts of GNA snowdrop lectin on predators and parasitoids have shown mixed results. When expressed transgenically in potato leaves, GNA confers partial resistance to two potato aphids, *Myzus persicae* and *Aulacorthum solani*. When female 2-spot ladybirds *Adalia bipunctata* were fed on GNA-fed aphids, impacts were found on fecundity, hatch rate and longevity, despite the fact that the ladybirds were switched back to a non-GNA diet halfway through the experiment (Birch *et al.* 1999). Another experiment on *A. bipunctata* fed GNA-fed aphids appeared to show no acute toxicity of GNA to the predator, although there was an indirect effect of prey size on ladybird development (Down *et al.* 2000). GNA-fed aphids have a suboptimal diet and are therefore small, so *A. bipunctata* could have been suffering from starvation or higher energy expenditure in gathering a larger number of prey items.

An endogenous parasitoid of aphids, *Aphelinus abdominalis*, could also be exposed to GNA during larval development. In one study, no direct detrimental effect of GNA on parasitoid success, development, size, emergence success, progeny survival and sex ratio was observed. However, there seemed to be an indirect host-size-mediated effect on sex ratio and size of parasitoids developing in GNA-fed aphids. GNA-fed aphids were smaller and produced a larger proportion of male parasitoids than the larger, non-GNA-fed aphids. The smaller size of parasitoids emerging from small GNA-fed aphids could have knock-on impacts on fecundity, which could in turn affect parasitoid populations in the field (Couty *et al.* 2001).

These findings demonstrate that laboratory-based tritrophic level studies are useful to assess the potential impacts of insecticidal GM plants on important invertebrates and their natural enemies.

Summary of evidence for toxicity of GM crops to non-target wildlife

The evidence presented above demonstrates that many GM pest-resistant crops, including some that are already grown commercially elsewhere in the world, have been demonstrated to exhibit either lethal or sublethal toxic effects on some forms of non-target wildlife. These effects include harm to organisms in higher trophic levels that consume plant herbivores feeding on toxic plant material. However, some of these effects may have been caused by a reduction in the quality or quantity of herbivorous prey rather than as a direct effect of the toxin itself – effects that would be a natural and inevitable consequence of any pest-resistant crop whether GM or not.

The published literature does not seem to contain any references for research on the possible toxicity of GM crops that do not contain pest- or disease-resistance transgenes. The most likely explanation is that such research is carried out as a routine element of GM commercial release applications but would not be reported in the scientific literature unless significant anomalous results were found.

The fact that some GM pest-resistant crops exhibit toxicity to non-target wildlife in the laboratory should not be considered surprising or alarming. These experiments are useful to indicate the most important organisms and interactions to test in population and ecosystem-level studies, which are discussed in the following section.

What are the likely impacts?

By carrying out experiments in the laboratory, it is relatively simple to demonstrate whether a particular organism is affected by contact with transgenic toxins. On the other hand, finding out whether there may be ecologically significant impacts is more complex and is always likely to involve extensive field-based research, not only to find out whether toxicity found in the laboratory occurs in the wild, but also to measure the exposure of organisms to the toxin under a range of conditions. Population dynamics of organisms in agricultural and semi-natural ecosystems are regulated by a number of different factors, so it does not necessarily follow that toxicity demonstrated in the lab will translate into a significant adverse impact in the field.

Impacts on herbivores, pollinators, predators and parasitoids

There is currently little published and peer-reviewed scientific information available on the ecological impacts of GM crops on non-target species that has been obtained from experimental field research that reflects commercial growing conditions. Most of the research has been carried out on small-scale plots in the United States where these crops are already commercialised. Several other studies involving impacts on non-target organisms in *Bt* crops in Europe are now under way.

Three small-scale field studies carried out in the US found no significant adverse effects on a range of beneficial insects in *Bt* (*Cry*1Ab) field corn and sweetcorn in comparison to unsprayed non-transgenic varieties. Two studies looked at predatory insects on hybrid field corn. One of these was carried out in Iowa on a very small scale (plot sizes between 22 and 45 m^2 with three replications) and found no significant differences in number of predators colonising *Bt* and non-*Bt* corn (Pilcher *et al.* 1997). The second was a larger-scale study in Michigan (plot size 4000m^2 with three replications). Population densities of *Orius insidiosus* (insidious flower bug), Coccinellidae (principally *Coleomegilla maculata*) and lacewing larvae were recorded on three days in August and September, and few significant differences were found. In addition, levels of larval parasitism of European corn borer *Ostrinia nubialis* by two ichneumonid wasps were not significantly different, suggesting that parasitism in these species is density-independent (Orr & Landis, 1997). The third was a very small-scale study in Minnesota to evaluate the impacts of *Bt* sweetcorn (*Cry*1Ab) and an isogenic non-*Bt* sweetcorn on beneficial insects. Few significant differences were observed in insect numbers between *Bt* and non-*Bt* plots. Only numbers of pink spotted ladybeetle *Coleomegilla maculata* in 1999 were significantly higher in non-*Bt* than *Bt* plots (1.17 per plant on *Bt*; 1.92 on non-*Bt*). However, the plots were small (four rows wide by 9m long) in the first year; 30 rows wide and 25m long in the second year) and density of predators was low, and with only four replications variance was high. The authors themselves recommend further research with larger sample sizes and spatial scales to investigate predator population effects of *Bt* corn (Wold *et al.* 2001).

A suite of studies was carried out specifically to test whether evidence of harm to a non-target herbivorous species demonstrated in the lab translated into an impact in the field (Sears *et al.* 2001). The experimental lab research carried out on event 146 *Bt* pollen and Monarch butterfly larvae was discussed in the previous section (Losey *et al.* 1999; Hansen & Obrycki, 2000). This research was based on a worst-case scenario that would be very unlikely to occur under natural conditions, so a major research effort was mounted to test whether significant

impacts on Monarch populations were occurring in commercially grown crops, involving a series of detailed assessments of both hazard and exposure.

The results demonstrated that Monarch larvae feeding on milkweed leaves in field plots of one variety of *Bt* corn (event 176) that is known to contain high levels of *Cry*1Ab in the pollen, had 60% lower survivorship and 42% lower weight gain than in control plots. However, there were no significant negative impacts in plots containing other *Bt* corn varieties. Larvae in non-*Bt* sweetcorn fields that were treated with insecticide suffered high mortality (91-100%) (Stanley-Horn *et al.* 2001). Significantly increased mortality was also observed in black swallowtail (*Papilio polyxenes*) larvae feeding on event 176 pollen, despite heavy rainfall that may have washed much of the pollen from milkweed leaves (Zangerl *et al.* 2001). Corn pollen is typically shed during a period of around 12 days, and the peak of the migratory Monarch generation and corn pollen shed were found to overlap by 15-62% depending on the region (Oberhauser *et al.* 2001). Overall, the studies show that event 176 *Bt* corn could have adverse effects on Monarch butterflies in the field, but that all other varieties studied have little or no impacts on Monarch populations. The studies did not examine Monarch population dynamics at the field scale throughout a whole season, so there is a possibility that the less toxic *Bt* varieties could still have chronic sublethal effects on Monarchs, although the overall impacts on populations would still probably be low or negligible (Sears *et al.* 2001). Other factors, including predation and agricultural activities, are likely to have a far more significant impact on Monarch population dynamics. This is an example of research that focuses on a particular species or group of species, rather than the agroecosystem as a whole. It is essential to test specific hypotheses where potential risks have been identified, but can tell us little about the overall impacts of transgenic insect-resistant crops on biodiversity compared to the impacts of the systems that they are replacing (Lövei *et al.* 2001).

Riddick *et al.* (2000) used 500m^2 paired plots on three farms in Maryland, USA over two years to compare the impacts of *Bt* potatoes (*Cry*3A) with non-*Bt* potatoes. Both treatments received insecticide applications to simulate commercial practices (including two applications of Esfenvalerate to nontransgenic crops to prevent total defoliation by Colorado potato beetle *Leptinotarsa decemlineata*). Plant-dwelling heteropteran predators and ladybirds (Coccinellidae) were monitored during both years using sweep nets, and surface-active generalist predators were captured using pitfall traps. For most taxa there was no significant difference in abundance between treatments. However, there were significantly more spiders on the ground in transgenic than in conventional treatments. In one year there were significantly higher numbers of *O. insidiosus* in transgenic fields. The authors suggest that observed differences may have resulted from a combination of a reduction in pesticide use and from the increased plant foliage associated with transgenic plants (which were damaged less than the conventional variety). There were significantly more *L. decemlineata* larvae in nontransgenic fields. The overall conclusion was that transgenic potatoes had no deleterious effect on the abundance of the plant- and ground-dwelling predators observed in this study.

Another set of field studies conducted in Oregon examined the impacts of *Bt* (*Cry*3Aa) and non-*Bt* potatoes on non-target arthropods under a range of treatments. Six treatments and six replications were used, with each plot measuring 337m^2. Visual counts and beat cloths were used to estimate the abundance of major arthropods on potato plants. The most abundant groups of generalist predators across all treatments were big-eyed bugs (*Geocoris* sp), damsel bugs (*Nabis* sp.), minute pirate bugs (*Orius* sp.) and spiders. The abundance of these predators on unsprayed *Bt* potato plants was either comparable to or significantly higher than any other

treatment. The abundance of secondary pests (not controlled by *Bt*) on unsprayed *Bt* potatoes was also higher than in other treatments (Reed *et al.* 2001) although in practice these would probably be controlled by use of systemic or foliar insecticides, reducing somewhat the environmental benefits.

A contribution to the GM Science Review website shows the results from the first year of a three-year study to compare the impacts of *Bt* cotton (*Cry*1Ac) and conventional cotton with and without insecticidal sprays on natural enemies in the southern United States (www.gmsciencedebate.org.uk/topics/forum/pdf/0088.pdf). The study found no significant adverse effects on non-target arthropods in *Bt* cotton fields, and *Bt* cotton fields often had significantly higher densities of non-target arthropods than sprayed conventional fields.

Impacts on soil organisms and processes

In gener*al.* there is a surprising lack of quantitative information on the total load of *Bt* in soil beneath transgenic crops (Evans, 2002). An unpublished field study carried out on *Bt* corn in Spain, submitted as a contribution to the GM Science Review website, demonstrated adsorption of *Cry*1Ab toxin by clays and retention of insecticidal activity against the target species *Trichoplusia ni* up to a period of eight weeks after the crop was harvested[2]. Root exudation does not seem to introduce as much *Bt* toxin into the soil as the incorporation of plant debris (Evans, 2002). No research was found in the published literature to examine the ecological impacts of plant-produced *Bt* relative to microbial *Bt* and/or routine chemical insecticide applications.

Cowgill *et al.* (2002) carried out field studies of transgenic nematode-resistant potatoes expressing cysteine proteinase inhibitors to test the effects on microbial community structure, soil microarthropods and litter decomposition. In the first year, the transgenic lines had no effect on the abundance, evenness or metabolic activity of the soil metabolic community, although one transgenic line influenced the structure of the community by favouring fungal growth relative to bacterial growth, while another transgenic line suppressed fungal growth. In the second year, microbial abundance in transgenic lines was reduced by 23% relative to the control. However, these observed changes did not result in changes in the rate of leaf litter decomposition. The transgenic lines had no significant effect on the abundance of soil microarthropods or free-living nematodes.

GNA lectin-containing potato plants were found to significantly alter the physiological profile of the rhizosphere community at harvest but effects did not persist from one season to the next (Griffiths *et al.* 2000).

Donegan *et al.* (1999) studied three types of alfalfa, either alone or in conjunction with GM nitrogen fixing bacteria, to examine their effects on the soil ecosystem. The alfalfa varieties studied were parental transgenic α-amylase producing and transgenic lignin-peroxidase producing. Lignin peroxidase is an industrial enzyme, used for large-scale lignin degradation and as a bleaching agent in the biopulping process. The alfalfa plants modified to produce this enzyme had significantly lower shoot weight, and higher nitrogen and phosphorus content. These changes in turn impacted on the soil chemistry. Soil pH was increased, and the activity of the soil enzymes dehydrogenase and alkaline phosphatase decreased. The soil biota also changed: microbial metabolic fingerprints of soil cores collected around uninoculated lignin peroxidase plants were significantly different from the parental alfalfa.

[2] GM Science Review Website. Costa 2003 www.gmsciencedebate.org.uk/topics/forum/0089.htm

This would indicate the presence of different levels and compositions of bacterial species. Lignin-peroxidase producing alfalfa inoculated with GM bacteria for enhanced nitrogen fixation also had the highest levels of culturable, aerobic spore-forming bacteria and cellulose-utilizing bacteria. Spore formation is often a response to adverse environmental conditions and has been used as an indicator of environmental stress or perturbation. The authors recommend broadened evaluation of the characteristics of transgenic plants to address such possible impacts on soil biota and processes.

Summary of evidence for ecological impacts of GM crops caused by toxicity to wildlife

No significant adverse effects on non-target wildlife resulting from toxicity of GM plants have so far been observed in the field, with the possible exception of Event 176 *Bt* corn which has since been withdrawn from the market. This suggests that the *Bt* crops that are currently grown commercially are generally beneficial to in-crop biodiversity in comparison to conventional crops that receive insecticide applications. However, this research is mostly based on small-scale field studies that have looked at densities of predators and parasitoids throughout the season, and there are no detailed studies on the population dynamics of target and non-target organisms. Additionally, we can predict that benefits may be restricted or even negated in situations where *Bt* crops require insecticide applications to control target or secondary pests that are not sufficiently controlled by the *Bt* toxin. Unfortunately there is little or no experimental evidence on these impacts in the published literature, although there is some anecdotal evidence that commercially grown *Bt* crops such as cotton often require additional sprays.

Studies on the impacts of transgenic crops on soil processes have shown some differences in soil microbial community structure, but so far there does not seem to be any convincing evidence to show that this could adversely affect soil health in the long term. This is because any potential for impact on soil ecology by GM plants must be seen in the context of natural soil variability and the currently accepted management practices that can have dramatic effects on soil microbial diversity and functions (ACRE, 2003).

Introducing potent and/or broad spectrum toxin(s) into crop plants may create novel ecosystem dynamics, by effectively removing the crop plant as a source of food for some herbivores, detritivores and higher trophic levels. Therefore, longer-term research that compares the population dynamics of key pests and their predators and parasitoids in transgenic pest-resistant and conventional sprayed crops will be very important from a basic-knowledge point of view, but is not a prerequisite or constitute an integral part of risk assessment (Hilbeck, 2002; Obrycki *et al.* 2001). For many species, GM pest-resistant crops are likely to provide significant benefits over conventional systems, but no direct comparisons have been made with alternative crop management practices such as organic farming or integrated pest management (IPM). If we want GM pest-resistant plants to contribute to ‘sustainable’ agricultural systems, their impacts on food webs and ecosystem dynamics must be understood and translated into integrated pest management practices that can be carried out by farmers.

6.3.4 Is there general scientific agreement?

A recent report from the International Council for Science (ICSU 2003) concludes that there is broad scientific agreement on a need for science-based environmental impact assessments and that the framework for such assessments is likely to be similar worldwide. Unlike in the area of food safety, there are no internationally agreed guidelines and standards for environmental assessments, so the interpretation of data and bases for comparison are subject to debate. It is this lack of agreed protocols for assessing hazards and exposure posed by GM pest resistant crops that has led to disagreements within the scientific community about the impacts of toxicity on wildlife.

An example of this is that the quality of some of the evidence used to determine applications for commercial release of *Bt* crops has been brought into question (Hilbeck *et al.* 2000). The main criticisms have been that the research does not always use ecologically relevant species and methods of exposure, and has only tested for acute toxicity rather than for chronic lethal and sub-lethal impacts.

In some cases, especially where regulators need to assess exposure of non-target organisms to a toxin within the crop, field scale trials should be essential components of environmental risk assessments of some pest-resistant plants. There may also be a need for field trials to assess the impacts of endotoxins relative to conventional insecticide use. Until recently these have not been required by regulatory systems, but requirements set out in the new EU Directive may lead to more comparative studies of this type.

There is a view among some scientists that the current generation of GM pest-resistant crops may have impacts on invertebrate community dynamics because they are designed to express high levels of a potent toxin throughout all plant tissues and throughout the season. The ecological impacts of this are expected to be different to the impacts of pesticide sprays and may be less, but we do not yet understand them sufficiently to be able to make predictions about the long-term implications for agroecosystems. There tends to be scientific disagreement about the amount of information that would be needed to demonstrate that growing GM pest and disease-resistant crops is sustainable in the long term. Some scientists would argue that reductions in pesticide use and increases in biodiversity compared to conventional crops are sufficient evidence to demonstrate absence of adverse impacts, while others advocate the need for a greater fundamental understanding of the underlying processes.

6.3.5 Is the issue unique to GM?

All plants have effective defences against herbivores, pathogens and parasites, which explains why most plants can only be attacked by a limited range of organisms. Defence mechanisms may be physically toxic to other organisms, or they may merely repel attacking organisms, by acting as physical barriers (e.g. extra lignin, waxes or hairs on leaves or stems), by inhibiting digestion (e.g. tannins) or reproduction (e.g. oestrogen mimics), or by being of suboptimal nutritional value. Considerable variation in these factors can exist within a plant species, and there is evidence that differences in host plant quality have the potential to affect long-term herbivore dynamics.

Conventionally bred pest-resistant crops can exhibit toxicity to beneficial organisms (Groot & Dicke, 2002). This can create dilemmas for plant breeders and practitioners of IPM. For

example, tomato plants bred for a high expression of the alkaloid α-tomatine were found to be toxic to a parasitic wasp, *Hyposoter exiguae*, in one of its larval hosts, a major agricultural pest *Heliothis zea* (Campbell & Duffy, 1979). This resulted in prolonged larval period, reduced pupal eclosion, smaller size and shortened adult longevity, which could potentially make this for of pest resistance incompatible with biological control programmes.

However, GM does have the potential to develop plants that express novel toxins (i.e. those not found in the crop and ancestor gene pool), including some of bacterial origin. First generation GM crops were almost always developed using constitutive plant promoters, which express the toxin throughout the plant tissues and throughout the season. The absence of endogenous mechanisms for fine-tuning of the expression of pest-resistance toxins makes these early GM plants rather blunt tools for pest management. However, the discovery of new promoters that can control timing and tissue specificity of transgene expression offers opportunities for fine tuning the delivery to minimise the threat of resistance development and potential for non-target effects.

The process of genetic modification theoretically has the potential to create unanticipated alterations in the levels or nature of toxic plant metabolites, for example by inserted transgenes disrupting metabolic processes in the plant. Such 'pleiotropic' effects could be caused either through the action of the transgene product, or through the transgene being inserted into a location that interferes with the transcription of another gene(s) (see Chapter 4). Assessment of toxicity is therefore carried out on all new transgenic plants, whether or not they were deliberately designed to contain toxins. However, conventional or mutational breeding can also result in unanticipated altered toxicity, and it could be argued that all new plant varieties should be tested for such effects.

6.3.6 Are there important gaps in out knowledge or scientific uncertainties, and are these important?

It is still largely unknown what happens to *Bt* toxins in non-target herbivores and/or whether these herbivores may act as intermediaries through which the toxins may be passed on to predators and parasitoids.

Agronomically realistic ecological studies comparing the impacts on biodiversity of the use of GM pest resistant crops with conventional insecticidal crop treatments should be undertaken for any GM pest-resistant crops that are being considered for commercial release in the EU. This research will be needed in future if lectins, protease inhibitors and other endotoxins are introduced into commercial crops especially for industrial end-use.

Studies of the impacts on vertebrates (especially birds known to eat crops) of commonly used GM-derived endotoxins are lacking in the scientific literature.

There is a need to develop better protocols for testing the impacts of GM crops on non-target species. Several authors have put forward their ideas (e.g. Lövei *et al.* 2001; Hilbeck *et al.* 2002; Hilbeck *et al.* 2000; Obrycki *et al.* 2001; Groot & Dicke, 2002).

The development of models able to predict the fate of plant endotoxins within natural and agricultural ecosystems would greatly increase the ability to be able to assess environmental risk.

More field research on the impacts of pest- and disease-resistant GM crops on soil microorganisms and processes should be carried out in advance of commercialisation.

6.3.7 Likely future developments

Because the EU regulatory system now requires more detailed information on the environmental impacts of GM crops and the way in which they are cultivated, field research may be a requirement of some new applications to commercialise GM pest resistant crops. Whether such research is needed prior to an application to release a GMO or is a requirement of post-marketing monitoring is for the regulatory system to decide. Some field scale research is likely to need large scale planting of crops and might only be possible after commercial release (as part of a post-market monitoring programme).

Small-scale field trials of GM crops to test for impacts on agroecosystems are unlikely to pose any long-term risks to the environment. Most of the possible negative impacts on biodiversity resulting from toxicity of the crop are likely to be reversible, e.g. by removing that crop from cultivation should any harmful impacts be observed.

However, there are some potential impacts of growing GM crops, either in field trials or more likely through commercial cultivation over several years, which could be irreversible. For example, gene flow from a pest-resistant crop to wild relatives that led to an increase in fitness could result in that plant increasing in density and/or expanding its range (see section 7.3). This could lead to the decline of less competitive plant species and/or declines in organisms in the food chain of those plants that were adversely affected by the toxin. Another potential irreversible impact could be the development of resistant pest populations through intense selection pressure, as happens now in non-GM agriculture. Therefore, it will be important to consider these risks (discussed in section 6.4) when deciding whether to proceed with field trials and/or commercial cultivation.

6.3.8 Where there is important scientific uncertainty, what is the way forward?

For the commercial cultivation of GM crops in the EU there is a legal obligation to include a post-market monitoring programme, aimed at testing the validity of assumptions made during risk assessment, identifying any unforeseen adverse effects on the environment or human health. Since our understanding of the impacts of GM crops on non-target species will never be complete, in cases where the environmental risks are assessed to be acceptably low, regulators are likely to grant commercial consent with the option of withdrawing consent if monitoring programmes identify significantly harmful impacts. Such monitoring programmes can be used to add to knowledge of the impacts of GM pest-resistant plants on the general environment.

Future advances in knowledge of the behaviour and fate of plant toxins in the environment should enable the development of predictive models that could be parametized by data from field or laboratory research. Such modelling may be the best way forward from predicting environmental risks from GM plants containing toxins.

6.4 DEVELOPMENT OF RESISTANCE

Could crops engineered with novel resistance genes lead to the emergence of new forms of pests, diseases and weeds that are resistant to chemical sprays? Will new forms of insects and diseases evolve which are able to bypass GM resistance genes?

Herbivorous insects, fungal pathogens, bacteria and viruses often get around disease resistance genes by mutating to new virulent forms. Is this more likely to happen with a GM-derived resistance gene than with a conventional bred resistance gene, and will the impacts be greater? Similarly weeds can develop tolerance to herbicide sprays. Is this likely to be a greater problem when herbicides are used with GM HT or other HT varieties?

6.4.1 Summary

Two key plant breeding aims, both of GM and other breeding technologies, are the development of varieties that are resistant to pests and diseases, and crops which are tolerant to herbicides. Disease resistant varieties, particularly if grown on large areas, provide a strong selection for *target organisms* (pests or pathogens) that can attack the new variety. Similarly new forms of pests, diseases and weeds can develop that are tolerant to any agro-chemicals applied to reduce incidence of disease or to kill weeds.

The time it takes for a virulent or resistant pest or pathogen to emerge depends on the nature and complexity of the genetic mechanism that makes the crop toxic to the pest immune to the disease and on the effectiveness of the management techniques deployed by the farmer. Current opinion is that 'single gene' mechanisms are less durable than immunity controlled by several genes. That said, some 'single gene' sources of resistance (GM or non-GM), including the *Bt* genes which confer resistance to insects, appear particularly robust and have not yet broken down in the field. However, all experience and science tells us that any gene-based resistance mechanism will eventually be overcome.

Similarly, weeds resistant to herbicides have been seen for various herbicides applied in association with herbicide-tolerant crops. This is the case whether the tolerance was introduced by GM or any other breeding technique. Weeds that are closely related and can hybridise freely with a herbicide-tolerant crop variety have the additional possibility of obtaining tolerance directly from the crop.

The conclusion is that, although new forms of plant pests, disease and weeds can be expected to emerge, there is no evidence to propose different responses depending on whether the resistance was introduced into the crop by GM or other breeding methods. The use of a diversity and/or combination of strategies for weed/pest control would expect to delay or even prevent resistance evolving.

6.4.2 Background

The evolution of virulence in plant pests and diseases allowing them to overcome resistant varieties of crops is a major concern for breeders; for example the insect targeted *Bt* crops or rice bacterial leaf blight targeted by the *Xa21* gene. Similarly, the emergence of new varieties of weeds resistant to herbicides used on herbicide tolerant (HT) crops is a concern. This has

been highlighted in the Review of Public Concerns with the question 'is it speeding up a natural process like survival of the fittest?' The issue of the emergence of herbicide tolerance in weeds has been addressed in a website contribution[1].

The development of resistance is not a novel phenomenon confined to agriculture, and certainly not to GM. The reciprocal selection pressures between a host and a parasite (termed co-evolution) are thought to be very important. The Red Queen hypothesis (Van Valen, 1973), derives its name from the character in 'Alice Through the Looking Glass', who tells Alice, "It takes all the running you can do, to keep in the same place", proposes that sexual reproduction persists because it enables species rapidly to evolve new genetic defences against parasites that attempt to live off them. Chromosome recombination and reassortment during sexual reproduction increases genetic variability in the population, which increases the chances of survival chances of some individuals in times of altered selection pressure. This is the basis of adaptation. It was Haldane that first argued that disease was one of the most important evolutionary agents, and its importance increases in line with its killing power.

These notions extend to the agricultural environment where chemical agents to control disease, pests and weeds are used extensively in modern intensive farming practices. Pesticides and herbicides are often effective for only a short time, until new cycles of co-evolution produce new forms of the target pest, weed or pathogen that can tolerate the chemical. A similar situation pertains with crop genetic resistance to pests and diseases, which, although usually more targeted to a specific crop organism, also exerts selection pressure that can results in new, more virulent forms of the pest or pathogen. This has been highlighted in the Review of Public Concerns with the question 'could harm take the form of new diseases? (D5- see Annex 1)'

This paper addresses issues associated with:

(a) The likelihood and speed of breakdown of GM resistance to pests or diseases, and of weeds evolving resistance to broad-spectrum herbicides used on GM HT crops, and the effectiveness of management strategies that can be used to reduce the speed at which resistance may evolve.

(b) The potential impact of resistance breakdown, and the development of herbicide tolerance in weeds.

(c) Whether we can expect differences between responses to resistance genes and herbicide tolerance genes incorporated in new varieties by GM or other breeding methods.

An understanding of the molecular and genetic basis for resistance is crucial in modelling the probability of parasites deriving resistance. Resistance may be determined by a single gene or controlled by many. See Box 6.2

It is important to note that although insect pests may reduce crop yields, associated and often more serious effects are caused by viruses that are transmitted by the insect, or by fungal or bacterial diseases that enter the plant through the insect puncture holes.

[1] GM Science Review Website. Hartzler 2003 http://www.gmsciencedebate.org.uk/topics/forum/0051.htm

Pest and disease resistance mechanisms in plants are of two types: 'gene-for-gene' specific resistance that we expect will be overcome by mutation and selection in the pathogen population and 'durable' (non-host) resistance that we expect to be more robust (see Box 6.2).

The probability of resistance breakdown arising will depend on the frequency of resistance alleles in the population, the method of inheritance (controlled by a single or multiple alleles which are dominant or recessive), the level of the selection pressure and, in insects, the mating ranges of the pests. In addition, resistance management strategies that aim to make the conditions as difficult as possible for the target organism to evolve resistance are often employed.

Box 6.2. Pathogen Type and Genetic Basis for the Evolution of Resistance

Genetic Basis for Resistance between host and pathogens

Gene for Gene – Race-specific resistance, conditioned single genes, is the best understood form of constitutive plant disease resistance and has been widely used in breeding. Each resistance gene in the host has a matching gene for virulence in the pathogen. It takes only one mutation in the pathogen's avirulence gene to create a protein that is not recognised by the host thereby laying it open to attack. This sometimes referred to as 'resistance breakdown'. It is important to note that resistance and avirulence genes tend to be dominant. Plant breeders have in the past concentrated on this sort of major gene resistance that is easier to select for. This has given rise to 'boom and bust' cycles (e.g. yellow rust in the UK wheats in 1970s).

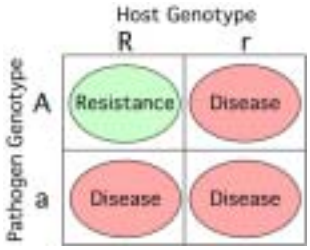


Fig. 1 Schematic representation of race specific, 'gene-for-gene' resistance

Durable Resistance – Resistance which is controlled by several genes is harder to circumvent because a mutation in any one of them is unlikely to confer resistance on its own. Durable resistance is generally broad spectrum as it does not rely on the host recognising the pathogen. This is less well understood than race-specific resistance, but is an important goal of plant breeding.

Types of Pathogens

Biotrophs – specialist interaction with hosts , generally obligate. Many fungal diseases fall into this group (rusts, mildews), but also some bacterial diseases. They generally have major 'gene-for-gene' resistance genes, but also have other, non-specific resistances that are generally durable.

Hemi-biotrophs – initially grow on plant without symptoms, then evidence of disease becomes apparent, e.g. *Septoria.*

Necrotrophs- non-obligate, saprotrophic stage, greater influence, have a role away from the host, generally not 'gene-for-gene' and thought to be polygenic.

6.4.3 Range of views and quality of evidence

The likelihood and speed of breakdown of GM resistance to pests and diseases, and of weeds evolving resistance to broad-spectrum herbicides used on GM HT crops, and the effectiveness of management strategies that can be used to reduce the speed at which resistance may evolve.

The probability of a pathogen overcoming resistance in a GM or non-GM transgenic crop and the crop management strategy deployed is very case-specific.

Evolution of resistance to herbicides in weeds is also expected. HT is sometimes discovered in weeds and wild crop relatives that have never been exposed to the chemical. There are many reports of HT arising in response to herbicide sprays and, more recently, in responses to sprays on both GM and non-GM HT crops. In the last 40 years more than 120 plant species worldwide have developed herbicide resistant individuals under modern agricultural conditions (see section 7.3.3).

Pathogens

There are now several examples of transgenic resistance to bacterial and viral pathogens that cause disease. Only a few will be discussed below. Note that, in addition to natural mutation in pathogen populations as a basis for breakdown of a resistance gene, importation of more virulent strains of pathogens from elsewhere are often the cause of breakdown.

Bacterial disease

Xa21 is a gene discovered in *Oryza longistaminata*, a wild relative of cultivated rice. It was introduced into rice by crossbreeding and found to be effective against all known races of bacterial leaf blight (*Xanthomonas oryzae*), in rice (Khush 1990). It was subsequently isolated (Song *et al.* 1995) and made freely available to public breeding programmes in developing countries.

The gene can now be manipulated in cross-breeding programmes and pyramided with other resistance genes (Huang *et al*. 1997) or used directly as a transgene. The gene has held up so far, either in transgenic or conventionally bred lines, and so may be durable.

Viral disease.

Viruses often have a devastating effect on crops and much agro-chemical pest control is directed at the insects, fungi, mites or nematodes that naturally transmit these viruses. The introduction of durable genetic resistance against many common, and often devastating plant viruses is seen as a more sustainable means of crop protection than frequent spraying with chemicals to control the pests that transmit them.

Virus resistance gene breakdown is commonplace, e.g. Tm1 and Tm2 *genes* overcome by Tobacco Mosaic Virus and the emergence of new strains potato virus X (PVX) in Latin America which overcame the natural *Rx* genes. Although PVX has been fully investigated most other examples remain empirical observation without full understanding of the molecular mechanisms involved.

Over seven years, several GM food crops expressing virus-derived sequences as novel resistance transgenes have been deployed commercially in the USA, China and Africa there

has been no reported case of any new strain of a virus "breaking" the GM resistance. Box 7.1 lists some seven successful applications of GM to give resistance to viral infection in papaya, wheat, rice, potato, chilli pepper and tomato. Perhaps this is because most viral R-genes function through a highly targeted and efficient plant defence/RNA degradation pathway related to RNAi-mediated gene silencing which inhibits the earliest stages of virus replication before large numbers of viral genomes accumulate to recombine or be mutated. Nevertheless, long history and experience tell us that, sooner or later, any single dominant virus-derived R-genes will be overcome by a new strain of the target virus.

Management and breeding strategies

There are several management strategies to delay resistance breakdown. These include the use of seed 'mixtures', different varieties carrying a range of resistance genes all mixed together (Wolfe and Barrett 1980, Mundt 2002). This is expected to reduce selection pressure on the pathogen within a field. On a larger scale, the deployment of a range of varieties with different resistance genotypes on a farm or within an agricultural area has been proposed (Priestley & Bayles 1982). Others however claim fungal spores are dispersed over such long distances as to make fields ineffective as barriers (Brown and Hovmøller 2002).

Pests

Insect pests are not a major problem in UK agriculture, however, elsewhere various insects are major pests themselves and, in addition, many transmit virus diseases. Natural insect resistance, for example to rice plant hoppers and gall midge, has been a major target in breeding programmes the world over. Effective alleles have been found in germplasm collections and in wild relatives of crops. Although the toxins involved in most of the resistances are unknown, gene-for-gene relationships are common as is resistance breakdown. Investigation of *Bacillus thuringiensis*, used as a sprayed insecticide led to the discovery of the *Cry* genes that underpin the transgenic *Bt* crops. The main differences between *Bt* transgenic crops and *Bt* sprays is the GM plant will express the toxin at a high dose throughout the growing season, which may decline at the end of the growing season making it easier for resistance to evolve. Sprays also involve a large numbers of toxins, whereas GM varieties use the products of only one, or more recently, two genes.

There are several strains of *Bt* which produce a range of *Cry* proteins and target a spectrum of insects, mainly lepidoptera, such as the European corn borer and boll worm, most of which are not pests in the UK. Therefore, *Bt* transgenic crops are unlikely to find application in the UK in the foreseeable future, it is useful to draw on the growing this experience of this group of transgenic crops.

As yet there has been no confirmed reports of breakdown of resistance in the field in the many crops that have been engineered with *Bt* genes, some of which have been planted since 1996. However, there is no reason to suspect that *Bt* will not break down. In fact break a decrease in sensitivity to certain *Bt* toxins by certain strains of target pests has been observed under laboratory conditions. In order to further delay breakdown, breeders are incorporating more than one gene at a time into new varieties, e.g. the two *Cry* genes now engineered into Australian Inguard cotton varieties (Peacock 2003).

In Australia, there are opinions that the addition of a second *Bt* gene will alter the balance of insect pests with increases in insects such as aphids green mirids and two-spotted mites which will demand more complex control measures (Fitt, 2001).

This is contested by Peacock, who insists no effects on 200 species of non-target insects have been observed in several years of monitoring (Peacock 2003). Also elsewhere diet feeding experiments indicate that the effects of the Cry1(c) protein on non-target insects are negligible (Sims, 1995)

Management strategies

The management of pest resistance currently favoured is the 'high dose/ refuge' strategy, in which farmers are required to leave small areas within the *Bt* area planted to susceptible varieties (Tabashnik, 1994; Huang *et al.* 1999). This strategy reduces the risk of resistant individuals surviving to mate (due to the high doses) and reduces the risk of doubly recessive individuals surviving to mate (creating a low percentage of these due to the compulsory use of refugia). See box 6.3. Whether or not the use of refugia is an effective strategy cannot be established until we have experience of resistance breakdown. The effectiveness of refugia has been questioned as has the possibility of applying the strategy on the smaller *Bt* cotton farms in, for example, South Africa, China and India (Jayaraman, 2002). Other critics of "high-dose/refuge" strategy argue that some of the assumptions to not hold for the European corn borer [2]. However *Bt* has been in the field now for many years so it may be durable.

Box 6.3 The principles of and assumptions underlying refugia

REFUGIA

Refugia are areas where susceptible insects may live, i.e. a 'refuge' from the insecticidal plants. It may consist of an area of non-insect resistant plants grown in the vicinity of the insect resistant crop, or dispersed amongst them. The aim of refugia is to keep a susceptible population for mating with resistant individuals, thus reducing the number of resistant alleles in the insect population.

The concept of a refuge relies on several three key factors: (a) resistance alleles are rare, (b) resistance alleles are recessive, (c) insects growing on the crop and the refuge come together to mate, and (d) crops contain high concentrations of the toxin in certain tissues.

Refuge requirements are designed on a case-by-case basis, considering the biology of the target pests and the nature of the cropping system. Examples of current refuge requirements for *Bt* corn are:

1) in the USA – in the "corn belt", growers must plant a 20% refuge area, which must be planted within one-half mile of the biotech field, and must contain non-Bt corn.
2) in Argentina – farmers are required to plant a 10% non-Bt corn refuge. This is smaller than the US because alternative host crops for the target pests serve to supplement the structured refuge. (Source, Monsanto contribution website)

Because of the conditions necessary for Refugia to be an efficient resistance management technique, it is only applicable to insect pests where mating partners grow on separate plants. It is important to ensure that insects from the main crop and refugia mate together and do not develop asynchrony, which has been documented in a few cases (Lin *et al.* 1999, Cerda and Wright, 2002)

Concern is sometimes expressed that GM makes it possible to introduce similar pest resistance genes into different crops (e.g. Bt for insect resistance) and that this therefore has a greater potential to select pests that overcome the resistance. However, different crops generally have a different spectrum of pests and diseases and therefore will usually require

[2] GM Science Review Website. Castanera 2003 http://www.gmsciencedebate.org.uk/topics/forum/0090.htm

different resistance genes to control them (e.g. different Bt genes or other resistance genes, or non-host resistance where a pathogen has no potential to infect a particular species).

Weeds

Genetically engineered crops resistant to a herbicide introduce the opportunity for routine use of broad-spectrum herbicide for weed control. Past case history in chemical weed control suggests that recurrent selection will frequently result in the emergence of resistant weed phenotypes. A range of herbicide tolerances have now been built into crop plants. Conventionally bred HT (atrazine) maize varieties were deployed in the US in the late 1980s. Roundup Ready (glyphosate tolerant) varieties of many crops, including maize, soybean, oilseed rape and cotton, have been bred by GM using two bacterial gene sequences. Increasingly non-GM sources of HT are being used, these include triazine and imidazolinone tolerance in oilseed rape and chlorsulfurun tolerance in wheat (Mazur and Falco, 1989). Tolerance to glyphosate is proving difficult to find or induce by mutation in crops. Nevertheless resistance in weeds is common and a defined spontaneous mutation for glyphosate resistance has been described in *Eleusine indica*, a wild weedy relative of finger millet (Ng *et al.* 2003).

The development of resistance to herbicide sprays is common. In some cases resistance can be found in crops, e.g. ryegrass and *Sestuca* (Johnston *et al.* 1989), emmer wheat (Snape *et al*.1987) and *Setaria viridis* (Wang *et al.* 1998), on which the chemical has probably never been applied. The first herbicide resistance weeds (to triazines) were reported in 1968. Since then, herbicide resistance has been observed worldwide and has been shown in at least 127 species covering 15 herbicide groups (Mortimer and Putwain, 1991). Characteristically, evolution to triazines in most species emerged after prolonged (typically 7-10 years) exposure to the herbicide. Highly resistant plants to chlorosulfuron occurred after three years in which the chemical was applied at 7-14 month intervals (Thill *et al. 1991*).

Emergence of glyphosate (Roundup) tolerance following repeated treatment has now been documented for a range of weeds, starting with ryegrass in Australia and now worldwide with a range of species[1]. In Canada volunteer oilseed rape with multiple tolerances to glyphosate, glufosinate and imidazolines (Liberty, Roundup and Clearfield - three of the herbicide tolerances used in Canadian canola varieties) has been found. They were first identified in Canada in 1998, only three years after GM HT oilseed rape was first grown (Downey, 1999). This resistance has presumably arisen from sequential crossing of several herbicide tolerant varieties and subsequent ‘gene stacking’ of an imported trait and not due to mutation. Although glyphosate has been used for more than 28 years, there have been a maximum of 4 reported cases of weeds developing resistance due to repeated exposure (mutation or other unknown mechanism of resistance). In theory, tolerance in some other weeds could also be derived through hybridisation with a HT related crop plant. No example of this sort of gene transfer has yet been observed.

The multiplicity of herbicides available ensures that HT gene-stacked volunteers are not an agricultural problem. Both 2,4D and paraquat (grammoxone) are being recommended by government agencies to control herbicide tolerant oilseed rape volunteers in Canada (Orson, 2002). However English Nature considered that if herbicide tolerance gene stacking arose in the UK, more paraquat and diquat use could result in harm to hares.

Management techniques

Management practices to avoid resistance could harm or benefit biodiversity (Orson, 2002). Theory suggests that for monogenetically inherited resistance, alteration of selection intensity by rotation of herbicides with different modes of action or the use of non-selective cultural methods will be effective in delaying the emergence of resistance. These management techniques (e.g. crop and herbicide rotation) are often implemented by farmers, but there is no regulation.

The potential impact of resistance breakdown, and the development of herbicide tolerance in weeds.

Disease resistance breakdown has been a common unfortunate event in varieties of, probably, all bred crops. In many cases alternative natural genes and alleles are available to the breeder to incorporate into the next wave of resistant varieties, which will already be in development. The major impact is economic, both at the level of the farmer who has lost yield and profit, and at the level of breeder with the costs involved in breeding effective alternative varieties. The economic impact of breakdown in transgenic crops is likely to be greater still because of the high costs involved with satisfying the present regulatory processes. There may also be environmental impacts where farmers resort to fungicidal sprays when genetic resistance becomes ineffective.

Pests

There will be similar costs associated with adaptation of pests to insect resistant varieties as for plant disease resistance breakdown. The breeding consequences might be more severe because effective alternatives to the Cry genes are probably not available.

The most likely immediate consequence will be that farmers will return to the previous insecticidal spray regimes with the associated economic costs (Conway 2003, National Research Council 2000).

HT weeds

Should target weeds become resistant to any single or several herbicides the most immediate effect is again likely to be an economic one, i.e. from loss of production due to excessive weeds or from the added cost of removing the HT weeds with a second herbicide. The actual economic impact is likely to be situation specific. Hartzler[1] describes different weed scenarios in glyphosate resistant crop that would involve the farmer in more or less expense.

The environmental impact could also be significant. Resistance to herbicides that are broken down rapidly in the soil, such as glyphosate, would mean that they would likely have to be replaced by herbicides such as 2-4D or diquat that can persist in soils for long periods. This increase in paraquat and diquat use could result in harm to hare populations in the UK (see above).

Whether we can expect differences between responses to resistance genes and herbicide tolerance genes incorporated in new varieties by GM or other breeding methods

There is no evidence, or reason to expect, that breakdown to GM-derived or conventionally bred resistance will result in different forms of disease to breakdown of natural genes. The

incorporation of, for example, *Xa21* into rice, by GM is unlikely to result in different consequences to its incorporation by GM. The use of such natural plant genes in transgenic programmes is likely to increase.

Herbicide resistance is developed to the herbicide, whether it is sprayed in association with a HT crop or not. GM HT crops could increase areas being sprayed with a particular herbicide (as in the US) and increase the frequency of the application. This could increase the likelihood of resistance developing[1].

6.4.4 Is there general scientific agreement?

There is agreement that resistance genes, introduced by GM or otherwise, are likely to be overcome by the evolution of resistance, especially where control relies on just a single gene.

There is debate as to whether the rates of co-evolution in response to, for example *Bt* genes, is likely to be more rapid than to resistances against other pathogens, such as viruses, or resistance to sprays including *Bt*[3].

There is considerable debate over the effectiveness of agricultural management methods to slow down breakdown, particularly for *Bt*. These arguments are unlikely to be resolved until actual breakdown occurs. There are also differences in opinion about the likely severity of impacts on agronomy and biodiversity where resistance develops, i.e. will resistance to glyphosate be a problem and whether it can be managed.

6.4.5 Is the issue unique to GM?

Not in the sense that disease and pest resistance genes have been bred into crops by both GM and other breeding methods. Similarly herbicide tolerance has been bred in several ways. In fact, non-GM HT is likely to become more common while the difficulties in progressing new GM varieties through the regulatory process persist. Resistance breakdown and HT development is expected to be similar in principle in either case. However, GM's ability to incorporate a toxin throughout the plant means potentially greater exposure and thus greater selection pressure, which is why for Bt the concept of refugia has been developed.

6.4.6 Are there important gaps in out knowledge or scientific uncertainties, and are these important?

- The nature of the toxins underlying the action of 'natural' insect resistance genes.
- The nature and mechanism of 'durable' (non gene-for-gene) resistance.
- Whether non-host resistance can be used as sources of durable resistance.
- The effectiveness of the refugia strategy.
- The relationships between 'fitness' and response to resistance genes.
- The ability to understand and predict weeds shifts associated with the widespread use of broad-spectrum herbicides over growing crops.

[3] Open Meeting. Hails 2003, http://www.gmsciencedebate.org.uk/meetings/pdf/170303-speaker-2.pdf

6.4.7 Likely future developments

Today more than twenty plant disease resistance genes have been isolated. Many of these share just a few similar functional domains, and the predicted products can be classified into just five groups - detoxifying enzymes, kinases, nucleotide binding sites/leucine-rich repeat and receptor kinases.

Swapping resistance genes from one species to another

As more native plant disease resistance genes become isolated it will become possible to transfer these between species. Successful reciprocal transfers of virus resistance gene between tobacco and tomato (Whitman *et al.* 1996, Thilmony *et al.* 1995, Rommens *et al.* 1995) indicate that signal transduction pathways are conserved and that further transfers may be effective.

Designer genes

It may soon be possible to design new disease resistance genes in the laboratory. Several groups are using the comonalties in functional domains of different genes to design genes with novel specificities by mixing and matching domains from diverse resistance genes. Similarly there are indications that transgenic plants with hairpin constructs of segments several topovirus N-genes can confer broad spectrum resistance to a range of topovirus[4] which may open up yet more designer opportunities

Gene pyramiding

The assembly of multiple resistance genes, both GM and non-GM, in single varieties may make resistance more durable but would probably make impacts on non-target organisms (and impacts on fitness of wild relatives via gene flow) harder to predict.

Reducing gene flow

Expression of resistance genes or herbicide tolerance genes in chloroplast rather than nuclear genomes[5] could eliminate the likelihood of gene flow via pollen.

6.4.8 Where there is important scientific uncertainty, what is the way forward?

Resolution of the areas of uncertainty outlined above will aid the development and effective deployment of improved and more predictably durable disease resistance for crops. Time and more targeted and co-ordinated research are necessary.

[4] http://www.embo-keszthely.abc.hu/
[5] GM Science Review Website. Birch 2003, http://www.gmsciencedebate.org.uk/topics/forum/0054.htm

6.5 NEW WEED CONTROL STRATEGIES OFFERED BY GM HERBICIDE TOLERANT CROPS

Will herbicide tolerant crops offer new weed control strategies and, if so, what are the likely impacts, positive and negative?

6.5.1 Summary

The introduction of genetically modified herbicide tolerant (GM HT) crops into the UK would, for the first time, give growers the possibility of using effective broad spectrum herbicides on crops where weed control has up until now been difficult to achieve. These changes could bring with them a range of environmental impacts, positive, negative and benign.

While there may be only modest declines in overall herbicide use, it is nevertheless likely that the more environmentally benign herbicides, glyphosate and glufosinate, could come to replace some of the more damaging herbicides currently in use. Inevitably, however, this benign characteristic does not extend to their impact on target organisms (weeds).

Weed control is potentially simpler for the GM HT grower than conventional cultivation and provides more flexibility, particularly in application dates. By delaying application dates growers could, in principle, use this flexibility to deliver more biodiversity by leaving weeds in fields for longer. However, there is only limited evidence that this can be done successfully, and the longer–term impacts on biodiversity have been questionable.

Evidence from the US suggests that GM HT cropping may favour reduced tillage that can itself deliver some environmental benefits; reduced soil erosion, increased carbon sequestration and potentially increased biodiversity. However, it is unclear whether GM HT cropping in the UK would lead to a renewed interest in reduced tillage, and some of the benefits of reduced tillage could be realised without GM cropping.

There has been a substantial decline in farmland biodiversity in recent decades and it is generally accepted that these declines have been caused by agricultural intensification. There is less evidence to indicate the relative contribution of herbicides *per se* in these declines but there is sufficient knowledge, particularly from studies of birds, to suggest that should weed populations decline further, then species that are dependent upon weeds may be adversely affected.

GM HT cropping may provided more efficient weed control than conventional regimes. However, because most comparative studies have been conducted within a single season, it is unclear whether reductions in weed populations would only be limited to that season or would further exacerbate the documented long-term declines in weed populations, or lead to shifts in weed communities over time.

There have been suggestions that GM HT cropping could be beneficial for biodiversity; these remain speculative. This is because the relative importance of the potential biodiversity gain (itself uncertain) from improved weed populations early in the season, and potential losses from reduced weed seed resources late in the season (generally accepted as likely), and reduced weed populations in the long-term (not yet studied in detail), are largely unknown.

There remains real scientific uncertainty over the impacts of GM HT crops on farmland biodiversity in the UK because few studies have been completed. The publication of the results of the UK government funded farm-scale trials of GM HT crops, established to investigate the impact of the management of these crops on farmland biodiversity, will clarify some of these uncertainties, though others remain to be studied.

Herbicide tolerant crops are being bred by both GM and non-GM methods. Some recent developments in non-GM breeding mal eventually lead to crops tolerant to broad spectrum herbicides. However, in the UK, the possibility of early commercial approval of GM HT crops represents the first major potential deployment of broad spectrum HT crops. Case-specific post-market monitoring and general surveillance is now a regulatory requirement which should provide information on this point for any crops which receive commercial approval for growing in the UK.

Real concerns remain that GM HT crops may represent a further ratchetting up of the intensity of UK agriculture in ways that will further reduce our depleted farmland biodiversity. Although these concerns remain, they are very far from being proven and so this area remains one of scientific uncertainty.

6.5.2 Background

The Corr Wilbourn Foundation Discussion Workshops indicated considerable public concern about the effects of new GM crops on the environment and wildlife in the UK. Amongst others, questions such as *'Is it [GM] destroying nature as we know it?'* and *'What will be the effects on wildlife?'* indicate the types of issues about which some members of the public feel concern over GM crops.

Some regard these worries as ill informed or scare mongering, and it is certainly true that worries about wildlife are anthropocentric in nature[1] and based on value-judgements. However, these concerns are backed up by legal obligations; the UK has international obligations under the EU Habitats Directive, the EU Birds Directive and the Convention on Biological Diversity to safeguard its native biodiversity. In addition, domestic legislation (notably the Countryside and Rights of Way Act 2000, which applies to England and Wales) confers duties on government departments and agencies to have regard to biological diversity throughout the landscape and to promote the conservation of important habitats and species.

6.5.3 Range of views and quality of evidence

The introduction of genetically modified herbicide tolerant (GM HT) crops would offer growers new weed control strategies largely unavailable under conventional agricultural regimes. Crop management involves the use of a broad spectrum herbicide on a growing crop that has been genetically modified to be tolerant to it.

A range of impacts, positive, negative and benign, has been proposed for GM HT crops. The potential benefits of GM HT cropping include: more simple weed control, more flexible weed control (for example delaying herbicide applications), a reduction in the use of persistent herbicides, a reduction in mechanical tillage and weed control, and reduced insecticide use as

[1] Prof. Sam Berry: http://www.gmsciencedebate.org.uk/topics/forum/0058.htm

pests are diverted to non-crop plants (weeds). Some of these potential benefits accrue to the grower, some to the environment more broadly, some to both.

The most important potential disadvantage is that weed control in GM HT crops may be so efficient that it will further exacerbate the declines of the non-crop flora, and those organisms that depend on it [2,3]. Other potential disadvantages, such as gene-stacking are discussed elsewhere.

There are developments in the production of herbicide tolerance by non-GM breeding, in some instances conferring tolerance to broad spectrum herbicides (e.g. glyphosate). Agronomic changes associated with the commercialisation of these could have parallel impacts on the environment. The issue is therefore not specific to GM crops although, in the UK, GM HT crops represent the first.potential major deployment of HT crops and this will remain the case for several years (see Chapter 6.6).

The following sections consider the potential advantages and disadvantages of GM HT cropping in more detail, and examine the evidence to support these assertions.

A quick introduction to GM HT crops

Control of weeds in crops has been a key goal for farmers for centuries. Initially, cultivation, crop rotation and seed cleaning were the principle options. Herbicides were introduced during the 20th Century in the UK, initially to control broad leaved weeds and later for control of grass weeds (Lockhart 1989). Selective herbicides - which kill target weeds but not the crop - have been in practical use in the UK for over 50 years. This selectivity was achieved by chemistry (testing novel compounds on weeds and crops), genetics (breeding from existing crop varieties for greater resistance to herbicides) and through mutation breeding. The advent of genetic modification has allowed the development of crops that allow the use of broad spectrum herbicides which had hitherto only been used in situations where all treated plants were to be controlled (killed).

No GM HT crops are grown commercially in the UK at present, but several are the subject of small scale or farm scale trials. A variety of crops are being studied, but those that are potentially closest to commercial release are maize, oil seed rape (spring- and winter-sown) and beet (sugar and fodder). In any one year, conventional varieties of these crops in Britain occupy around 170,000 ha for sugar beet, 10,000 ha for fodder beet, more than 100,000 ha for maize (forage), and 60,000 ha for spring- and 470,000 ha for winter-sown oilseed rape (Nix 2001, HGCA 1999). However, as these crops are grown as breaks within cereal rotations, the total area of land on which these crops are grown could be at least three times greater.

Each of these crops has been made to be tolerant to a broad spectrum herbicide, most commonly either glyphosate or glufosinate ammonium. When sprayed with these herbicides, the weeds are controlled, but the crop is not harmed. The advantage of using a broad spectrum

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[2] The UK government's statutory nature conservation advisors, English Nature, the Countryside Council for Wales and Scottish Natural Heritage, have a joint position on GM crops (http://www.english-nature.org.uk/news/statement) which states that:

> *'…the use of transgenic techniques, incorporating new combinations of genes into crops and other commercially valuable organisms, may pose additional risks to our natural heritage due to potential impacts on ecological food webs. In addition, there is potential for GMOs to enable changes in agricultural, forestry and fisheries management, which could be detrimental to wildlife.'*

[3] Royal Society Meeting, 2003. Lord May http://www.gmsciencedebate.org.uk/meetings/pdf/110203-transcript.pdf

herbicide is that a range of both broad-leaved weeds (dicotyledons) and grass weeds (monocotyledons) can be controlled simultaneously, rather than using several different herbicides to control these different components of the non-crop flora.

Crops have been modified to be tolerant to a range of other herbicides. For example, oilseed rape and cotton have been modified to be tolerant to bromoxynil, and cotton and flax to sulfonyl urea. In the longer-term, the impacts of herbicide tolerant cropping in the UK may not be limited solely to the effects of glyphosate and glufosinate ammonium or to the products of GM plant breeding.

The potential impacts of weed control strategies offered by GM HT crops

Changes in the use of persistent herbicides

The environmental impact of broad spectrum herbicides

The two broad spectrum herbicides used most commonly in association with GM HT crops – and those that would most likely be used in the UK if approved – are glyphosate (e.g. 'Roundup') and glufosinate ammonium (e.g. 'Liberty'). Glyphosate is a systemic herbicide used for post-emergence, broad spectrum control of annual and perennial broad-leaved and grass weeds, and acts by inhibiting an amino acid metabolism pathway that exists in higher plants and micro-organisms, but not in animals. Glufosinate ammonium similarly provides post-emergence broad spectrum control, but of annual grasses and broad-leaved weeds, and acts by inhibiting an enzyme responsible for ammonia detoxification ultimately leading to the cessation of photosynthesis.

Glyphosate is already widely used in Britain, for example to clear weeds in stubbles before cropping or as a dessicant in oilseed rape, as well as in gardens and industrial sites. Glufosinate is used very occasionally for weed control on oilseed rape and potatoes. Crops that are tolerant to these herbicides allow the use of a single herbicide rather than a combination of several narrow spectrum herbicides, some of which are persistent in the soil. Both herbicides act mainly through contact with foliage, and are broken down rapidly in most soils (i.e. are non-residual).

The environmental impact of glyphosate is considered very low compared to many other herbicides on the market. Two studies (Dewar *et al.* 2003, Hin *et al.* 2001) have used the Millieumeetlat ('environmental yardstick') system (Reus & Leendertse 2000) to guage the environmental impact of glyphosate. In both studies, this system, which considers the toxicity, biodegradability and persistence of pesticides in the soil, rated glyphosate's environmental impact as very low compared to the herbicides used in a conventional beet or soybean weed control programme.

While glyphosate may itself be relatively harmless, some of the surfactants with which it has been formulated (to prevent the glyphosate from forming into droplets and falling off leaves) were somewhat more toxic, acting as irritants. More recent surfactants have none of these toxic effects.

These results broadly confirm those of field and laboratory toxicological studies which have shown that both glyphosate and glufosinate ammonium have low direct toxicity to invertebrates and vertebrates (Breeze *et al.* 1999, Haughton *et al.* 2001a & b, Edwards &

Bohlen 1996). Glyphosate may leach into watercourses, however, where it may present some fairly low risks to water-borne organisms (Hin *et al.* 2001), while glufosinate may be toxic to some soil micro-organisms (Quinn *et al.* 1993, Ahmed & Malloch 1995). Their toxicity is in general much lower than the several herbicides they may replace in controlling weeds in non-GM crops.

Changes in pesticide use

Determining whether chemical pesticide use declines, or is likely to decline, upon the introduction of GM HT cropping is complex (Heimlich *et al.* 2000, Carpenter *et al.* 2002, Hin *et al.* 2001, Phipps & Park 2002). It is even more problematical determining the environmental impacts of these changes in pesticide usage. Not only have different studies adopted different analytical methods, but what should be measured and how? Should it be changes in total herbicide use, or changes in the 'conventional' herbicides that the GM HT system replaces? Both are valid questions. Similarly, should use be quantified as, for example, total area treated or total quantities of active ingredients per unit area, and how can chemicals with different environmental impacts be compared meaningfully?

In Europe, the use of GM HT crops is projected to reduce the overall amounts of herbicide used (Coyette *et al.* 2002), however to examine the actual (rather than projected) impact it is necessary to look to the US or Canada where GM HT soybean and canola (oil seed rape) has been grown commercially since 1996.

The USDA[4] Economic Research Service estimates that overall (ie glyphosate plus conventional) herbicide use - measured as pounds of active ingredient per acre - increased by 3% due to the adoption of glyphosate-tolerant soybean (Lin *et al.* 2001). Between 1995 and 2000 the percentage of the total soybean acreage treated with glyphosate (use rate ~630 g/ha) rose from 20% to 62%, while that of the most commonly used 'conventional' herbicide (Imazethapyr, use rate ~70g/ha) declined from 44 to 12%. Similar trends were noted with other herbicides such as pendimethalin and trifluralin (Carpenter *et al.* 2002). The environmental impacts of these latter two herbicides were rated substantially higher than glyphosate by the Millieumeetlat system (Hin *et al.* 2001).

A separate study (Fernandez-Cornejo & McBride 2000), again of soybean, suggested that glyphosate use (pounds per acre) rose from 0.17 in 1996 to 0.43 in 1998, while all other herbicides combined fell from 1.0 to 0.57; the net result of this was that total herbicide use fell by about 10%. A review of various studies (Hin *et al.* 2001) suggested that change in overall herbicide use on GM HT soybean in the US during 1995-98 varied from a 7% increase to a 40% decrease, depending on the study concerned and the analytical method adopted. In Canada, a detailed analysis of grower experience with a range of HT canola varieties indicated a 39% reduction in herbicide costs compared to conventional cropping (Canola Council of Canada[5] 2001).

There is emerging evidence that weeds in GM HT crops may develop resistance to glyphosate (e.g. soybean; Carpenter *et al.* 2002). For example, within three years of using glyphosate in GM HT soybean at a site in Delaware, horseweed, an annual broad-leaved weed, developed resistance to it (VanGessel 2001). Herbicide resistance is not unique to GM cropping, however, and more than 200 weeds have been reported to be resistant to the herbicides that

[4] United States Department of Agriculture

[5] **Canola Council of Canada** .2001. An agronomic and economic assessment of transgenic canola. http://www.canola-council.org

once controlled them (Carpenter *et al.* 2002). In addition, some weeds, for example late emerging ones such as waterhemp in soybean, are not controlled well by glyphosate (e.g. Baldwin 1999).

Given that one of the potential benefits of GM HT cropping is a reduction in pesticide use (both overall, and of more environmentally damaging pesticides), such problems may lead farmers to increase the rate and frequency of glyphosate application, or to re-introduce older, less benign herbicides alongside it (Bridges 1999, Duke 1999). Indeed, there is some evidence from the US that this is already happening (Baldwin 1999, Owen 1997, 2000); for example, the residual herbicide atrazine is sometimes used alongside glyphosate in controlling pernicious weeds in GM HT corn. The situation in the US, however, may not be the same as in the UK, particularly as agricultural rotations are more diverse in the UK, where farmers are unlikely to adopt US-style rotations with crops that are all tolerant to the same herbicide. The extent to which resistance to broad spectrum herbicides might become a problem in the UK remains unclear.

Thus, the evidence of whether herbicide use overall declines with the introduction of GM HT cropping is somewhat equivocal, although on balance it does seems that modest declines have occurred in the US and Canada in the short term. More importantly perhaps, it is likely that the more environmentally benign herbicide glyphosate has replaced some with higher environmental impacts. In principle, resistance to broad spectrum herbicides could, over time, counter some of these potential beneficial effects; whether or not this would happen in the UK is unclear. However impacts of herbicides on non-target biodiversity are not necessarily related to the amount of herbicide used, but to the efficacy and timing of use of the herbicide concerned, so we must be careful not to confuse inputs of herbicides with their impacts.

The simplicity of weed control

While there may remain some doubt whether GM HT cropping always increases farmers' yields or profits, it seems to be widely accepted that one reason farmers in North America favour GM HT crops is because weed management is simpler (Owen 1997, Firbank & Forcella 2000, Carpenter *et al.* 2002). Instead of using several herbicides to achieve adequate weed control, farmers can use a single herbicide to control a broad spectrum of weeds. On soybean in the US, farmers using GM HT cropping used fewer active ingredients (Benbrook 2001) and made fewer trips over each field, both of which made for easier management (Carpenter & Gianessi 2002). The fewer passes over a field brings with it other potential environmental benefits such as reduced energy costs and emissions. In a survey in Canada, half of all growers suggested that the main reason they chose to grow GM HT canola was because it was easier and provided improved weed control whereas less than 20% did so for higher yields and profits (Devine & Buth 2001).

The flexibility of weed control and potential biodiversity gains

Glyphosate and glufosinate ammonium are sprayed after weeds emerge (post-emergence) and may be more effective in controlling larger weeds than existing herbicides in the rotation. Because of this, the timing of their application is less critical than for conventional herbicides. This gives farmers increased flexibility in weed management; for example not worrying if spray dates are missed due to bad weather. In addition, because these herbicides are applied post emergence, farmers could choose only to spray those areas that most need it, rather than spraying the entire field. In principle, a farmer could wait to see if weed burdens were low in a particular year, and might decide not to spray at all. Such a strategy is less practical with

pre-emergence conventional herbicides. There is little evidence to support this assertion, however, as most farmers seem to follow the herbicide manufacturer's labels, which suggest one or two applications.

Two separate studies, one in Denmark (Strandberg & Pedersen 2002) and one in England (Dewar *et al.* 2003) have used the flexibility in application dates provided by glyphosate to attempt to deliver biodiversity gains. Each of these studies compared the biodiversity associated with plots of conventionally grown beet, with that associated with plots of GM HT beet with varying glyphosate application dates.

In the Danish study of fodder beet, the GM 'Roundup Ready' plots held improved populations of weed flora and arthropod fauna compared to conventionally treated plots early in the season. The greater the delay in application of the first glyphosate spray, the greater the improvements in flora and fauna. Conventional plots held fewer weeds, but at least a proportion set seed; in the Roundup plots no seeds were set following applications of glyphosate. This study was restricted to a single season, so the impact on the weed seed bank, and on weed populations in following crops were not measured, although lack of seed set suggests that routine cropping using Roundup Ready beet could have a dramatic effect on weed population as there would be no recruitment from that season's crop. In addition, food resources for seed predators (e.g. some invertebrates and small mammals and granivorous birds) would be markedly reduced.

The English study (Dewar *et al.* 2003) used band-spraying, in which glyphosate was sprayed along, but not in-between, rows of glyphosate-tolerant sugar beet. By band spraying, those weeds that directly compete with the crop could be controlled - thus ensuring yield losses are reduced - while allowing weeds to grow in the rest of the field. Theoretically, by band spraying over GM HT beet, weeds in between rows could be allowed to grow to a substantial size, before being killed by a second spray of glyphosate over the entire crop. The occurrence of abundant weeds in the crop, it was argued, could provide resources for invertebrates important in the diet of declining farmland birds. Although the design of this study was complex, and the statistical treatments sometimes unclear, there was evidence – across treatments – that when more weeds were left in a plot, numbers of some arthropods were higher. However, this was not true of all faunal groups, nor did the results hold at all study sites. In addition, the evidence that band-sprayed plots specifically held greater weed and invertebrate abundance than conventional plots was weak.

The most potentially interesting result of this study was that band spraying allowed subsequent spraying of the entire crop to be delayed without significant reductions in yield compared to conventional treatments. However, yield was nevertheless greatest in those plots with normal GM management (i.e. early application of glyphosate and no band spraying), which contained lower weed and invertebrate populations than conventional treatments. Thus, despite media coverage of this study - which suggested that GM HT crops would benefit skylarks – it actually demonstrated that unless farmers were willing to risk a yield loss, then the management that they would most likely adopt would reduce weed and invertebrate abundance compared to conventional management. The greater flexibility afforded by such GM HT crops provides an opportunity to explore more creative management regimes, provided they can be enforced.

Unfortunately, this study did not investigate the impact of treatment on seed set and on following crop weed populations.

Changes in the extent of mechanical tillage

Traditionally, mechanical tillage has been used to control weeds and prepare seedbeds. However, it also leaves the ground exposed to wind and water erosion that can carry fertile soil and agricultural chemicals into watercourses (Carpenter *et al.* 2002, Fawcett & Towery 2002). It is argued that GM HT cropping could favour a reduction in mechanical tillage (Carpenter *et al.* 2002, Fawcett & Towery 2002, Firbank & Forcella 2000) as weeds are controlled with broad spectrum herbicides instead, and the subsequent crop could be planted into the stubble of the previous crop without ploughing. Such reduced tillage, it is argued, could provide a wide range of environmental benefits, ranging from reduced soil erosion, through carbon sequestration into the soil to increased biodiversity.

Arguably, the most obvious benefit of reduced mechanical tillage is that it leaves more crop residue on the soil surface, protecting the soil from the erosive impacts of wind and rain (Laflen *et al.* 1985). 'No-till' systems that leave nearly all plant residue in place and can reduce erosion by 90 percent (Hebblethwaite 1995, Fawcett 1994). Because tillage increases the availability of oxygen, it speeds the decomposition of soil organic matter and releases CO_2 – a greenhouse forcing gas – into the atmosphere. A reduction in mechanical tillage increases the ability of soil to sequester carbon, thus reducing CO_2 emissions. A ten-year study in the US showed that the emissions of greenhouse gases were about eight times higher on conventionally tilled land than on no-till land (Robertson, Paul and Harwood 2000). The biodiversity benefits of reduced mechanical tillage are less clear, although some studies have suggested it could be beneficial for wildlife, too. Tillage harms earthworms by burying food sources and destroying burrows, thus earthworm populations increase as tillage is reduced (House and Parmalee 1985). Similarly, several studies have shown that no-till row crops may hold higher densities of birds than conventionally tilled crops (Basore *et al.* 1986, Warburton & Klimstra 1984). Whether or not reduced tillage favours biodiversity more broadly is unclear; some taxa may be favoured others disfavoured. The biodiversity impacts of reduced tillage is currently an active area of research.

There is some evidence that 'no-till' acreage increased following the introduction of glyphosate-tolerant soybean. In the US, no-till soybean acreage rose by 35% (Conservation Technology Information Center 2000) or 111% (American Soybean Association 2001) between 1995 and 2000. In Argentina, it rose by 57% between 1996 and 1999 (James 2001). In each study, the rise in no-till was attributed to the adoption of glyphosate tolerant soybean. In the US, however, the overall level of conservation tillage (of which no-till is the most soil conserving form) has not changed. In Canada, an analysis of grower experience with a range of HT canola varieties indicated a 12% reduction in operational costs associated with less tillage (Canola Council of Canada 2001)

Whether or not GM HT cropping would lead to reduced tillage in the UK is unknown. The use of differing tillage regimes in UK is influenced by soil type (Cannell *et al.* 1978) and so reduced tillage may not be practical in some soil types, even were GM HT cropping practiced. In addition, some of the benefits of reduced tillage (e.g. carbon sequestration) are only apparent when practiced throughout the whole rotation and over long periods. While this may occur in the US, it would be unlikely in the UK where, in the short to medium term at least, only one crop in the rotation is likely to be GM HT - the break crop.

While GM HT cropping may favour it, reduced tillage nevertheless brings a range of benefits – outlined above - that could influence its adoption in the UK in the absence of GM HT cropping, i.e. within conventional agriculture.

Changes in damaging agricultural operations

It has been suggested that GM HT cropping, could favour a reduction in agricultural operations (such as mechanical weeding or number of tractor passes) that may be damaging to ground nesting birds during the breeding season. While it may be the case that agricultural operations can be damaging to nesting birds (e.g. Green 1988), and that fewer herbicide applications are needed in GM HT crops (see above). Studies are needed to deterimine whether or not these changes can lead to increased nest survival.

Pest diversion to non-crop flora

One study (Dewar *et al.* 2000) has suggested that, as GM HT cropping may allow weeds to be maintained for longer than under conventional cropping, aphids may be provided with a larger non-crop food resource, thus potentially reducing damage to the crop and usage of aphicides.

Wildlife food web impacts

Arguably, the most important concern surrounding the introduction of GM HT crops in Britain is that their weed control programs may be so efficient that they will further exacerbate the declines of the weed flora, and the farmland wildlife that depend on it (English Nature[6] 1998, 2000, Hails 2000, Dewar *et al.* 2003, Andreasen *et al.* 1996, Buckelew *et al.* 2000, Watkinson *et al.* 2000). Given its importance, a summary of the evidence of these declines over the last few decades, and their causes, is provided here.

Declines in farmland biodiversity

Evidence

The decline of arable biodiversity has been well documented (Robinson & Sutherland 2002). While the most detailed information comes from birds, there is considerable information on declines in the arable weed flora and growing information on declines of invertebrates.

The long-term (post 1970) declines in population and range of farmland birds first became apparent in the late 1980s (Baillie *et al.* 2001, Gibbons *et al.* 1994, Fuller *et al.* 1995) and subsequently (Siriwardena 1998a, Chamberlain *et al.* 2000). The UK government's headline 'Quality of Life' wild bird indicator summarises this decline very succinctly[7] (Gregory *et al.* 2003) and it continues, albeit at a slower rate than in the 1970s and 1980s.

Although the information on declines in population of arable plants is less detailed, if anything their contractions in geographic range have been even more dramatic (Preston *et al.* 2002), and there are well documented recent changes in plant diversity (Barr *et al.* 1993; Haines-Young *et al.* 2000) and abundance (Smart *et al.* 2000, Wilson 1992, Wilson 1999,

[6] **English Nature.** 1998. Government wildlife advisor urges caution. http://www-english-nature.co.uk/news/story.asp?ID=139 Peterborough: EN.
English Nature. 2000. English Nature continues to back trials of GM crop. http://www-english-nature.co.uk/news/story.asp?ID=195 Peterborough: EN.

[7] http://www.sustainable-development.gov.uk/indicators/headline/h13.htm

Wilson *et al.* 1999, Firbank & Smart 2002). Non-crop plant communities have changed so that, generally, broad-leaved weeds have declined and grasses increased (Chancellor 1985, Firbank 1999). Fourteen of the 62 vascular plants in the UK. Biodiversity Action Plan are exclusive to farmland. Some of these species are now extremely rare in the countryside, whereas a few decades ago they were regarded as important economic weeds of arable systems. In addition, the overall size of the seed bank in arable soils has declined markedly in Britain (Robinson & Sutherland 2002). Such declines in arable flora are not restricted to the UK (e.g. Andreasen *et al.* 1996).

Information on population and range trends among invertebrates are less well documented. The most detailed study of long-term trends in invertebrate numbers – from Sussex (Aebischer 1991, Ewald & Aebischer 1999) – shows that most groups declined. While another long-term study from Rothamsted shows that farmland moths have declined similarly (Woiwod & Harrington 1994), butterflies that occur widely on farmland have increased (even though those with more restricted distributions have declined; Greatorex-Davies & Roy 2001, Asher *et al.* 2001). Bumblebee populations also declined over the last half-century (Williams 1986).

Broadly, across all taxonomic groups, the available evidence suggests that there have been widespread declines in the populations of many organisms associated with farmland in Britain, and that these declines have been most marked among those that are farmland habitat specialists; many of those still common on farmland are habitat generalists (Robinson & Sutherland 2002).

Causes – focussing on the link between biodiversity loss and herbicide use

There is a growing body of evidence that suggests that these declines in biodiversity are related to intensification of agriculture (Robinson & Sutherland 2002). Again, some of the best examples come from studies of birds (Krebs *et al.* 1999, Chamberlain *et al.* 2000, Donald *et al.* 2000), although there is also evidence from plants (Wilson & King 2000, Wilson 1999).

Given that the most direct change in management upon the introduction of GM HT crops would be the more widespread introduction of broad spectrum herbicides, it is important to be able to tease apart the impacts that herbicides *specifically*, rather than a range of other factors (e.g. autumn sowing, loss of mixed farming, loss of farm features such as hedges and ponds), have had on these biodiversity declines. This is not straightforward, as teasing apart these various effects – even within the chemical inputs alone – is best undertaken by experimental studies which are frequently lacking.

The increase in use (particularly in the 1970s and 1980s) and effectiveness of herbicides specifically aimed at removing weeds from cropped areas has resulted in reduced weed populations (Aebischer 1991, Campbell *et al.* 1997, Cooke & Burn 1995, Wilson 1992) and resulting soil seed banks (Jones *et al.* 1999, Robinson 1997, Robinson & Sutherland 2002). The use of herbicides is frequently associated with reduced species diversity and reduced abundance of non-crop herbaceous plant species on agricultural land (Marshall 1991, Jobin *et al.* 1997). Experimental evidence for the effects of herbicides has suggested that they lead to a reduction, rather than an elimination, of weed populations (Cousens & Mortimer 1995). Conversely, experiments in which herbicide inputs are reduced show that the seedbank and flora can recover (Moreby & Southway 1999, Squire *et al.* 2000). Many species that remain common on farmland are either resistant to, or difficult to target with herbicides, or have

prolific persistent seed banks, suggesting that herbicides are likely to be responsible for declines of the remaining species (Robinson & Sutherland 2002).

A wide range of organisms depends on the non-crop plants within cropped areas, and there is evidence that changes in the arable flora can affect these other taxonomic groups. The non-crop vegetation provides resources directly to herbivorous insects, as well as to seed predators such as birds and beetles. It supports many invertebrates that themselves provide food for vertebrates (e.g. Potts 1986, Pollard & Relton 1970) and other invertebrates (Bohan *et al.* 2000). Numbers of some invertebrate groups, particularly carabids and staphylinids can be greater with increasing amounts of non-crop vegetation (Lorenz 1995, Dewar *et al.* 2003, Strandberg & Pedersen 2002) and in the absence of herbicides (Raskin *et al.* 1994). Experimental studies have shown that increases in herbicide applications to cereal crops led to a decline in grey partridge chick survival through the removal of chick invertebrate food host plants (Potts 1986, Sotherton 1991), and this has been the primary cause of the partridge's decline.

The declines of populations of seed-eating farmland birds have received much attention recently. Declines of these species have largely been driven by changes in over-winter survival (Siriwardena *et al.* 1998b, Siriwardena 2000), a period when these species rely heavily on non-crop seeds for food (Moorcroft *et al.* 2002, Robinson & Sutherland 2002). Experimental provision of seed food at this time can increase over-winter survival (Hole *et al.* 2002).

Direct experimental evidence linking the declines of farmland birds to increased levels of herbicide use is available only for the grey partridge. The chain of evidence linking declines in broad-leaved weeds as a consequence of herbicide use, the use that birds make of weed seeds, and the declines in bird populations, however, make a strong circumstantial body of evidence that suggests that further declines in weed seed resources are likely to exacerbate farmland bird declines as well as those of other species dependent on this resource.

Efficiency of weed control

A range of studies in the UK, Netherlands and US have shown that broad spectrum herbicides used in conjunction with GM HT beet (Read & Bush 1998, Strandberg & Pederson 2002, Wevers 1998), maize (Read & Ball 1999a), oilseed rape (Read and Ball 1999b) and soybean (Buckelew *et al.* 2000,Culpepper *et al.* 2000) can provide substantially more efficient and more reliable (e.g. less dependent on weather conditions) weed control than conventional herbicide regimes. By contrast, one study has suggested that weed control is sometimes less successful when growing GM HT varieties than when cultivating conventional varieties (Firbank & Forcella 2000).

Herbicides can also effect the vegetation of field boundaries. One study in England has shown that glyphosate damaged hedgerows and field margins, removing perennial species, allowing colonisation by annuals (Sweet & Shepperson 1998). The application of broad-spectrum herbicides over GM HT crops occurs during the peak growing season in field margins, when they are at their most vulnerable to the effects of spray drift, although, compared to selective herbicides, the timing of application is less critical allowing more choice in weather conditions.

Evidence from the US suggests that GM HT cropping can lead to marked changes in the overall weed community with time, for example favouring those that seed before the broad

spectrum herbicide is applied, or which germinate after herbicide application (Derksen *et al.* 1999, Forcella 1999, Owen 2001). Thus, weeds populations may not be reduced by GM HT cropping, rather they may be changed. The impact that such chronological shifts might have on the taxa that rely on weeds as a resource is unknown.

Effects on other taxa

These changes in the efficiency of weed control could have important knock-on effects on other taxa that are dependent upon them. Few studies have investigated the effects of GM HT cropping on biodiversity; most studies have been small scale with equivocal results (Buckelew 2000, Jasinski *et al.* 2001, Ruiz *et al.* 2001). Given the extent of GM HT cropping in North America, it is a great pity that there are so few published studies on its biodiversity impacts. The likely reason for this is that in North America agriculture and wildlife are catered for in geographically distinct areas, whereas in the UK wildlife and agriculture are expected to share the same ground.

The two most important effects on biodiversity of broad spectrum herbicides could act in opposing directions. The delay in herbicide application could allow more weeds to live in fields for longer early in the season; this might favour invertebrate populations some of which might be important prey for vertebrates. Conversely, the efficiency of control may reduce the number of weeds late in the season, and the number that set seed, thus reducing important food resources for seed predators and reducing weed populations over time.

As outlined earlier, there is a small amount of evidence that delayed weed control can lead to improvements in some invertebrate populations at some sites early in the season (Strandberg & Pedersen 2002, Dewar *et al.* 2003). There is no evidence that these modest increases in invertebrates favoured taxa at the next trophic level. Neither study specifically investigated impacts on those elements of the invertebrate fauna important in the diet of vertebrates, such as birds, even though their diets are well known (e.g. Wilson *et al.* 1999). Neither study investigated the effects on vertebrate populations directly, thus the assertion that improved invertebrate populations might lead to more birds remains hypothetical. In addition, GM HT cropping does not always favour invertebrates; a study in the US showed that invertebrate numbers were lower in plots of glyphosate-tolerant soybean than in conventional plots (Buckelew 2000).

More concerning is the impact on weed seed resources and weed populations. Few studies have compared seed set in conventional and GM HT treatments. In the Netherlands (Strandberg & Pedersen 2002), the effect was dramatic with no seed set at all in GM HT treatments, but some in conventional treatments. No studies have examined weed seed bank and weed populations in following crops to determine the long-term effects of GM HT cropping however, in cases where no seed is set at all then the seedbank will eventually be depleted.

The potential impact of reduced weed seed resources and weed populations on vertebrates has been modelled (Watkinson *et al.* 2000) using the skylark *Alauda arvensis* and the weed fat hen *Chenopodium album*, the seeds of which are an important component of the skylark's diet (Wilson *et al.* 1999). The model allowed calculation of the impact of herbicide use on weed seed production and thus skylark numbers, and concluded that effects on local field use by birds might be severe as fat hen populations declined due to the use of glyphosate. The model showed that the greater the degree of weed control in GM HT cropping compared to conventional, then the greater its deleterious impact on skylarks. More subtly, it also showed

that the pattern of uptake of GM HT crops would greatly affect the overall impact on farmland biodiversity, as the results were dependent on whether farmers with weed-rich or weed-poor fields were more likely to adopt GM HT cropping.

While this model has been criticised for its simplicity (e.g. Firbank & Forcella 2000, Carpenter *et al.* 2002), it provides an elegant insight into the concerns for biodiversity conservation, and modelling of this sort could provide a powerful tool to assess the long term possible impacts of GM cropping on UK biodiversity.

The central issue as to whether GM HT cropping will be more or less harmful to wildlife than conventional cropping revolves around the relative importance of these two contrasting impacts. Will delayed application in GM HT crops allow more weeds, more invertebrates and, for example, improved breeding productivity of birds? Or, will the efficiency and reliability of weed control mean fewer seed resources for seed predators such as granivorous birds, and declining weed populations in the long term? Expressed more simply for birds, is there any point in providing insect food for chicks in the summer that will subsequently starve as adults over-winter because of lack of seeds. Given that populations of seed-eating farmland birds seem to be limited largely by winter food resources (Siriwardena *et al.* 1998, Siriwardena 2000, Hole *et al.* 2003, Robinson & Sutherland 2002), ensuring that abundant weed seeds are retained in the arable environment could well be more important than improving the availability of chick food.

6.5.4 Is there general scientific agreement?

There is general scientific agreement in some areas regarding the impacts that the changed weed control strategies resulting from GM HT cropping will have on the environment, but not in others.

It is generally accepted that the broad spectrum herbicides (e.g. glyphosate) used in association with broad spectrum HT (whether GM or non-GM) crops are more environmentally benign than many of the conventional herbicides that they would replace. This benign character does not extend to the impact on their target organisms (i.e. weeds) and dependent food webs.

There is some debate about whether overall herbicide usage will decline consequent upon the introduction of GM HT cropping, although the emerging scientific opinion – mostly from North America - seems to be that modest declines are likely. Evidence, again from North America, that the more environmentally damaging conventional herbicides will be phased out over time is stronger.

It is generally accepted that GM HT weed control strategies are simpler for the grower than conventional cultivation. Similarly, they seem to offer the grower more flexibility, particularly in application dates. There is no evidence that farmers alter their GM HT weed control strategy based on observed weed burdens, even though this is possible in principle.

While delayed herbicide application dates in GM HT crops could deliver enhanced non-crop biodiversity, there is only limited evidence that it does, and substantial disagreement about whether what it can deliver is important to biodiversity conservation or not.

There is general agreement that reduced tillage can deliver a wide range of environmental benefits, and there is evidence from the US that GM HT cropping can favour reduced tillage techniques. It is entirely unclear whether GM HT cropping in the UK would lead to a renewed interest in reduced tillage as its application is constrained by soil type and other factors.

There is no evidence to judge whether GM HT cropping increases the productivity of ground-nesting birds through reduced agricultural operations.

There is general agreement that there has been a substantial decline in biodiversity in recent decades. The evidence is stronger for birds and plants than for invertebrates. There is growing scientific acceptance that these declines have been caused by agricultural intensification. There is less evidence (particularly experimental), and therefore less general agreement, to indicate the relative contribution of herbicides *per se* in these declines. There is, however, general agreement that the decline in weed seed resources has played a major causal role in the dramatic declines of seed-eating farmland birds.

There is general agreement that GM HT cropping can provide more efficient and reliable weed control than conventional regimes. Crucially there are differing views about how farmers would use GM HT crops in terms of frequency and timing of herbicide applications, so it is unclear whether GM HT cropping will result in more effective weed control. The Field Scale Evaluations may shed some light on this issue.

There is substantial disagreement about the biodiversity impacts of GM HT cropping. This is because the relative importance of the potential biodiversity gain from improved weed populations early in the season, and potential losses from reduced weed seed resources late in the season and reduced weed populations in the long-term, are largely unknown.

6.5.5 Is this issue unique to GM?

The potential changes in weed management strategies outlined above are due to the introduction of different herbicides as alternatives for farmer decisions, and not to the genetically-modified herbicide tolerant crops *per se*. Herbicide tolerant crops have been developed using conventional plant breeding techniques, and thus herbicide tolerance is not unique to GM. For example: atrazine tolerance in corn, triazine tolerance in rape, imidazolinone tolerance in corn and wheat, and chlortoluron tolerance in wheat were all developed using conventional methods (Mazur & Falco, 1989). Recently there is the development of a glyphosate tolerant rygreass by non-GM breeding (Johnston *et al.*1989) that could present similar challenges to those being considered here.

However, GM techniques have allowed the development of several crops that are tolerant to several broad spectrum herbicides, whereas conventional breeding techniques have not so far allowed such radical developments. Because of this, many of the issues surrounding crops that are tolerant to broad spectrum herbicides are currently primarily relevant to GM.

Some of the environmental benefits that may accompany GM crops, such as low-tillage farming and a reduction in use of more harmful pesticides can be accomplished without GM crops. For example, interest in, and use of, low-tillage farming is increasing in the UK

regardless of the GM debate[8]. Similarly, there are alternative ways to reduce the use of some of the more environmentally damaging herbicides that may be replaced if GM crops are given commercial approval. These include introducing pesticide taxes or greater regulation.

6.5.6 Are there gaps in our knowledge or scientific uncertainties, and are these important?

Unquestionably, the largest gap in our knowledge is the impact that GM HT cropping would have on biodiversity. Given the lack of studies in the US and Canada (and, in any case, the different species of wildlife and different approaches to farming that are involved) there is insufficient information from other parts of the world to form a scientifically valid assessment of the impact of the introduction of any particular GM crop on UK biodiversity. Nor would studies from elsewhere in Europe, should they exist, necessarily provide a sufficiently detailed picture to inform UK approvals since species differ in distribution, ecology and status across the EU. Thus, the large-scale crop-specific field trials carried out are important to assess any impacts (positive or negative) of GM crops on UK biodiversity, and ongoing monitoring should form an important part of the process of commercial approval of individual GM crops in the UK.

Given that the UK government is increasingly favouring demonstrably sustainable forms of agriculture, and that it has committed itself – via Public Service Agreements – to reverse the fortunes of farmland wildlife, a much better understanding of the biodiversity impacts is required. Studies that examine the impacts of GM cropping at a field or farm scale and over several seasons are clearly required. Such studies should be undertaken increasingly on land away from agricultural research establishments, thus allowing a better approximation of day-to-day farming practices.

The case for the introduction of GM HT crops would be strengthened if the evidence of reductions in overall herbicide usage were less equivocal. Such data will become available with longer runs of data from North America. Similarly, there is a need for further analyses of long-term changes in usage of conventional herbicides, and importantly the impacts these might have on the biotic and abiotic environment.

It would be useful to further quantify the extent to which GM HT cultivation allows for simpler weed control. It would be valuable, for example, to obtain information from more crops of the number of applications, the number of active ingredients used and the number of tractor passes needed.

An analysis of the likely uptake of reduced tillage consequent upon the commercial introduction of GM HT crops in the UK would be informative, in particularly how this might be influenced by soil type. Further evidence of the biodiversity impacts of reduced tillage would help inform this debate.

Improved monitoring of, particularly, non-avian taxa would strengthen arguments about declines of species associated with the arable environment. In some cases this may be the introduction of new schemes, in others the analysis of existing data. Arguably, much of this is

[8] see e.g. http://www.gct.org.uk/research/icm/frameset.html , http://www.fwag.org.uk/LocalGroups/Gloucestershire/news.html and http://www.pan-uk.org/pestnews/pn24/pn24p3.htm

already in hand. Should GM crops be commercialised, this monitoring must continue to ascertain their impact on biodiversity.

With the exception of maize, the GM HT crops that are nearest to commercialisation in the UK are grown as break crops. If more than one GM crop was grown in a rotation the effects could be cumulative. Because of this a much better understanding of changes in weed populations throughout an entirely GM rotation is needed.

Were GM HT crops commercialised in the UK, it is largely unclear how they would be adopted. Would only farmers with particularly heavy weed burdens adopt the technology, or would it be adopted more broadly? In the US there is evidence of a widespread uptake of GM cropping irrespective of weed burdens (Fernandez-Cornejo & McBride 2002). It would be valuable to ascertain the likely adoption of GM cropping in the UK, only then can its likely overall impacts be predicted.

6.5.7 Likely future developments

The immediate future will see the publication of the initial results of the UK government funded farm-scale trials of GM HT crops (Firbank *et al.* 2003, Perry *et al.* 2003). These trials were established to investigate the impact of the management of these crops on farmland biodiversity in Britain. Three separate crop types have been investigated, beet (sugar and fodder), maize, and oilseed rape (spring and winter-sown). The trials have concentrated on the effects of the broad spectrum herbicides associated with the GM HT crops and contrasted this with the weed management of comparable conventional varieties. The experimental design involved halving fields and sowing half with a conventional variety and half with a GM HT variety of the same crop. Measures of abundance and diversity of a wide range of taxonomic groups were obtained from within the field and at field margins before, during, and after crop growth and in following crops. Fieldwork was undertaken during 2000-03, and results of the spring-sown crop studies are due in late summer 2003, with the results of winter-sown oilseed rape following in 2004.

The results of this study will help provide answers to many of the questions related to the impact of GM HT crops on biodiversity (and more broadly). In particular, the large scale of the FSEs, both spatially (with ca 60-75 fields of each crop type planted throughout Britain on farms of varying intensity) and temporally (over several seasons, with measures taken in following crops) will overcome many of the problems associated with previous studies of the impact of GM HT cropping on biodiversity.

Despite this, the FSEs will not answer all outstanding scientific questions. The FSEs have only studied break crops and maize, and not an entire GM HT rotation, so cumulative effects over many seasons cannot be investigated (although the FSEs will provide some information on continuous GM HT forage maize). The FSEs only studied normal GM practice, i.e. by following the manufacturer’s labels to ensure cost-effective weed control; they do not examine novel techniques (such as band-spraying) that could be developed to favour biodiversity but nor do they study non-compliance of recommended procedure. They compared current conventional herbicide regimes with GM HT cropping. Should conventional regimes change (for example with potential EU legislation phasing out more environmentally damaging herbicides) then the relative impacts of GM versus conventional

may also change. In addition, some of the wider environmental impacts, such as a reduction in tillage, could not be studied.

Some of these outstanding issues are being investigated within the BRIGHT[9] project (Sweet & Griffith 2002). This six-year trial, commenced in 1998, while mainly exploring agronomic issues such as the persistence of HT volunteers and the evolution of multiple tolerance in oilseed rape, is also investigating the impact of broad spectrum herbicides on botanical diversity across rotations.

Given the paucity of UK information on the impacts of GM HT crops on biodiversity, and the imminence of the publication of the initial findings from the FSEs, we will return to this issue in more detail in our second report.

The GM HT crops currently under consideration for UK commercial approval could be followed by GM HT wheat and grass (for both amenity and livestock farming) in several years time. Wheat and grass together cover more than half of UK farmland and therefore much larger areas of land would be concerned. In the case of wheat, GM HT wheat could potentially lead to a reduced need for break crops such as oil seed rape, peas, beans etc in UK agriculture and this would reduce landscape variety, and, almost certainly biodiversity, in the countryside. In addition, being able to grow GM HT wheat in rotation with GM HT oil seed rape could lead to very dramatic and rapid further reductions in non-crop arable flora. GM grasses, particularly but not only GM HT grasses, could also greatly reduce floral diversity on livestock farms. Such potentially major changes to farming practice would be likely to have impacts on biodiversity and these would need to be assessed under current regulations before commercial approval could be given.

6.5.8 Where there is important scientific uncertainty, what is the way forward?

Research

There remains scientific uncertainty over the impacts, positive or negative, of GM HT crops on UK biodiversity simply because few studies have been published to address this area of concern. The establishment of the FSE programme indicates the type of study needed to assess biodiversity impacts at the farm scale. However, as noted above, it is unlikely that the FSE programme will address all of the concerns about GM crops outlined at the beginning of this chapter. The regulatory process includes risk assessment, risk management and post market monitoring steps. The post market monitoring could be an important contributor to the overall understanding of environmental effects as the products are used in practice, in the event of commercial approval. Appropriate measures and indicators for simple and robust monitoring systems could prove valuable both for practical application and to test and improve generalisable mathematical models.

Only a few studies have endeavoured to develop novel management techniques for GM HT crops that specifically favour biodiversity. Should GM crops be commercialised in the UK, then it would be valuable to investigate such techniques further.

[9] Botanical and Rotational Implications of Genetically Modified Herbicide Tolerance

Although it is hoped that the FSEs will provide information on the likely impacts of GM HT cropping on higher vertebrates, for example birds, there would still be merit in developing the Watkinson *et al.* (2000) model, using parameters obtained from the FSEs, specifically. Such a model could potentially provide a powerful general predictor of the likely impacts of new cropping systems on wildlife and farm productivity.

Regulatory

It is possible to imagine situations where harmful impacts on wildlife have not been established beyond doubt (perhaps because none really exists) and yet concerns remain in the minds of the public and some scientists. Cautious commercial approval might be a way forward, which would involve post-release monitoring.

It is sometimes suggested that if GM crops are higher yielding and/or more profitable for farmers to grow then areas could be set aside from active production in order to deliver biodiversity benefits. Such measures would rarely be in the individual farmer's economic interest but could be imposed through the regulatory process through 'cross-compliance'.

6.6 HORIZON SCANNING

Apart from herbicide tolerant crops, what are the major new traits that might give rise to significant environmental impacts, positive or negative?

6.6.1 Summary

Assessment of the timescale and magnitude of new product introductions and their effects becomes more difficult the longer the timescale being considered. AEBC has carried out a thorough horizon scan[1] which illustrates the range of possibilities whilst highlighting the uncertainties inherent in such an analysis.

In the shorter term, most of the products in current registration processes for possible use in the UK are for import use (for food, animal feed or fibre) or for herbicide tolerance. This reflects the international nature of agriculture and food. The environmental impact of these crops in their country of growing is also of interest in informing the public debate. Potential positive impacts from virus and insect resistance are reductions in pesticide use; this is significant and well documented in cotton, more marginal in maize for corn borer resistance and yet to be measured for corn rootworm resistance. Potential negative impacts in development of resistant insect populations are dealt with in 6.4.1 of this chapter. Potential negative effects on non-target insects (Monarch butterflies) have so far been demonstrated to be minimal. Within a 10 year period, there is the possibility of introduction of crops resisting fungal attack (wheat, potato) or viruses (sugar beet, tomato, cucurbits or potato). Potential positive impacts are reduction of pesticide use. Potential negative impacts on non-target organisms such as soil fungi. Crops with improved quality (shelf-life or nutrition) are most likely to be imported.

The potential products from work currently at the research stage cover a much broader scope, but with a longer development time. Arable, minor or tree crops designed to produce specific non-food products (pharmaceuticals, speciality or bulk chemicals, biomass for energy or paper-making) are anticipated. Positive impacts in terms of renewable sources of industrial feedstaocks and diversification of farm crops and sources of rural income might ensue. Negative impacts could arise from direct effects of the novel crops on wildlife, or indirect effects on patterns of land use arising from large-scale adoption of such crops.

Traits with the potential to improve crop production in marginal environments (eg tolerance of drought, heat or salt stresses) could be anticipated to have major benefits to growers in those environments, including the developing world. Potential negative impacts could be direct, from making crops more successful as weeds or indirect from the changing the economic drivers to improve and cultivate areas with wildlife and conservation value. An example could be a highly productive grass which changed hill farming productivity.

The horizon scan has identified the paucity of baseline data and models at different scales from field to landscape agro-ecological systems as the basis for future assessment of larger scale environmental effects which could be useful across a broad range of policy making issues relating to land use and the rural economy. Most of the issues foreseen are not unique

[1] **AEBC.** 2002. Looking Ahead: An AEBC Horizon Scan.
http://www.aebc.gov.uk/aebc/reports/horizon_scanning_report.htm

to GM and will be driven by the economic decisions relating to the context of UK farming and food production. These are largely political rather than technical factors.

6.6.2 Background

This section addresses the potential environmental impacts of new[2] (at least to the UK) GM crops and products. The Agricultural and Environment Biotechnology Commission (AEBC) has undertaken a horizon-scanning exercise in 2002, to examine future developments (AEBC. 2002), much of which is still very relevant. Many website contributions have addressed this area as well as being covered in the public meetings[3] .

New crops and plant products are considered in three groupings.

- Crops and traits already commercialised or in late registration somewhere in the world.

- Crops and traits for which some product-related information is already available and there will have been some history of environmentl release. These are likely to be commercialised later in the 10 year horizon.

- Finally, traits which are currently in the research or experimental phase are more most likely to be commercialised, if at all, on a longer time horizon. .

In each of these areas the amount of field information from the UK is likely to be limited or non-existent. We have not considered GM microbes or animals.

6.6.4 Range of Views and Quality of Evidence

Earliest commercialisation: crops and traits already commercialised or in late registration elsewhere

The crops and traits in the earliest category of potential commercialisation listed on the agbios website[4]. Apart from herbicide tolerance traits they are likely to be :

(i) Maize with insect resistance (European corn borer and other Lepidopteran pests)
(ii) Maize with resistance to corn rootworm (soil coleopteran pest[3])
(iii) Cotton with insect resistance
(iv) High yielding oilseed rape hybrids
(v) Squash with virus resistance

The major environmental impacts anticipated with these developments are considered below.

Insect resistance (traits i-iii):

At present these traits are not directly relevant to the UK

[2] All traits considered are new to the UK, but are not confined to those of potential UK commercial importance. Some of the traits considered are currently being grown commercially by other countries.
[3] Refer to GM Scienec Review http://www.gmsciencedebate.org.uk/topics/forum/default.htm for full list.
[4] http://www.agbios.com/dbase.php?action=ShowProd&data=MON63

Potential Positive Impacts: reduced insecticide use The existing information from multi-year field and commercial experiences with cotton (USA, Australia) has supported the contention of reduced insecticide use (Phipps *et al.* 2002**,** Gianessi *et al.* 2002[5]) and web contributions [6, 7, 8.] This has also been seen in South Africa (Thomson J. 2001) and India[9]. In addition, higher yields of maize and better returns to farmer where insecticide use to control European corn borer is uneconomic (Gianessi *et al.* 2002). Higher yields of cotton and better economic return to cotton farmers in US, china, South Africa and India .
Potential Negative Impacts: Development of resistant insect populations (see 6.4 which deals specifically with this issue). Insect resistance management tools have so far avoided this eventuality in USA and Australia (Tabashnik *et al.* 2002, Monsanto. 2003). Potential Effects on non-target insects and predators has been a major cause for concern, based on lab studies (Losey *et al*. 1999), but subsequent field-based research has shown a neutral or positive impact, for instance in sweetcorn (Musser *et al.* 2003), maize (Pimental *et al*. 2000), and cotton (Carriere *et al*. 2003).

High yielding varieties (iv)

The high yielding oilseed rape is designed for increased productivity from hybrid vigour. The trait itself provides cost-effective production of hybrid seed through a sterility mechanism (Mariani *et al*. 1990).
Potential Positive Impact: Increased productivity and farmer income, more efficient land use.
Potential Negative Impact: Gene flow of sterility system components. Section 7.3 in the gene flow addresses this issue, where two key questions were raised. (i) Is the segregation of the sterility genes in pollen from the F_1 hybrid plants going to lead to an enhanced gene flow? And (ii) Could the sterility genes cause harm to populations of wild relative?

Virus Resistance (v)

Squash with virus resistance has been commercially grown in USA for some years. The potential is there for varieties to be developed for EU markets by backcrossing from the same events .
Potential Positive Impacts: Indirect effects could be seen in (a) reduction in pesticide use to control insect vectors (tomato/cucurbits/potato) and potential to work alongside biological control methods in glasshouse/ contained crops.
Potential Negative Impacts: Virus recombination leading to new diseases. The specific issues (primary effects) for virus resistance are dealt with in section 6.4.3.

Potential commercialisation within 10 years

Agronomic traits: Virus resistance (Sugar beet, Tomato, Cucurbits, Potato)

The specific issues (primary effects) for virus resistance are dealt with in Gene Flow topic five and above. An additional potential benefit in broad acre crops such as beet, potato or field

[5] Gianessi LP, Silvers CS, Sankula S, Carpenter JE. 2002. Plant biotechnology: current and potential impact for improving pest management in US agriculture: an analysis of 40 case studies. http://www.ncfap.org/40CaseStudies.htm
[6] GM Science Review Website. Halford 2003 http://www.gmsciencedebate.org.uk/topics/forum/0049.htm
[7] GM Science Review Website. Michael 2003 http://www.gmsciencedebate.org.uk/topics/forum/0086.htm
[8] GM Science Review Website. Monsanto 2003 http://www.gmsciencedebate.org.uk/topics/forum/0076.htm
[9] GM Science Review Website. Leaver 2003 http://www.gmsciencedebate.org.uk/topics/forum/0037.htm

tomatoes is in greater choice of rotations and options for crop growth where limited by virus disease at present (eg beet/rhizomania). Virus resistance is seen as potentially beneficial in developing world agriculture, and is the subject of research using locally adapted cultivars of rice (Pinto *et al*. 1999), potato (Murray *et al*. 2002) and cassava.

Fungal tolerance: Wheat and Potato.

Potential Positive Impacts

disease resistance could impact on fungicide spray regimes and provide more robust and long lasting control in the face of evolution of resistant pathogen strains. Commercial development of fungal resistance has lagged behind insect and virus resistance, and has proved technically challenging (Stuiver *et al.* 2001).

Potential negative impacts

Non-target effects are potential impacts of a fungal resistance trait on soil microbes and mycorrhizal fungi during crop growth have been investigated in field trial situations, where no effects were detected (Impact Consortium. 1999, Glandorf *et al.* 1997) however, there is not a major literature in this area. Gene flow giving rise to altered fitness of weeds. is dealt within section 7.3.

Quality/End Use Traits:

- Potato: industrial starch; highly digestible grass and maize
- Nutritionally enhanced vegetables (Tomato)
- Shelf life extended banana
- highly digestible grass and maize
- 'Designer' oil and fat molecules in oilseed rape

The direct effects of these traits are likely to be small because agronomic and growth characteristics are not targetted. However, this category covers such a large range of possible products and transformations, that it is hard to make generalisations about environmental impacts. One web contribution has emphasized the perils of any generalisations in this area[10]. Increased feed digestibility of forage grasses and maize might have benefits in terms of productivity and reduced wastage in animal nutrition, extended shelf life banana could have benefits in reduced wastage and transport costs but these potential benefits have yet to be quantified. Transfer of a gene altering major structural components or slowing maturation to wild relatives of a crop that can out-cross might be of ecological significance.

Later commercialisation: traits and target areas in research

The pace of scientific research at a fundamental level has accelerated over recent years. Publication of whole genome sequences for the model plant *Arabidopsis thaliana* (The Arabidopsis Genome Initiative. 2000) and rice (Goff *et al*. 2002), and various microorganisms enables a more complete cataloguing of genes involved in any particular process. Potential application are also being explored both from a classical breeding and biotechnology approaches. Web contributions were received which considered future potential applications across a wide range of targets from crop productivity, yield and quality through to the improvement of human nutrition or the production of industrial and pharmaceutical products[11, 12, 13, 14, 5]. Dunwell's contribution[15] highlighted the use of IP databases (IP

[10] GM Science Review Website. Tester 2003 http://www.gmsciencedebate.org.uk/topics/forum/0065.htm
[11] GM Science Review Website. Klurfeld 2003 http://www.gmsciencedebate.org.uk/topics/forum/0013.htm
[12] GM Science Review Website. Murphy 2003 http://www.gmsciencedebate.org.uk/topics/forum/0011.htm
[13] GM Science Review Website.Cummings 2003 http://www.gmsciencedebate.org.uk/topics/forum/0031.htm

Database) and field trial applications (Biotechnology and GMO information website) as sources outside the standard literature for understanding what might be in early research. Nonetheless it is impossible to provide definitive answers to the request [16] for clarity on what is possible and will be delivered for the UK citizen. The potential for developing countries was also the subject of web contributions [12, 17].

Seven areas of research which might have environmental impacts, positive or negative, are described below to demonstrate some of the breadth of potential outcomes and issues.

Resistance to abiotic stresses

Plants have evolved a wide array of approaches to respond to abiotic stresses such as frost, heat, drought cold or salt. Research to understand these mechanisms can also proved new options to transfer these traits into crops, as well as providing novel genes for yield improvement. Examples include improvement of salt-tolerance in tomato, although not relevant to UK (Zhang *et al*. 2001), improvement of tolerance of aluminium in soils (Lopez-Bucio *et al*. 2000), improvement of yield from altering photosynthesis or grain starch synthesis (Ku *et al*. 2001, Smidansky *et al*. 2002) Changing the tolerance of crops to abiotic stresses could allow new crops to be grown in the UK, for instance cold-tolerant sunflowers. In addition, predictions of climate changes in the UK (Downing *et al*. 2003) suggest that UK crops will need to become more tolerant of drought in the future.

Potential Positive Impacts

Crops with enhanced tolerance of different stresses enables more flexibility within agriculture and leads to more productivity in problem soils or situations (this may be particulary important in developing countries where poor soils are widespread (Thomson. 2001, Morris. 2003), also relevamt to degraded or desiccated soild in the UK. More attention could then be paid to other objectives such as maintenance of biodiversity.

Potential Negative Impacts

Could enables agriculture to move into new areas that were previously marginal and thus might also be of ecological interest – salt marsh is a key example (although in UK this is very unlikely as the majority of these areas have statutory protection). Adaptive traits such as salt and drought tolerance might also confer on crops, and sexually compatible relatives, an ability to become weeds in these marginal areas

Plants as Renewable sources of industrial feedstocks and energy crops

Cost effective agriculturally-sourced materials to provide renewable supplies of industrial feedstocks such as bulk and speciality chemicals or energy crops are the subject of active research within the EU and elsewhere[18].

Potential Positive Impacts

Replacement of fossil fuel sources of energy and feedstock; new sustainable crops and income for farming communities.

Potential Negative Impacts The potential areas required for growth of such crops could be large which would mean changes to the pattern of agriculture and indirect effects on wildlife and landscape (positive or negative).

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[14] GM Science Review Website. Lamb 2003 http://www.gmsciencedebate.org.uk/topics/forum/0066.htm
[15] GM Science Review Website. Dunwell 2003 http://www.gmsciencedebate.org.uk/topics/forum/0020.htm
[16] GM Science Review Website. Gene Watch UK http://www.gmsciencedebate.org.uk/topics/forum/0008.htm
[17] GM Science Review Website. Harris 2003 http://www.gmsciencedebate.org.uk/topics/forum/0068.htm
[18] Biomatnet , Murphy. 2003 http://www.oit.doe.gov/agricu lture/

Plants as Factories for Pharmaceuticals

As well as being potential production systems for large scale chemicals and feedstocks, plants are also being considered for production of pharmacetical and other high value proteins. Examples of these applications are the production of antibodies, cytokines and edible vaccines[19, 9, 10, 11]

Potential Positive Impacts

Development of high value speciality crops to improve crop choices and increase farm incomes. Potential to develop rural livelihoods where processing is co-localised. Use of non-animal production systems reduces likelihood of spread of animal disesases; cost effective production of vaccines or antibodies for developing world uses.

Potential Negative Impacts

Containment of the genes and segregation of the speciality crops will need special consideration to keep separate from crops in the food chain. This is a major concern of web contributions [16, 11] as well as being recognised in a number of broad-based science and regulatory reviews (ICSU. 2003), although not without it's critics (Miller, 2003). Impact on wildlife of eating the speciality crop might be harmful, although many would be grown in containment. Potential positive or negative biodiversity effects from introduction of introduction of specialised minor crops for production purposes.

Forest biotechnology

Crops which are particularly slow or difficult to improve through breeding, such as trees, have the potential for improvement through biotechnology. Research is underway to understand and modify the reproductive cycle of trees, to improve tolerance to some herbicides and to change the quality of wood to improve the quality for paper making. (AEBC. 2002, O'Connell *et al.* 2002, Pilate *et al*. 2002, Weizel *et al*. 1995). The environmental impact assessments of GM trees both positive and negative, will raise similar questions to other crops, but there may be special considerations also.

Potential Positive Impacts

GM trees might be a more productive and a renewable source of fuel and forest products, reducing pressures on native forests. Better paper making quality could reduce the environmental impact of this process (Pilate *et al*. 2002).

Potential Negative Impacts

Changing the economics of forestry might encourage extension of managed forestry to previously marginal or ecologically significant areas. Wildlife and amenity aspects of new forest areas are likely to require careful consideration given the scale of land which could potentially be used. Repoductive characteristics of modified trees could also provide important challenges; pollen and seeds are important sources of food for wildlife, and on the other hand, pollen and gene transfer to related species could be an issue. Genetic isolation mechanisms that involve sterility could therefore have adverse effects on wildlife.

Phytoremediation

Using plants to reclaim or clean up pollution is a growing research and commercial area. Plants modified to metabolise, accumulate or tolerate polluted soil, for instance containing arsenic or TNT have been described at a research phase (Dhankar *et al*. 2002, Hannick *et al.* 2002, Biomatnet). Regulatory challenges are being considered (Flechas *et al*. 2003).

[19] GM Science Review Website. Genewatch http://www.gmsciencedebate.org.uk/topics/forum/0009.htm

Potential Positive Impacts

Plants may be able to tackle pollutants that are not susceptible to non-GM remediants. Phytoremediation techniques are particularly pertinent on soils which are contaminated with metals and organic compounds: in the UK this applies to 50% of contaminated soils.

Potential Negative Impacts

These are related to final disposal of the plants grown in contamined soils. (AEBC. 2002). There might also be a concern that efficient cleanup techniques could lessen the regulatory pressure on control of pollution in the first place.

Grasses in agriculture and amenity uses

Grazing, golfing and gardening all have significant environmental impact; many species of grasses are of economic and environmental importance. Application of GM technology to grasses has been reviewed (Wang *et al*. 2001). Targets for modification have included productivity traits for commercial grasslands, herbicide resistance to improve golfcourse management (AEBC. 2002 ref 105,106) and the removal of a major pollen allergen from rygrass (Bhalla *et al. 1999*) . An interesting benefit/risk scenario to explore might be drought-resistant turf grasses – good in terms of reducing water use, but potentially bad if crossing to wild relatives occured.

Potential Positive Impacts

Improved productivity for animal production could make marginal farming more economic; improved management of amenity grass areas such as reduction in mowing or weed control costs, reduction of water use associated with drought tolerance.

Potential Negative Impacts

Highly productive grasses might as an indirect effect encourage pasture improvements and more intensive methods in marginal areas; traits with selective advantages such as drought or salt tolerance might alter grassland ecology if genes introduced into wild grasses. All grasses are wild in the sense that they outcross to the same species; some are very promiscuous and outcross to many other species and genera (Wipff and Fricker, 2000).

Horticultural and minor or exotic crops

Improving the current major crops has been the early target of GM research, however, improving horticultural and minor or marginal crops with special properties (eg flax, lupins and new oilseed crops such as Lunaria) such that they can become a real economic option in a diverse and successful rural economy is a significant research possibility. An interesting example of this is the maintenance of the papaya growing economy in Hawaii and elsewhere in the face of the spread of Papaya ringspot virus was achieved by introduction of virus resistant lines (Ferreira *et al*. 2002). Horticultural crops such as tomato, banana, strawberry and peppers have a high demand for quality in the market place. Modification of ripening characteristics has been widely studied (AEBC. 2002, James. 2003). Modification of fruit trees could be a route to preserve local varities with specific heirloom quality traits while bringing improvement to specific agronomic weaknesses limiting their current potential[20].

[20] GM Science Review Website. James 2003 http://www.gmsciencedebate.org.uk/topics/forum/0029.htm

Box 6.4 Case study of Potato Cyst Nematode (PCN) research and benefits

Potato cyst nematode (PCN) in the UK is a very serious problem occurring in 64% of potato fields in England and Wales and causing annual yield losses of approximately £43 million between 1990-1995. Resistance to nematodes is a very strong example of the clear benefits of a GM technology for the UK.

- The current control of PCN in the UK is based on oxime carbamates/carbamates that are highly toxic to most animals. One (Temik or aldicarb) will be withdrawn by the EU in a few years. The future of the other main chemical (oxamyl/vydate) is uncertain. Aldicarb is very water-soluble, becomes stable in groundwater and kills soil animals e.g. earthworm populations and have the potential to kill birds if not used correctly. There is even more concern about nematicides in a developing world context.

- A GM approach developed by the University of Leeds involves a plant gene naturally expressed in rice seed. Similar proteins are found in maize seed, egg white and saliva. These proteins inhibit cysteine proteinases and they are termed cystatins. They interfere with the nematode's ability to digest its dietary protein.

- Several field trials in the UK have established that the cystatins provide a useful level of resistance when expressed in potato (Urwin *et al,* 2001), The resistance has recently been shown to stack with natural partial resistance and obtain full control of PCN (Urwin *et al.* 2003)**.** Preliminary biosafety studies indicate that soil microbes, earthworms, aphids and leafhoppers are not affected by plants expressing cystatins.

- The technology has potential against many nematodes worldwide. It is donated for many developing world applications. It could provide benign control of nematodes that reduce current yields of subsistence growers and reduce exposure of agricultural workers to hazardous compounds

6.6.4 Is there General Scientific Agreement?

There is most scientific agreement in relation to the possibilities of particular technological goals being achievable at an experimental scale. The pace of scientific developments is such that many things are possible, and laboratory proof of principle has been achieved for a great many traits.

This broad scientific agreement on possibilities breaks down when possibilities are turned into products.

Firstly, the likelihood, timing and scale of a particular product being developed in the UK is subject to a wide variety of views as to likelihood, timing and desirability [21,16, 11] .There is an enormous gap between a proof of principle which might be written up in a patent application or publication in a refereed journal and a product being placed on the market. The nature and pace of introduction of GM plants into UK agriculture and horticulture is therefore hard to predict. This aspect of uncertainty is covered in the study of economic impact of the Strategy Unit. The most far off products have the most uncertainty as to likelihood of commercialisation. The pace of development of UK-specific expertise in both developing crops for specific environmental applications and testing in real life is not rapid at present so much of the progress and direction will be from elsewhere with the UK reacting to developments and being a secondary market.

Secondly, there is less agreement on the environmental impact of potential future products. Hazard, likelihood and benefit are all subject to argument, both in terms of significance in relation to any regulatory system and in terms of the appropriate tests to carry out for regulatory clearance. There are no international bodies analogous to the Codex alimentarius Commission for food regulation (Miller. 2003).

Hazard: what are the hazards, how broad to cast the net in relation to indirect effects; how much environmental value to place on weeds and pests which are part of an ecological food web. What tests and trials should be used to monitor and assess these effects? How can the tests be made non-discriminatory between technological approaches?

Likelihood: What meaningful tests can be adopted when recognising that proving zero likelihood is not possible as a regulatory or scientific goal

For the products in catagories A and B, the key scientific uncertainties are the extent to which experience elsewhere in the world is a good guide to the environmental impact in the UK. Subsidiary issues where views differ is the extent to which the environmental impact of crops which are imported to the UK should be considered at all (for instance in considering insect-resistant maize where the targeted pests are not found in the UK), and the question of how EU regulations impact decisions in developing countries (Morris. 2003, Miller. 2003). A case-by-case evaluation still seems to be the most robust approach in considering each potential product.

6.6.5 Are the Issues Unique to GM?

Not in most cases. All the targets listed are largely independent of the technology approach and are addressed currently through conventional approaches to improvement (even for pharmacetical production, the environmental impact of current production systems is a regulated aspect of the manufacturing process). What is different is the potential of significant step change outcomes which could move the trait/crop properties “forward” in a dramatic way. Another aspect of this is that a GM trait would be visible to the regulatory regime in a way that a conventionally bred salt tolerant variety or an exotic introduction (eg new interspecific willow or grass hybrid) may not be. In such a case, the issues would be addressed in the regulatory review process (see chapter 3).

6.6.6 Are there gaps in our knowledge or scientific uncertainties, and are these important?

One web contribution specifically lists uncertainties and gaps[21] after assessment of the literature in 2000. This study identified Controversies (questions not answered unequivocally) and or Gaps (questions not receiving adequate attention) in relation to the following topics.

- Effects on biodiversity
- Effects on sustainability

[21] GM Science Review Website Greenpeace http://www.gmsciencedebate.org.uk/topics/forum/0026.htm http://www.gmsciencedebate.org.uk/topics/forum/0083.htm

- Effects between neighbouring agro-ecosystems +/- GM crops
- Predictability of environmental effects

Other more detailed science questions have been suggested during the review process, and have considerable overlap with this list:

- Agro-ecological data and models of farming systems as baselines for assessing potential changes and impacts.

- Indirect and non-target effects of pest, disease and abiotic stress resistance traits: methodology and simple, robust assays for early stage evaluation.

- Soil ecology and function data and models as baselines for assessing potential changes and impacts

- Scale up: understanding of impact of gene flow and other effects from commercial-scale crop use particularly when applied to crops beyond the well-studied current crops. Use of this information in development of robust testing approaches in research and development.

- Impact of introduced genes upon ecological fitness of wild species.

6.6.7 Likely Future Developments

This topic area is concerned entirely with future developments. Suffice it to say that it is likely that the pace of scientific developments will continue to be rapid, generating more demonstrations of principle both of how plants work and of ways they could be changed for the benefit of humans and the environment either through genetic modification or a range of "smart" breeding approaches enhancing current tools and methods. The regulatory system will need to be able to assess the impacts of combinations of gene effects and also traits providing more profound changes to crop biology.

6.6.8 Where there is Important Scientific Uncertainty, what is the Way Forward?

Science

The scientific opportunities to research the gap areas listed above are quite open ended and challenging, and not really unique or specific to GM.. On the one hand case-by-case review is recommended for particular environments, and on the other general background data, models, methods, protocols and approaches are required to underpin a science-based international regulatory regime. And many of these factors are simply not available for environmental assessment, unlike food and animal feed safety

Baseline data and models of agricultural ecosystems would help in policy and decision making within the UK agri-environment context.

Simple robust tests of relevance to environmental impact would be useful in research and commercial practice

Regulation

The experience from centuries of conventional agriculture and the existing science base allows the regulatory system to foresee some of the general principles to be considered in assessing environmental impacts of new GM crop developments. However, science has been developing rapidly over the last century, and correspondingly, the ability for new approaches and methodologies to understand environmental impact at a deeper level has brought the opportunity to re-evaluate understanding and accepted practices.

Nevertheless, the key uncertainties around environmental impact are likely to beprincipally indirect. Economic factors (at micro and macro level) will drive decisions at farm and regional level and hence lead to potential indirect effects. Changes in EU agricultural regimes are likely to be far more significant causes of such indirect effects.

6.7 CHANGES IN AGRICULTURAL PRACTICE

Might GM crops change agricultural practice in the UK? If so, what might be the likely consequences?

6.7.1 Summary

It is widely acknowledged that modern (non-GM) agriculture has already had significant negative impacts on biodiversity and the wider environment in the UK. Large changes over the last century, including recent decades, in the way that farmland is managed have resulted in a decline in both on- and off-farm plant, invertebrate and bird abundance and diversity. The species that have been hardest hit are specialists of the arable environment, which thrive in very particular habitats, though intensification has made some commonplace species much rarer.

GM technology might have the potential to increase biodiversity and reduce some environmental impacts of farming, such as pesticide applications although as yet these benefits have not been demonstrated in the UK. Alternatively it may intensify agriculture with detrimental effects on biodiversity.

It is impossible to state categorically what will happen to agricultural practices following the adoption of GM crops in the UK. Overall, the consequences will depend on the nature of each individual product and what farmers, the public, and policy makers decide. Due to this uncertainty, many of the potential changes it could bring about in agriculture are speculative. There is a major need for policy makers to understand how these factors are likely to interface with the new technologies, because they will need to predict outcomes from the environment if targets are to be delivered.

If GM crops are grown in the UK, the farming system most likely to be affected by the technology will be the sector that benefits the most economically. At present it is thought that this is likely to be arable and mixed lowland farming, because they are currently the most productive and potentially profitable sectors of agriculture.

GM technology might have the potential to increase biodiversity and reduce some environmental impacts of farming, such as pesticide applications. It may intensify agriculture, with possible detrimental effects on biodiversity. Alternatively, this intensification may have the effect of reducing the amount of land dedicated to crops, leaving the rest of the land for other purposes, such as nature conservation.

6.7.2 Background

Almost every habitat in the United Kingdom is affected by farming. Of the 24 million hectares in the UK, 19% are crops and bare fallow, 48% grass and rough grazing, 3% other farm use, 11% forest and woodland, and 18% urban land and that used for transport, recreation and non-agricultural use (e.g. sand dunes, inland water, grouse moors).

Farmland is therefore a very important habitat for wildlife. Any changes in agricultural practice in the UK, whether GM or non-GM (crops and varieties grown, rotations, intensity of

agriculture, herbicide and pesticide applications, cropping patterns, number and nature of field operations) will have effects on the wildlife within and surrounding that habitat.

Drainage and increased fertilizer use have led to losses of floristically-rich meadows and an increase in grass monocultures, overgrazing of uplands by sheep and deer has reduced species diversity, herbicides have reduced diversity of flowering plants in arable fields and led to some formerly abundant arable weeds now being classified as extremely rare (Wilson and King, 2003). Farmland birds have particularly suffered: the populations of nine species fell by more than a half between 1970-1995 (Pain and Pienkowski, 1997; UK Biodiversity Steering Group, 1995, 1998, 1999). This was discussed at the Royal Society meeting[1].

This is the backdrop for further technological change in agriculture – whether it be GM or non-GM. Farming has always shaped rural biodiversity and the countryside, and has already had far-reaching and fundamental effects (Jenkins, 2002; Pretty, 2002; Robinson and Sutherland, 2002). Some 25 of the 200 species of British arable plants are now nationally scarce, and a further 24 are of conservation concern (Johnson, 2000). Farmland bird diversity and biomass has fallen, with the populations of at least 13 species now considered so low that they need special protection (Siriwardena *et al.* 1998). The key question is: would the adoption of GM crops (and the crop management choices they provide) increase, slow down, or reverse the rate and direction of change while contributing to improvements in farm productivity and efficiency?

6.7.3 Range of views

The main purpose of the first generation of GM crops is to give farmers more, easier and cheaper options for control of pests, diseases and weeds. Giving greater control could mean either benefit or harm to biodiversity, depending on the farmers' objectives, and market and policy drivers.

It may be possible to manage GM crops in such a way that some weeds and the insects associated with them are left for birds but the evidence for this is at present limited (Dewar *et al.* 2000); such methods may have associated crop yield losses. But it is equally possible that GM HT or insect resistant crops may produce even more weed-free and invertebrate-free fields. If GM crops are introduced to the farms that already have very low residual weed numbers in their fields, it will have little impact on bird populations. However, if those remaining farms with weed-rich fields or field margins grow GM crops, they may become weed-free and pest free, thus decreasing the reservoirs of food and cause bird numbers to drop even further (Watkinson *et al.* 2000). It is because some of the current GM HT crops currently under consideration for commercial approval in the UK, (e.g. fodder beet), are known currently to be weed-rich compared with others such as autumn-sown wheat that wildlife conservationists have concerns about their use. (See section 6.5 for detailed discussion on new weed control strategies offered by GM HT).

While some GM technologies may lead to reduced agrochemical use, benefiting biodiversity and water quality, others could result in greater use of agrochemicals (ERS-USDA, 1999; Dewar *et al.* 2000; Elmore *et al.* 2001; Huang *et al.*2002; Pray *et al.* 2002). There may be an increased uptake of environmentally beneficial farm methods, such as zero or minimum

[1] Royal Society Meeting, Watkinson, Vickery, http://www.gmsciencedebate.org.uk/meetings/pdf/110203-transcript.pdf

tillage, which though requiring more herbicide, will lead to improved soil carbon storage and reduced run-off pollution (Renwick *et al.* 2002).

This section is based on an analysis of the potential changes to three UK agricultural sectors. The farming system most likely to be affected by GM technology is likely to be the one that benefits most economically (Countryside Agency, 2002). All traits are considered in a post-commercial approval scenario, which will differ in their timing and impact from crop to crop.

Arable and Mixed Lowlands

These systems are likely to show the greatest changes, largely because they are the most productive and potentially-profitable sectors of farming, and so would be the target of commercial enterprises developing GM crops. Over the first few years after commercial approval[2], the following changes could occur in farming systems:

- Herbicide-tolerant (HT) oil seed rape and fodder beet would come into common use;
- Non-GM alternative crops, as import substitutes, could also become more common, with soya, lupins and beans/peas replacing GM products from the USA and Latin America. Alternatively these protein crops could be imported from other non-GM growing countries.
- Trees with altered lignin/ cellulose ratios for paper production.
- High-value pharmaceutical and nutraceutical crops could be grown, but only on a relatively small scale.
- Fungal-tolerant potatoes and wheat could come into commercial use, thus reducing the need for fungicide applications.
- GM cereals could be more common, particularly those with HT and insect resistant traits. Their use would be greater if they have been shown to reduce the use and impacts of herbicides and insecticides, and if reduced-tillage systems become more popular, thus leading to benefits for the environment and for lower farm costs.
- High nitrogen-use efficiency in wheat and potatoes (currently far from development as a commercial possibility) could reduce the need for nitrogen fertilizers, so benefiting the environment through reduced nitrate leaching and nitrous oxide emissions, as well as reducing farm costs.
- Increased cultivation of insect and disease resistant vegetables and flowers is possible, thus reducing some pesticide use.

Crop rotations might become more diverse, as GM traits could increase the economic value of some crops (e.g. oats and legumes) and might therefore increase the likelihood of farmers cultivating them in rotations although lack of markets might constrain this likelihood. Alternatively if some GM crops currently used as break crops in rotations, such as oil seed rape, have substantial yield and economic advantages, then they may become even more

[2] Refers to commercial approval, should this be granted. Approval is very case specific and each trait differs in timing, given its current state of development. See chapter 6.6 for further details.

common as break crops in cereal rotations which could lead to a less diverse landscape, with the majority of farmers opting for them. Another possible scenario is that resistance to biotic stresses and better weed control via HT traits would mean no need for break crops – so continuous cereal cropping could be a result.

The consequences of such adoption of GM technologies over the decade after commercial approval may include the emergence of some new agronomic problems, such as HT volunteers in crop rotations and the emergence of secondary pests and weeds. UK farmers may become more globally competitive, through lower costs for inputs; reduced insecticide use, and reduced water pollution; and increased uptake of zero or minimum tillage systems, with some benefits for soil moisture retention and reduced soil erosion.

Lowland dairy and Beef Systems

On current estimates, these systems are likely to show an intermediate level of change with the projected adoption of GM crops. The most likely candidate for early commercial cultivation is HT maize. It is unlikely that more productive forage grasses will be approved for release in the UK in the near future.

It is not clear what would occur as a result of widespread adoption of HT fodder maize. At present maize fields are almost entirely weed free because of atrazine use. Atrazine has been banned from most uses in the UK because of its effects on human health and use on fodder maize is one of its few legal uses in the UK. It remains to be seen whether the use of HT maize would offer new opportunities to control weeds, making the fields more wildlife friendly.

Of greater concern, however, would be the introduction of new forage grasses that could be substituted for traditional or `unimproved' grasslands and meadows. Where they would substitute for existing intensive grasslands, then the marginal effects on landscape would be small. But if farmers are tempted, or permitted, to use more productive GM varieties to spread further the process of intensification, then there will be additional biodiversity and landscape losses. As there are very few remaining unimproved meadows in the UK, these may require further protection (Robinson and Sutherland, 2002), if not already protected as SSSIs[3]. Some GM forage grasses may, however, reduce the likelihood of farmers de-intensifying their grassland systems, or adopting new management intensive rotational grazing, both of which have substantial benefits for landscape diversity and farm incomes. However, given the current climate of GM regulation it is unlikely that more productive forage grasses will be approved for release in the UK, because of the concern that the GM trait would be transferred to the large number of wild relatives. Traditional breeding approaches to this target continue in the meantime.

Given market acceptance, lowland livestock systems at the aggregate level could become more diverse, as the number of profitable options for farmers would increase, including GM oats and legumes, more productive grasses, HT and/or insect resistant fodder maize, and grasses with reduced nitrogen requirements. Once again, though, a particularly economically beneficial GM technology could come into widespread use very rapidly (as is the case for many other agricultural technologies).

[3] Site of Special Scientific Interest

Upland Livestock and Permanent Pasture

These landscapes are likely to see much less change than arable and lowland livestock systems after commercial approval of GM crops. More productive grasses could lead to greater intensification of grazing, leading to more animals per hectare. The same could happen if GM acid- and cold-tolerant grasses were developed, so permitting farmers to expand the current limits of intensive production to higher altitudes and latitudes, possibly leading to losses in biodiversity.

6.7.4 Is there general scientific agreement?

Some GM crops could speed the process of agricultural intensification, so contributing to further losses of farmland biodiversity and valued landscape features, if applied broadly. But GM products could also result in a more diverse landscape, with the adoption of niche crops and new high-value options, such as energy crops (Nuffield Council on Bioethics, 1999; House of Lords, 1999; Royal Society *et al.* 2000; Pretty, 2001), although in the short term this is unlikely to happen because GM crops currently under consideration would mostly replace non-GM varieties.

The extent to which a new agricultural technology alone can bring about significant change is uncertain and will probably depend on the economic, agronomic and other advantages that the new technology delivers to farmers. What is not in doubt is that agricultural policy subsidies and support also play a huge role in defining the possibilities of uptake of new technologies. Recent incentives for maximising agricultural production provided by the Common Agricultural Policy were the backdrop to the removal of hedgerows from the countryside (a change not dependent on any new technology) but also a massive switch from spring-sown to autumn-sown cereals (only possible through the availability of new herbicides and new conventionally-bred cereal varieties). Thus the uptake of any GM crop will depend critically on its advantages to the farmer as well as the policy background.

A further important factor in the uptake of GM technology by farmers will be their acceptability to the consumer. Consumer choice between GM and non-GM produce will override any agronomic advantage or disadvantage that GM crops may or may not have for the farmer.

6.7.5 Is the issue unique to GM?

Yes, in the sense that GM crops offer new agronomic possibilities, such as the widespread use of herbicide tolerances, and the adaptation of crops to difficult environments. However, it is widely acknowledged that modern (non-GM) agriculture has adopted new technology and processes to improve productivity and competitiveness from many sources and that this has had significant negative impacts on biodiversity and the wider environment in the UK and that these are greater than in many other parts of Europe (Conway and Pretty, 1991; Campbell *et al.* 1997; Pretty *et al.* 2000; EA, 2002; Robinson and Sutherland, 2002). Large changes over the last century, including recent decades, in the way that farmland is managed have resulted in a decline in both on- and off-farm plant, invertebrate and bird abundance and

diversity. The species that have been hardest hit are specialists, which thrive in very particular habitats, though intensification has made some commonplace species much rarer.

Modern conventional agriculture has already produced a landscape in which many fields have very few invertebrates and very few weeds, providing little food for other types of wildlife, especially birds. In the course of the 20th century there was a 95% decline in the number of weed seeds in the environment. From 1900 – 1930 there was a range of plants, many of them annuals, which were fairly widespread. By the 1960s some had become very rare, such as *Agrostemma githago* (corncockle). This was formerly widespread, but has since declined to extinction. All those now seen in the countryside have come from wild flower seed mixes. Other plants that have dwindled in number or disappeared include the cornflower, corn cleavers, red hemp nettle and pheasant's-eye. All are wildflowers associated with arable farming (Robinson and Sutherland, 2002).

Thus, one important environmental issue surrounding GM crops, particularly GM HT crops, is whether they might make a bad situation for biodiversity even worse. There are many other changes in agricultural practice, which over the same timescale could also have deleterious effects on biodiversity, and many of these are currently subject to less scrutiny than GM crops. Examples would include the future development of conventionally bred HT crops, the expansion of biomass crops on a large scale in the UK countryside and the continuation of land drainage practices which affect SSSIs and the wider countryside.

This issue is therefore not unique to GM crops, but part of the wider consideration of agriculture, environment and the rural economy, which is at the heart of the debate over review and reform of the Common Agricultural Policy

6.7.6 Are there important gaps in out knowledge or scientific uncertainties and are these important?

There are many uncertainties in the topics covered here, and necessarily so because of the huge uncertainties in this area. Some of these gaps are scientific but many are social or economic. The complex ecological interactions between all the components of agro-ecosystems are not yet fully understood, including those related to the effects of schemes designed to produce benefits for the countryside and biodiversity (e.g. Countryside Stewardship). Therefore, it is not possible to predict with certainty all the consequences of ecosystem change on biodiversity brought about by small or large changes in agricultural technologies.

There is a major need for policy makers to understand how these factors are likely to interface with the new technologies, because they will need to predict outcomes from the environment if targets are to be delivered.

6.7.7 Likely future developments

More laboratory and field experiments, combined with better ecological knowledge of all the side-effects of farming, will increase scientific knowledge of the potential impacts of a wide range of GM crops on all possible crop-environment combinations. However, it is important

to note that the effects on agriculture are more likely to be from political, economic and social change than growth of scientific knowledge.

With biotechnology, farmers' practices could get more complicated, with separation distances, volunteer management, refugia (see box 6.3), etc. Because of this environmental management could become more difficult for farmers.

6.7.8 Where there is important scientific uncertainty, what is the way forward?

Scientific uncertainty centres on the effects of GM crops on specific agricultural environments, particularly with respect to the effects of farmers' practices. If GM crops are given commercial approval then their impact on the environment, either positive or negative may be influenced considerably by any guidelines for management of the crop (or other areas), which accompany them. For example, if the provision of insect refugia were seen to be an important concomitant measure to accompany insect-resistant crops then the extent to which farmers complied with such guidelines would influence their benefits. This could act in either direction – those farmers acting with particular care could deliver more than the expected benefits and any falling short of full implementation could reduce any such benefits. The confidence with which it was felt that guidelines would be implemented by farmers as a whole would influence the extent to which regulating authorities would consider guidelines to be voluntary measures or whether they should be made a condition of commercial approval. There is a clear need for more research in these areas to monitor uptake and application of new technologies in general and GM crops in particular.

6.8 LIMITATIONS OF SCIENCE

Is the science available to predict the environmental impact of GM plants?

6.8.1 Summary

The main approaches for determining and predicting the environmental consequences of GM crops are: comparisons with non-GM crops, experience with comparable traits, experiments, field experience of GM crops and ecological modelling. A combination of comparative, experimental, observational and theoretical approaches is typically used to consider the implications of a given trait.

Most of the environmental issues raised by traits resulting from currently developed GM crops do not differ qualitatively from those associated with conventional crops.

Models are important for placing any anticipated changes in context, and are important for scaling-up from experiments to landscape-level impacts.

A major conclusion of this review in relation to currently available GM crops is that the issue of greatest environmental concern is the potential consequence of changes in herbicide management of GM HT crops which might reduce weed populations and hence impact of seed eating birds and other groups. The underlying ecology of the weeds is reasonably well understood and the herbicides involved are well studied. The current farm-scale evaluations have been devised to examine this very issue of the consequences of management of GM HT crops upon wildlife and should provide an excellent basis for understanding the consequences. Thus should then be one of the best understood potential changes in the agricultural landscape.

The environmental effects and implications of various agricultural weed-control strategies have been observed over the last century and experimental work has analysed the impact of various strategies. The FSEs will show any environmental implications specific to GM herbicide tolerant crops. If the results suggest that there may be implications from the GM HT crops, then it is important to understand the groups of farmers who are likely to take up the technology if we are to predict the consequences on a landscape scale. Fields differ greatly in weed density and a critical issue is whether the small proportion of fields with high weed density are likely or unlikely to be planted with herbicide tolerant crops.

6.8.2 Background

There is a range of possible environmental concerns related to the use of GM crops, and it is essential to evaluate the possible impacts of these. This requires predicting the ecological response to novel environmental conditions. In this section we review the methods that are adopted and the strengths and limitations of each.

In order to predict the environmental impacts of GM crops we first need to develop testable hypotheses about the kinds of impacts that might occur. It is logically impossible to predict and/or quantify the impact of an unknown risk.
Over the last 20-30 years risk assessment frameworks for genetically modified organisms have gradually been developed and refined by the scientific community and regulatory

authorities. These frameworks are based on, among other things, our understanding of how plants interact with the physical environment and with other organisms, how transgenes are likely to affect these interactions, the behaviour of transgenes within the host genome and their ability to move into other genomes, and the way in which agricultural management practices affect the wildlife and natural resources in and around farmland. For example, the UK regulations on GMO release require applicants to answer a series of detailed questions that cover direct impacts of the crop itself on the environment, the potential for gene flow to lead to ecological disruption, and indirect effects of the way a GM crop is managed by farmers.

Most scientists today are confident that we have a good understanding of the main types of environmental risk that could arise from the release of GM crops (even though we may lack the data or modelling capability to adequately quantify all of these risks). However, some commentators have argued that since genetic modification is a relatively new technology, there may be environmental (or other) risks that our knowledge of genetics, ecology or ecotoxicity does not yet enable us to predict. For example, scientists in the 1940s lacked the knowledge to predict that the insecticide DDT would reduce the thickness of the eggshells of peregrine falcons. It is important to acknowledge that we may still not be asking all of the right questions, let alone have the science to provide answers to them.

The results described and discussed here are restricted to the impact of GM crops on the UK environment.

6.8.3 Range of views and quality of evidence

There are five main approaches used in predicting the environmental impacts of GM crops. In practice it is usual to use a combination of a number of these methods.

This section does not consider the issue of gene flow, which is the subject of Chapter 7, while Chapter 5 considers the safety of GM food and feed.

Comparisons with non GM crops

This entails comparing the GM crop with existing crops to determine the differences. Thus if there are crops that are widely used and accepted by society, then it is clearly unreasonable for regulatory systems to question those characters that are shared by both the GM and conventional crops. Risk assessment must concentrate upon those traits that differ between GM and conventional crops. This may lead to three possible outcomes

(i) The GM crop may not differ in expected environmental impact from existing crops. For example, where the crop management will not differ significantly from current practices and it can be demonstrated that the transgenic phenotype is unlikely to change the interactions with other organisms in the field. In these cases it is clear that the ecological impacts will be insignificant.

(ii) The GM crop may be similar to conventional crops except for certain specific traits. It is then necessary to consider the implications of these traits. If the crop contains several transgenic traits then the comparison will need to consider the interaction between the traits.

(iii) The GM crop has no equivalent crop that is comparable. This would clearly involve a challenging and detailed assessment. We do not believe this applies to any of the environmental issues that we discuss in this section however it may be possible in the future as our ability to make more radical transformations increases.

A crop or product is expected to differ from its conventional counterpart only in the transgenic trait which it has been engineered to express, therefore risk assessments concentrate upon the impacts of the trait of interest. This approach has been criticised by some (e.g. Millstone *et al.* 1999) especially where there may be some uncertainty as to the phenotypic consequences of changes in the genotype. For example *Bt* maize was found to contain elevated levels of lignin (Saxena & Stotzky 2001) and Roundup Ready soyabean was observed in the field to have higher levels of stem splitting in hot weather – perhaps due to higher lignin levels (Gertz *et al.* 1999). The majority of unpredicted significant changes in phenotype, particularly if detrimental to crop morphology or development, would be detected during agronomic field trials at the research and development stage.

Comparison with non GM crops is best considered as the preliminary stage to guide risk assessment and to be followed by some of the following approaches. The amount and type of data required to carry out a risk assessment on a particular GM crop will depend largely on the crop species, the nature of the transgene(s) and the extent of prior experience with other similar transgenic crops.

Experience with comparable situations

Although a given trait may be novel, experience from comparable situations may provide useful insights. For example, the experience of using more efficient herbicides in conventional agriculture can be used to predict the consequences of the use of GM herbicide tolerant crops. As another example, the experience of the introduction of conventional novel crops and varieties with enhanced pest resistance can be used to give insights into the likelihood that there will be problems with toxicity to wildlife of GM crops. As a third example, the behaviour of conventional crop varieties that have escaped from cultivation can be used to assess the likelihood that GM crops with traits that are unlikely to enhance fitness outside cropped habitats will become invasive. See section 6.2.

An important question is what is considered to be comparable. For example, the current programme of Farm Scale Evaluations (FSEs) is assessing the impacts of specific crops and herbicides on biodiversity in and around fields, for example GM maize resistant to the herbicide glufosinate ammonium. At some time in the future, it is likely that other combinations of GM HT crops and herbicides will be considered for commercial release (for example, glyphosate-tolerant maize) and in this situation regulatory authorities might need to assess whether the impacts of the glyphosate and glufosinate could be considered comparable or whether further large-scale field trials would be required for an adequate risk assessment.

Experiments

Experiments can be a very powerful means to predict ecological responses to changed conditions and can be carried out in the laboratory and in greenhouses (under contained conditions) and in the field (deliberate release). Laboratory experiments are the usual initial

stage before considering field experiments of GM crops. They are easier to carry out as they avoid the spatial and temporal variability associated with field studies. Containment is also much easier for laboratory experiments.

Well-designed scientific experiments allow the manipulation of variables under reasonably controlled conditions. Laboratory and greenhouse studies offer the possibility of close control over environmental conditions and enable detailed comparisons between different crops and traits. However, it may be difficult or impossible to accurately replicate field conditions and therefore predict the actual impacts on biodiversity.

Field experiments may be carried out at a variety of scales: in general larger plot size combined with higher numbers of replications will enable a wider range of environmental conditions (including both temporal and spatial variation) to be studied and therefore give more accurate predictions of impacts. However, including too many environmental variables in field experiments may mean that it is difficult to separate out the impacts of the transgenic trait(s) under study. Therefore, the design of field experiments usually involves a compromise between the degree of accuracy required and the ability to control environmental variables (e.g. split field or paired field plots in FSEs) and also the cost of field research – a major consideration. Additionally, field research involves a deliberate release of GM crops into the environment and as such may involve greater risks of environmental impacts such as gene flow from trial sites (depending on the species under study). Therefore a decision to proceed from contained to field research must be backed up by evidence from laboratory studies to show that risks of invasiveness or gene flow are acceptably low.

Field experiments can be an excellent means for examining likely responses but can be expensive and contentious. They are strongest when they replicate realistically conditions in the field. For example, for insect-resistant crops, most of the experimental research on impacts of crop-produced toxins on non-target organisms has been carried out in the laboratory. Research at the field-scale has been very limited. Lab research can be useful in identifying potential hazards or impacts but these can only be tested reliably by agronomically realistic field-scale experiments. Once such case was the Monarch butterfly (Losey *et al.* 1999, Hansen & Obrycki, 2000) which suggested that pollen from a particular line of *Bt* maize with high expression level could increase mortality in Monarch butterfly larvae. Laboratory studies do not necessarily mean a real risk arises in the field. Later research indicated that Monarch migration and *Bt* pollen show does not coincide; that pollen does not travel far (90% falls in the first 5 metres); that larvae on milkweed are not adversely affected by *Bt* pollen; and that most milkweed tends not to be found close to maize fields. (Hellmich *et al.* 2001; Oberhauser *et al.* 2001; Pleasants *et al.* 2001; Sears *et al.* 2001; Stanley-Horn *et al.* 2001; Zangerl *et al.* 2001). See Sction 6.3 for a more extensive discussion of this issue.

Experiments can examine components of fitness (e.g. survival and fecundity) and see how these are affected by management and field conditions (Parker & Kareiva 1996). Thus, after field experience showed that conventional rape plants only persisted ephemerally outside agricultural land, experiments were used to determine whether the GM plants were more invasive (see section 6.2). The PROSAMO experiments showed that a selection of GM herbicide-tolerant crop plants were never more invasive than their conventional counterparts in any of eight experimental treatments at any of 12 locations (Crawley *et al.* 2001).

If experiments do identify environmental differences resulting from GM crops compared to conventional crops, then models are required to predict their long-term and large-scale

implications because experiments usually only last one or two years. For example models of the type outlined by Watkinson *et al.* (2000) can be used to predict long-term changes in weed and bird populations rather that the response over just one or two years. The current farm scale evaluations should provide convincing evidence on the implications of herbicide tolerant crops for weed and invertebrate population ecology. However they are too small to assess the impacts on birds (Chamberlain *et al.* 2002). The results will need to be incorporated into population models in order to predict the long-term changes rather than the response over one or two years, to predict the changes over landscape scales and to attempt to predict the implications for wide-ranging taxa such as birds (Watkinson *et al.* 2000).

Field experience of GM crops

Examining the actual consequences of growing GM crops in the field under commercial conditions is a useful tool in risk assessment. The approach may be either to examine the experience from growing the same or similar varieties elsewhere (e.g. North America) or monitor the consequences of GM crops if they are introduced into the UK.

Examining the consequences of the same or similar varieties grown elsewhere has the advantage that the consequences of realistic, and sometimes large-scale, planting can be assessed before the crop is actually introduced to the UK. Although likely to produce useful insights, there is an issue that agricultural ecosystems often differ between countries, so there is a possibility that responses may differ. For example, rotations in North America are often less diverse than in the UK, so the ecological impacts of GM HT crops may be exacerbated there; on the other hand, wildlife in the UK is more reliant on the cropped environment and so may be more severely affected by increases in herbicide efficiency than in North America. EU risk assessment requires that field trials must be conducted in European environments or that adequate bridging studies be carried out other wise.

Comparison with experience elsewhere is obviously a very useful approach (e.g. Owen 2000) but there has been surprisingly little work studying existing commercially-grown GM crops, probably because farmland wildlife does not have the same significance in the countries where GM crops are currently commercialised. If there were dramatic affects then it seems probable that these would have been detected.

If GM crops are introduced into the UK, then monitoring any impact on biodiversity within farmland and associated habitats will be important in confirming the validity of the risk assessments but difficult. Although the current UK bird population monitoring organised by the British Trust for Ornithology is perhaps the best in the world, it would have difficulties detecting small persistent changes from annual variability, especially due to weather. Furthermore, it would not be straightforward to determine the impact of GM crops within the existing monitoring as it would presumably be necessary to question the farmer as to which crops are GM while much of the current surveying, including identifying crops, is done from public footpaths. Furthermore, if the critical change is the winter food supply then it will be difficult to relate changes in breeding population to changes in farming practice over a wider area. Despite intensive research on farmland birds it has been very difficult to determine the mechanisms behind the decline as a suite of changes has occurred simultaneously (Robinson and Sutherland 2002). In the future there are also likely to be suites of changes, so that determining any causal role for GM crops is likely to be difficult unless there is a detailed programme to examine this specifically.

Ecological modelling

Models are a standard methodology that underpin much of science. A mathematical description of the world can provide a rigorous understanding and is essential for quantitative predictions (with certain limitations – see later). Population models use a series of equations to describe the ecological interactions. The most basic model comprises understanding the birth, death, immigration and emigration rates and how these are affected by population density. It is then possible to predict the expected population size.

Models have the considerable advantage that they can make use of pre-existing information. For instance pre existing models and existing field studies can be employed to predict changes in management with GM HT crops by incorporating the possible changes in plant survival and could consider possible changes in seed survival (for example as a result of changes in tilling or subsoiling operations).

Ecological modelling is usually explanatory and confirmative rather than predictive but is increasing in its ability to make predictions. For example, for the bitterling (a freshwater fish), Smith *et al.* (2000) quantified how the birth rate depended upon the number and species of mussels in which they bred, while the death rate of the young depended upon an interaction between the density within nursery habitat and the density of predatory perch. It was then possible to predict the density of bitterling within a range of lakes given the extent of the nursery habitat, mussel density and perch abundance and tests showed that these predictions fitted reasonably well (Smith *et al.* 2000).

By understanding the underlying processes it is possible to predict the responses to novel conditions. Stephens *et al.* (2002a) used behavioural data on alpine marmots to predict the underlying population ecology and by testing the output of these models showed that the models appeared to perform well. They could then be used to predict the response to novel conditions such as changes in exploitation (Stephens *et al.* 2002b). Stillman *et al.* (2000) predicted the mortality of oystercatchers in relation to their density by quantifying the fundamental components of their ecology and behaviour. This model provided a good fit to the actual change in population density.

Muir and Howard (2001) evaluated the likely ability of transgenic fish to persist by measuring differences in components of fitness (juvenile and adult viability, age at sexual maturity, female fecundity, male fertility, and mating success) and then incorporating these into a mathematical model that integrates them into a single prediction of risk. This approach has not yet been tested on other organisms.

The ability to create predictive models will vary between subjects. For weed populations the data and understanding are good. The link to bird populations is better in the winter when feeding upon seed that in the summer when most feed on arthropods. There is the theoretical framework for studying the ability of genes to spread in the population but much depends upon determining the selection pressure. The current knowledge is insufficient to model the impacts of insect/ disease resistant crops on non target species.

Limitations to predictions

Predictions are dependent upon understanding the underlying processes and determining sufficiently accurate parameters. There are, however, examples in which insufficient understanding of the processes confounded predictions. The disease *Myxomatosis* was

experimentally introduced onto the island of Skokholm, off the Welsh Coast, but did not persist and it thus was considered an unlikely control measure in the UK. However, *Myxomatosis* was subsequently introduced by farmers and it massively reduced the population of rabbits in the 1950s (the numbers have partly recovered since). The explanation was that, in the UK, *Myxomatosis* was spread by fleas, rather than by mosquitoes, and that, unusually, the rabbits on Skokholm do not have fleas (Lockley, 1954). As a second example, the parasite *Cyzenis* has been shown to play only a minor role in regulating winter moths *Operophtera brumata* in the UK, yet it acted as a very effective means of biological control in Canada where winter moth was previously a pest. The difference has been shown to depend upon the details of the predation of pupae in the soil (Hassell, 1980).

There are also examples in which species responded in an unexpected manner showing that the underlying processes were not fully understood. Brent geese were scarce in the UK and restricted to intertidal habitats where they fed particularly upon the plant *Zostera* spp. The *Zostera* had declined and there were a number of proposals to develop the areas of mudflat that they frequently used which was thought likely to greatly affect the geese. However, following a number of good breeding seasons in the Arctic, the numbers of geese increased and they then adopted the novel behaviour of feeding upon crops over the sea wall.

The confidence in the ability to predict will vary with the taxonomic group being considered. For example, the understanding of weed population dynamics seems good as there are only a narrow range of important weed species, their ecology is uncomplicated and it is reasonably straightforward to carry out experiments altering management or density (Freckleton and Watkinson 2002). It is possible to make reasonable predictions about the consequences of GM technology upon weed populations. However our understanding of invertebrate ecology is much poorer as there are a huge number of species, their ecologies are more complex and much less well understood and even measuring basic ecological information such as birth rate, mortality rate and density dependence is not straightforward.

Where there is the ecological knowledge available, as with weed populations, then models will be an important method for extrapolating to larger scales and to longer time periods. However in many cases, such as soil ecology, invertebrate ecology and breeding ecology of birds there is not yet the scientific background to use this approach with confidence.

The main environmental issue identified by this report is the consequences of GM HT crops. The herbicides under consideration are widely used, well researched and shown to have relatively small side effects. These broad spectrum herbicides have considerable potential for reducing weed populations with the potential for impacts on seed eating birds and species dependent upon the weeds (see Section 6.5). The results of the farm scale evaluation, due to be published in the autumn of 2003 and spring of 2004 will provide invaluable information on the consequences of these crops.

One response to uncertainty is the precautionary principle. For example, the European Commission communication on the precautionary principle states that "*Recourse to the precautionary principle presupposes that potentially dangerous effects deriving from a phenomenon, product or process have been identified, and that scientific evaluation does not allow the risk to be determined with sufficient certainty.*" (Brussels, 02.02.2000 COM (2000) Communication from the Commission on the precautionary principle). However, our scientific review in this report of the environmental issues associated with proposed GM crops have not identified 'potentially dangerous effects'. The risks identified are comparable

to those within existing practices for example from the escape of garden plants or changes in conventional agricultural practice. The exception is potential consequences of changes in management resulting from GM HT crops. After the farm scale evaluations have reported their results the implications of GM HT crops will be one of the most thoroughly researched ecological issue in the UK.

6.8.4 Is there general scientific agreement?

The range of approaches used for predicting the response to GM crops are the same as used in all branches of science. Scientists differ somewhat on the weight they would place on the different approaches and their confidence in the ability to predict, but there is widespread acceptance that these are the main standard methods. The approaches are reasonably well developed but the understanding of the underlying processes and parameter values vary considerably between subjects from good (e.g. weed ecology) to poor (e.g. soil ecology). There is a need for predicting the response to change to be a central component of biology and especially ecology.

6.8.5 Is the issue unique to GM?

Limitations in our ability to predict ecological changes within complex systems apply to a wide range of ecological issues and to many aspects of agriculture. For example, a wide range of agri-environment schemes exist with the purpose of improving biodiversity. Although often based on research their success is very mixed (Kleijn and Sutherland, in press). Although there were great concerns over the loss of hedgerows in the last few decades, in practice the change from spring sown cereals to autumn sown cereals and the greater stratification in farming with arable in the east and pasture in the west were probably of greater importance (Robinson and Sutherland 2001).

It should be pointed out that current widespread changes in agriculture with new crops, varieties, chemicals, equipment, and operations are also likely to affect biodiversity, yet typically receive negligible scrutiny.

The issue of GM crops becoming weeds is often considered alongside the comparable issue of the likelihood of the spread by alien species, especially from gardens. Predicting whether alien plants, of usually unknown ecology, will be invasive is probably considerably more difficult.

6.8.6 Are there gaps in our knowledge or scientific uncertainties and are these important?

In general, the potential level of harm will dictate the quality and quantity of information needed. For example, if protected species could possibly be affected, different information could be needed than if impacts could only be affecting common species. For example, changes to agroecosystems from the introduction of GM HT crops have a real possibility of affecting bird species on the biodiversity action plan priority list, and this was a driver behind the resources invested into the Farm-scale Evaluation programme.

A key issue is the pattern and extent of uptake of GM crops; i.e. "what farmers do". Models of the consequences of GM herbicide resistance showed that the pattern of uptake is critical (Watkinson *et al.* 2000): as only a small number of fields contain high densities of weeds the critical issue is whether these fields are particularly likely to be sprayed (for example to overcome the weed problem) or particularly unlikely to be sprayed (for example because the social, economic or ethical position that results in the farmer having high weed densities makes it unlikely that GM crops will be used).

There are clear gaps in our understanding of farmland ecology. However the research in GM HT crops is expected to be one of the most comprehensive analyses of ecological change. Furthermore the herbicides involved are widely known and well researched. The research shows that the side effects of these herbicides are generally less than selective herbicides (a number of which will be banned in 2003) but they are effective in killing non tolerant plants. The main direct impact is upon the weed populations and the ecology of these is well understood. The indirect impacts upon species feeding on these weeds are less well understood although the farm scale evaluation should reveal the implications for invertebrates. It is hard to predict the impacts that GM antifungal/antimicrobial crops might have on soil organisms and processes.

6.8.7 Likely future developments

With hindsight it is obvious that research being done now on herbicide resistant crops should have been done many years ago. It is similarly obvious on a global scale that the introduction of crops capable of growing in saline soils will lead to a wide range of questions and concerns (Sutherland and Watkinson 2001). There is a clear need to ensure that the science is done so that decisions may be made in an informed manner. This requires literature reviews, mathematical models and in some cases new observations and experiments.

Should the farm scale evaluations reveal significant environmental concerns then there are a range of policy options including allowing a mix of GM HT crops and measures to improve biodiversity. If this is on the agenda then it will obviously require forward planning and research if it is to be effective. There will also be the need for monitoring to see if such measures are effective.

6.8.8 Where there is important scientific uncertainty, what is the way forward?

Experiments are clearly essential to determine ecological impacts of some types of GM crops. However ecological results will often need to be placed within a theoretical framework to predict the wider consequences. For example, much of the concern relates to bird populations, yet the field experiments are not long enough nor on a large enough spatial scale to detect any direct impact on bird populations. This clearly requires models to consider the wider implications, especially for species such as birds whose ranges are enormously larger than the scales of experimental plots. Crop weeds have been declining over at least the last century (Robinson and Sutherland 2001) with obvious benefits to farmers, but costs to biodiversity. Detecting changes in weed populations over a short period will be difficult and this really needs to be placed within a theoretical framework to predict long-term responses.

For the issue identified as being of greatest current concern, the change in management resulting from the introduction of GM HT crops, a major source of information will be the

farm-scale evaluations which compare split fields with half treated with GM HT crops and the other half managed conventionally with selective herbicides. The results from the crops planted in the spring should be available in the autumn of 2003 while the results for the autumn sown crops should be published in the spring of 2004. These should greatly improve our understanding of the subject.

Chapter 7

GENE FLOW, DETECTION AND IMPACT OF GM CROPS

7.1 INTRODUCTION

This Chapter of the Science Review considers the state of our knowledge on issues related to the transfer of genes (*gene flow*) from GM crops to plants and other organisms, the environmental impacts that might arise, methods for gene detection and means of controlling gene flow.

Whether gene flow matters will depend on its consequences (whether from GM or non-GM crops). However, if GM crops are to be grown on a commercial scale in the UK, gene flow will be an important factor in determining the terms on which non-GM, organic and GM agricultures might co-exist.

The dispersal of plant material does not necessarily constitute gene flow *per se*. Gene flow results in the combination of genetic material from individuals with different genetic backgrounds – for instance between different plant varieties or populations. A critical factor is whether these genetic combinations will persist in individuals in future generations and if they do, what significance if any, they might have. Gene flow from crops has implications for the maintenance of genetic integrity, an issue that has been raised with respect to gene flow from GM crops to semi-natural plant populations in this review.

Genes are transferred between sexually compatible plant species when they hybridise (cross) with each other - this is facilitated by pollen dispersal and cross-pollination. Genes can also be moved in seed and sometimes by other plant material that is capable of giving rise to new plants (e.g. potato tubers). In the UK, some crops can exchange genes with certain agricultural weeds or with plants living in semi-natural environments with which they share a close genetic relationship. Whether we have the knowledge to predict the extent to which transgene flow from crop plants to related species and genera could occur and the potential consequences for agriculture and the wider environments (e.g. increased invasiveness) is addressed in this Chapter.

Pollen is released in enormous loads into the atmosphere, and can travel over very great distances - therefore plants will be dusted with pollen from a diversity of sources. However, the vast majority of this pollen will not result in successful cross-hybridisation for any number of reasons e.g. sexual incompatibility, it does not land on the female parts of the plant, it cannot successfully compete with other pollen grains or because it is unviable. This Chapter does not deal with the presence of pollen (that does not pollinate the plant that it lands on) or dust from GM crops on non-GM plants as this does not constitute gene flow - although for some people it does constitute a form of GM ‘contamination’. Whether such GM plant material is likely to be more toxic and/or have greater allergenic potential than non-GM plant material is considered on a case by case basis in risk assessments. The science behind these questions is discussed in Chapter 5. In addition, sections 5.4 and 7.4 of this Review consider the potential for genes from GM crop material to transfer to microbes in the gastro-intestinal tracts of the humans or animals that consume it and to microbes in the soil.

A possible consequence of gene flow between different GM (and non-GM) varieties is 'gene stacking' (the accumulation of genes encoding different traits resulting from cross-pollination between different varieties of the same crop). This has been well documented in oilseed rape in Canada. The effect that stacked traits such as herbicide tolerance, but also in future a wide range of other traits, might have is an issue that has been raised in this Review.

Spilt seed or vegetative tissue (e.g. tubers) remaining after a GM crop has been harvested may act as a reservoir for transgenes. As GM volunteers (plants growing adventitiously from this residual seed or from vegetative material) can grow several years after the original GM crop is harvested they could mediate transgene transfer over time. GM material may also become mixed with non-GM and other GM varieties after crops have been harvested - in storage or further down the production and transport chain. This raises the issue of detecting unintended GM presence, which is also addressed in this Chapter.

The transfer of genes between plants by cross-pollination is sometimes referred to as vertical gene transfer (VGT). This contrasts with horizontal gene transfer (HGT), which refers to the non-sexual, non-parental-to-offspring processes by which genetic material can sometimes transfer between organisms with distant genetic relationships. In this Review the potential for gene transfer from GM crops to soil microbes and to viruses has been raised. There is concern from some quarters that this could lead to adverse effects on ecosystems and to the generation of new viruses. However, this supposes that gene transfer between plants and microbes/ viruses, actually occurs - the evidence will be discussed in this Chapter. The transfer of transgenes to gut microflora is not addressed here, this topic is covered in section 5.4.

Public concerns about GM were reflected in a report, produced as a result of a series of foundation discussion workshops under the GM Public Debate strand of the GM dialogue (see Chapter 2 - methodology). The questions of particular relevance to this Chapter are:

Could harm be caused by cross-contamination?
What will the effect be on 'natural' (non-GM) crops / wildlife?
What are the real experience of US farmers and consumers?
What controls and regulations/ legislation are in place?
What legacy are we leaving future generations?

More specifically, issues on gene flow, detection and impact of GM crops were raised under the Review at various open meetings, in contributions to the Science Review website and by GM Science Review Panel members at their meetings.

We consider four types of gene flow and the potential consequences of it occurring in this Chapter. Text in italics aims to encapsulate many of the public issues and concerns that have been raised on gene flow, detection and impact of GM crops during this review:

7.2 Gene flow between crop varieties

Can the extent and consequences of gene flow from GM crops to other crop varieties (GM and non-GM) be predicted and controlled? Is co-existence between GM and non-GM crops possible and can we detect unintended GM presence?

7.3 Gene flow from GM crops to agricultural weeds and wild relatives.

Can the extent and consequences of gene flow from GM crops to agricultural weeds and wild relatives be predicted and controlled? Could gene flow from GM crops generate superweeds or eliminate wild plant populations?

7.4 Can genetic material in GM plants transfer to soil microbes?

In nature, how important and prevalent is horizontal gene transfer between plants and microbes in the soil, and does the presence of transgenic DNA make this more likely to occur? To what extent are the ecological effects of horizontal gene transfer from plants to soil microbes predictable?

7.5 Can genetic material in GM plants transfer to viruses?

Can plant-virus-derived transgenes recombine with, and be transferred to viruses? If horizontal gene transfer is possible between GM plants and viruses could this result in new viruses that could cause irrecoverable damage to the ecosystem or to crops?

7.2 GENE FLOW BETWEEN CROP VARIETIES

Can the extent and consequences of gene flow from GM crops to other crop varieties (GM and non-GM) be predicted and controlled? Is co-existence between GM and non-GM crops possible and can we detect unintended GM presence?

7.2.1 Summary

Transgenes and native plant genes are dispersed in pollen and seed. For the most part gene flow takes place within a few metres of the plant, but it can occur over several kilometres. Seed is typically moved over much greater distances than pollen, both by natural seed dispersal and intentionally and unintentionally by humans. In addition, once seed is dispersed, it is much more likely to result in the establishment of a plant containing the genes in question.

Pollen-mediated gene flow and the separation distances employed to minimise it typically generates more public interest than the movement of genes in seed and this has been the case in this Review. However, the implementation of agronomic practices that minimise the dispersal of genes through seed is essential for maintaining gene flow below set thresholds, for example by limiting gene flow through volunteers, preventing unintended mixing of different seed lots and reducing the transportation of seed on agricultural machinery. However, the complete genetic isolation of most crops grown on a commercial scale, either GM or non-GM is not practical, at least in the foreseeable future.

Distance from a pollen source and cross-pollination frequency with neighbouring crops can be predicted for most major crops. Separation distances based on these predictions and agricultural practices that minimise seed-mediated gene flow have been employed to successfully minimise gene flow between non-GM crop varieties for economic (e.g. certified seed crops and identity preservation schemes for different types of maize) and safety reasons (i.e. separation of non-GM oilseed rape varieties containing high or low erucic acid levels). However, restricting gene flow between non-GM and GM varieties will potentially be on a much larger scale than anything that has preceded it.

The amount and potential consequences of gene flow are considered on a case by case basis for GM crop varieties and this forms part of a risk assessment on which the decision on whether to issue consent for release into the environment is made (please refer to Chapter 3).

The levels at which gene flow can be maintained for different crop varieties are significant in determining whether co-existence of different types of agriculture is feasible. There is little available evidence on how the different factors (seed purity, cross-pollination, the contribution of volunteers and the effects of seed mixing) affecting co-existence will combine if GM crops are grown on a commercial scale in the UK, this makes prediction difficult. Political decisions may ultimately affect whether co-existence is practical, in particular what thresholds are set for maximum GM presence in non-GM crops (and their products), whether conventional or organic. For some crops, maintaining thresholds of gene flow may be relatively straightforward, by employing separation distances and, more importantly, by reducing gene flow through seed. However, in other cases it may be difficult, and perhaps impossible.

In order to enforce maximum threshold levels of transgenic DNA in non-GM crops, the tools for accurately sampling, detecting, quantifying and identifying unintended transgenic DNA presence must be in place. Absolute thresholds for detecting specific fragments of DNA depend on the crop. As there are limits of detection, even though these are extremely low, zero transgenic DNA presence cannot be guaranteed using these methods.

Detection and identification of GM presence may be limited if genetic markers which identify the GM crop aren't available (e.g. in the case of unapproved GMOs). Genetic elements that are commonly used in GM technology may show that transgenic DNA is present but these will not identify its source.

'Gene stacking' (accumulation of genes conferring different traits as a result of hybridisation between different varieties) is not unique to GM crops. However, 'transgene stacking' could result in the combination of genes that would not be brought together in non-GM crops and were not intended to be brought together in approved GM crop varieties (although the potential and consequences of these traits combining would have been addressed in risk assessments). The potential consequences of transgene stacking is already a consideration in the release of new GM crop varieties in the UK but this is likely to become more complex if a range of different GM crop varieties are grown on a commercial scale.

The advent of GM crops that produce novel products such as pharmaceuticals, bioplastics or biofuels pose a new problem for regulators. However, this is not unique to GM agriculture, some oilseed rape varieties produce oil that is toxic to humans so must be separated from varieties that produce oil for food products. The existing practice of assessing each new GMO on a case by case basis is appropriate for regulating these new types of GM crops.

7.2.2 Introduction

Gene flow from GM crops to other crop varieties was discussed at open meetings held at the Royal Society of Edinburgh[1], the Royal Society[2] and the Institute of Grassland and Environmental Research in Aberystwyth[3].

Gene flow between neighbouring crop varieties has been taking place almost since agriculture began. Consequently, it has been important for growers to maintain agronomic characters specific to certain varieties and for seed producers to maintain purity standards for certified crop varieties. However, the advent of GM crops has significantly increased public and scientific interest in gene flow.

Genes are transferred between sexually compatible plants through pollen. Typically, there is an rapid decline in the amount of pollination that occurs as distance from a plant increases, however this decline becomes much less pronounced as the probability of pollination nears zero (Champolivier *et al*., 1999). Therefore, the vast majority of pollination will occur within a few metres of a plant, but there may be rare occurrences of cross-pollination at distances of a kilometre or more (Rieger *et al.* 2002).

[1] http://www.gmsciencedebate.org.uk/meetings/pdf/270103-transcript.pdf
[2] http://www.gmsciencedebate.org.uk/meetings/pdf/110203-transcript.pdf
[3] http://www.gmsciencedebate.org.uk/meetings/pdf/170303-transcript.pdf

The distance that pollen travels depends on a number of factors, for example: the type of pollen (i.e. how much is produced and how heavy the grains are) and the mechanism by which it is dispersed (wind/ insects or a mixture of both). If insects are involved, their behaviour will affect pollen dispersal. Climatic conditions will significantly affect dispersal (i.e. temperature, humidity, light, wind and rain) as will natural barriers such as surrounding vegetation and topography.

Although pollen dispersal provides a guide to the actual distances of gene flow there are a number of other important factors that must be taken into consideration. These include:

- The breeding characteristics of different crop varieties. For instance, some crops predominately self-pollinate (e.g. wheat and barley) whereas others have a higher degree of out-crossing (e.g. maize).
- Cross-pollination can only occur between plants that flower at the same time.
- Different types of pollen vary markedly in their ability to remain viable under different conditions (such as temperature and humidity). Therefore, the pollen may travel a long distance but it will not necessarily have the capacity to cross-pollinate.
- Competition between pollen from different sources (e.g. large amounts of local pollen versus small numbers of pollen grains arriving over long distances).

The potential for gene transfer is very different depending on the crop and the variety. For example, Hucl (1996) examined variability in the amount of self-pollination (in-breeding) of 10 wheat cultivars and found rates varied between 97.7% (cv. CDC Makwa) and 93.95% (cv. Oslo). Maize on the other hand is more likely to cross-pollinate (under normal field conditions at least 95% of the ovules are fertilised by pollen from other plants, Poehlman, 1959). Many plant varieties have self-incompatibility mechanisms that restrict self-pollination (Kao and Mc. Cubbin, 1996; Takayama and Isogai, 2003), however varieties grown for grain or seed often have high degrees of self-pollination.

The relationship between distance from pollen source and the cross-pollination of neighbouring crops can be predicted. In a report for the then Ministry of Agriculture, Fisheries and Food, Ingram (2000) identified robust, representative data sets and applied them to typical farm situations. The report proposed recommended separation distances to restrict cross-pollination frequencies to below 1%, 0.5% and 0.1% for non-seed crops of sugar beet, maize and oilseed rape. These recommendations form part of the basis for current assessments of gene flow.

The movement of genes in seed may be a more significant factor than cross pollination in contributing to gene flow but this hasn't, so far, received the public interest that separation distances have, possibly because most GM field trials, including the large-scale farm-scale evaluations, have been managed to minimise the possibility of viable seed being set. Agricultural practices that minimise the movement of seed are very important in maintaining levels of genetic purity.

As with pollen, seeds can transport genes away from their source, however unlike pollen, seeds can also mediate gene flow over time. Depending on the type of seed, it may lie dormant in the soil for many years before germinating. Plants that grow from seed, or from vegetative structures (e.g. beet tops or roots) left by a previous harvest are referred to as volunteers (Downey 1999). Volunteers and plants growing from stubble are a potential source of transgenes to crops in future harvests.

The unintended presence of GM seed in non-GM seed lots may also facilitate the transfer of transgenes into conventional crop varieties. This adventitious GM presence may be the result of hybridisation events between different varieties, or the accidental post-harvest mixing of seed. The practice of saving a proportion of seed from one harvest to sow in subsequent years (so-called 'farm-saved seed'), rather than buying seed produced commercially, is likely to increase the likelihood of seed mixing – particularly on farms growing GM and non-GM varieties of the same crop.

A harmonised EU system for tracing and labelling GMOs and products derived from them at all stages of their placing on the market is being developed. From a technical standpoint it is important that methodologies are in place that can accurately and reproducibly detect unintended GM presence at levels dictated by the legislation. Annex V describes the proposal for new legislation concerning the traceability and labelling of food and feed derived from GMOs.

The thresholds proposed for the unintended presence of approved GMOs in conventional crop seed are between 0.3 and 0.7% depending on the crop (Commission proposals on thresholds for the adventitious presence of approved GMOs in seeds, document: SANCO/1542/02July2002)[4]. These were calculated to broadly support the food labelling threshold. It is important to note that the thresholds set for GM presence are pragmatic as their role is to give consumer choice – this contrasts with other 'contaminants' which may have safety implications above set thresholds.

Ultimately, for this legislation to be effective there must be internationally approved monitoring, sampling and detection methods for all crops (and products derived from them) that are capable of facilitating the detection and quantification of GM presence at, or below any threshold levels that are set. Although it is outside of the remit of this review paper, it is important to emphasise that technical ability is not the sole consideration in determining whether legislation can be enforced – there are many political considerations such as economics and liability.

7.2.3 Range of views and quality of evidence

To what extent does crop to crop gene flow occur and how predictable is it?

This section is principally concerned with pollen-mediated gene flow because of the interest expressed in this issue during the GM Science Review. However, it is a widely held view of experts in this area that the implementation of agricultural practices that limit the movement of seed is critical in minimising gene flow from GM varieties.

A point that is often made is that once genes are transferred out of GM crops they cannot be 'recaptured', or once the genie is out of the bottle it cannot be put back. The next section in this Chapter (7.3) considers the potential for transgenes to persist if transferred from GM crops to sexually compatible weedy and semi-natural plant populations.

There are polarised viewpoints on gene flow from GM crops. One group considers that any amount of gene flow is unacceptable as they do not want the food they eat to be derived from

[4] http://www.defra.gov.uk/corporate/consult/approvedgmos/sanco1542.pdf

GM crops, whether it contains transgenic DNA or not (highly processed products such as sugar and oil do not contain DNA or protein). Conversely, others consider that gene flow is only a problem in particular instances, for example if there is gene flow between high erucic acid varieties of non-GM oilseed rape and low erucic acid varieties used in food products (Bilsborrow *et al.* 1998). Since the risk is specific to the varieties involved and not a generic problem of gene flow *per se*, and the amount of gene flow differs in different crops under different circumstances, the case by case assessment of each crop/trait combination is appropriate.

There is a large body of evidence on gene flow over relatively short distances (i.e. hundreds of metres rather than several kilometres), for major crop plants. This comes from:

(i) Practical experience in limiting gene flow between non-GM varieties

Certified seed schemes (require seed production above set levels of genetic purity). However, the amount of detailed information from this source is generally limited e.g. minimum separation distances are adhered to but the actual separation distances used are not recorded. In addition, it is possible that in some cases, the presence of unwanted genotypes (so called 'off-types') have been under-estimated since they are often screened on the basis of visual characteristics.

Non-GM field crops separated to maintain product purity. There are limited examples where measures have been taken to restrict pollen-mediated gene flow between different non-GM varieties of the same crop. These have been employed in protecting the characteristics of sweetcorn from other types of maize and in preventing contamination of low erucic acid varieties of oilseed rape (the oil is used in food products) with pollen from high erucic acid varieties as they produce oil that is toxic.

(ii) Scientific studies

There is a large body of evidence from scientific experiments on pollen-mediated gene flow and this has increased in recent years due to the interest in GM crops (reviewed by Treu and Emberlin, 2000[5]; Ingram 2000; Eastham and Sweet, 2002). There are different views on whether separation distances based on these data are adequate to maintain cross-pollination rates below specified levels. The results of some cross-pollination studies may appear inconsistent with the separation distances that have been set. This is because they must be extrapolated for whole field situations (Ingram 2000). Also, in setting separation distances, the variety of the crop used (e.g. whether it contains male sterile plants), the environmental conditions, including the possibility of extreme weather are taken into account. However, even though variability is inevitable, all the evidence suggests that cross-pollination between fields declines very rapidly with distance and that separation distances are very effective in reducing pollen-mediated gene flow to low levels.

The main view of experts in this area is that there are sufficient data available to predict the separation distances required to limit pollen-mediated gene flow to below 1% for most, and below 0.5% for many crop varieties. However, for certain varieties e.g. varietal associations and partially restored hybrids of oilseed rape there is insufficient information to predict separation distances needed to reduce cross-pollination below 0.5% (Ingram 2000).

[5] http://www.soilassociation.org/pollenreport

The typical pattern of decline in cross-pollination over relatively short distances (up to a few hundred metres) may not apply at greater distances (many hundreds of metres to several kilometres), where the pattern is defined by very rare events (Perry, 2002). There have been a small number of studies involving mathematical models that predict gene flow on a landscape scale (Squire *et al*. 1999; Perry 2002). In 2000, a non-GM herbicide tolerant oilseed rape variety was grown for the first time in Australia and this provided an opportunity to study gene flow on a landscape scale without the need for mathematical modelling (Rieger *et al.* 2002). Forty eight million oilseed rape plants were examined within 5km of the source fields. The results showed that, in most cases pollen-mediated gene flow occurred within the source fields - less than 1% of pollination events took place in adjacent fields containing oilseed rape varieties without the herbicide tolerance trait. A small amount of gene flow was detected up to 3km from a pollen source and the distribution of these isolated long-distance pollination events was more variable than would have been predicted from small-scale experiments.

A contributor to the GM Review website has highlighted the results of the Nature paper by and Quist and Chapela (2001). Although the experimental design of this study was flawed (Kaplinsky *et al*., 2002; Metz M. and Fütterer, 2002) it is generally accepted that there has been gene flow between GM maize and maize that is native to Mexico (landraces). However, this is very unlikely to be evidence of unexpected gene flow over extreme distances (i.e. from North America) – it much more probable that cross-pollination has occurred between the landraces and GM plants grown in the same field[6].

Another contributor to the GM review website has highlighted the findings of a report representing the combined findings of two separate Defra monitoring contracts on gene flow from large scale releases of GM oilseed rape between 1994 and 2000 (Monitoring large scale releases of genetically modified crops, EPG 1/5/84. Incorporating report on project EPG 1/5/30: monitoring releases of genetically modified crop plants)[7]. The results showed that with fully fertile varieties, cross-pollination events declined rapidly with distance from the source and most occurred within the first ten metres. However in some cases, cross-pollination levels exceeded 0.5% at distances of 100 – 200 m and the amount of outcrossing associated with varietal associations was considerably higher than that found in samples of fully fertile rape. These results are within the expected range but emphasize the importance of recognising varietal differences when considering separation distances.

What impact do volunteers have on crop to crop gene flow?

Residual seed remaining after a crop has been harvested may germinate in subsequent years producing volunteer plants that can transfer genes to other varieties of the same crop grown at the site. This may be a significant route for gene flow between varieties, for example if oilseed rape volunteers are not controlled it is likely that they could contribute more to impurities in crops than gene flow by pollen movement from other varieties. The length of time that the seed from different crops can remain viable in the soil varies considerably. For example, seed from oilseed rape varieties persists for around six years (although this can be up to ten years in exceptional circumstances) whereas maize seed remains viable for less than a year.

The ACRE's view is that it is good practice, to keep seed shed from GM crops such as oilseed rape and potatoes, on the soil surface and to encourage it to germinate - this allows volunteers

[6] ACRE's advice: http://www.defra.gov.uk/environment/acre/advice/advice14.htm
[7] http://www.defra.gov.uk/environment/gm/research/epg-1-5-84.htm

to be controlled and limits accumulation of seed in the soil. The period before the same crop (either a GM or non-GM variety) can be grown on the same site is assessed. This depends on the potential for seed to remain viable in the seed bank and whether volunteers continue to germinate at the site. Consents for small-scale research and development trials of GM crops require that the sites are monitored after the plants are removed for a minimum number of years, or until volunteers no longer emerge. Removing volunteers before they flower will prevent gene flow to sexually compatible plants.

A view expressed in a contribution to the GM review website is that consents to release GM crops for trial purposes are inadequate from the point of view of volunteer control. The evidence cited was the occurrence of GM oilseed rape volunteers at a site at least four years after the original GM crop was harvested (monitoring large scale releases of genetically modified crops, EPG 1/5/84)[8] and studies that have shown that oilseed rape can germinate 8 years after seed shed and that seeds can remain dormant for around 10 years. It is well known that oilseed rape seed can persist in the soil for these periods and can give rise to volunteers. Therefore in order to prevent these volunteers mediating gene flow, they must be removed before flowering, or at least before seed set if there are no sexually compatible plants in the vicinity.

Gene flow between crop varieties is inevitable, whether mediated through volunteers or not. It can be restricted to low levels but if the GM crops themselves, or gene flow from them, poses a hazard they should not be released into the environment. Gene flow therefore, represents exposure not risk; to assess risk, the potential consequences of crop to crop gene flow must also be considered.

What are the potential consequences of crop to crop gene flow?

Crop to crop gene flow results in transgenes being transferred between extremely similar genetic backgrounds. For this reason the consequences of transgene presence in GM crops will mainly be dealt with in Chapters 4 and 6. This paper considers issues that are unique to plants receiving transgenes unintentionally through cross-pollination.

Pollen-mediated gene flow from GM crop varieties results in transgene presence in the seed of recipient non-GM plants but not in other parts of the plants. Therefore in the first instance, seed crops such as oilseed rape and cereals will contain transgenic DNA as a result of cross-pollination, whereas root crops such as potatoes and sugar/fodder beet[9] will not. However, seed that germinates from these plants will be hybrid and this will contain transgenic DNA in all cells.

'*Transgene stacking'* i.e. the accumulation of transgenes (encoding different traits) resulting from cross-pollination between different GM varieties, was raised during this Review. If several GM varieties of a crop were to be given commercial approval for cultivation in the UK, and were grown widely, then the strong possibility exists that transgene stacking would occur. This might involve transgenes conferring resistance to several herbicides, raising the possibility of multiple herbicide resistance (Orsen, 2001 and Beckie *et al.* 2001), as has happened in Canada - for further details please refer to Dr Linda Hall's presentation to the

[8] http://www.defra.gov.uk/environment/gm/research/epg-1-5-84.htm

[9] Sugar/fodder beet generally flowers in its second year. Roots are harvested in the first year before flowering occurs and therefore seed production is uncommon. Premature flowering can occur and it is good practice to remove these premature bolters before they set seed.

Royal Society discussion meeting.[10] The possibility of generating GM crop plants that are invasive of semi-natural habitats (see section 6.2) as a result of transgene stacking is conceivable in the more distant future, especially if a range of GM crop varieties with resistance to different pests, diseases or other environmental stresses (see section 6.6) are grown on a commercial scale in the UK. However, it should be noted that crops containing such transgenes have not been approved for cultivation in the UK, and are unlikely to be approved in the near future (refer to Chapter 6). In addition, gene stacking is not unique to GM crops. Non-GM varieties have been bred that are resistant to different pests and diseases and have different tolerances to environmental stresses, however the combination of such traits has as yet, not resulted in plants that are invasive of semi-natural habitats.

In Canada, crop varieties with novel traits such as herbicide tolerance fall under the same regulatory framework whether they are GM or not. Once approved, farmers can plant these crops where they choose. In western Canada, farmers have rapidly adopted the use of herbicide resistant oilseed rape and grow the different varieties in close proximity - gene flow between them is therefore inevitable.

The advent of multiple herbicide tolerant volunteers in Canada has necessitated changes in management practices in subsequent crops. There is some concern about the implications that this might have on farmland biodiversity in the UK. In 2002, English Nature published a report that considered what could be learned from the Canadian experience of herbicide tolerant oilseed rape volunteers (Orson, 2002). The conclusion was that if GM varieties of oilseed rape with tolerance to glyphosate (Roundup Ready) and glufosinate (Liberty Link) were introduced into the UK on a commercial scale, stacking of these traits would be inevitable, but that this would have little impact on other agricultural practices. The report suggested that the main implication for herbicide use was likely to be increased usage of paraquat +/- diquat predrilling, which might have an impact on hares. However, studies of European hare populations suggest that this is unlikely to be the case (Edwards *et al.* 2000). The English Nature report recommends that methods for controlling multiple herbicide tolerant volunteers of oilseed rape should be put in place that have minimal or no impact on biodiversity.

ACRE considers the possibility of gene stacking through gene flow and its consequences when assessing the potential impact of releasing a GM crop into the environment. This involves a consideration of what transgenes are present in other GM varieties of the same crop that already have approval for release. Assessing the ecological behaviour of a phenotype that has resulted from the stacking of different traits (in GM or non-GM plants) can be difficult however, as it often relies on evidence other than direct field data. ACRE has the power to require field evidence of the behaviour of novel phenotypes derived from transgene stacking or to invoke the precautionary principle, but such situations have not yet arisen in the UK. There have been some studies that have looked at the potential effects of transgene stacking e.g. Senior *et al.* (2002) deliberately introduced tolerance to glufosinate and glyphosate into the same plants and looked for interaction between them (and did not find any). However, possible transgene combinations must be assessed on a case by case basis irrespective of whether stacking is deliberate or unintentional.

There is a widely held view that transgenes that are used to produce pharmaceuticals or other GM products (e.g. bioplastics and biofuels), that might adversely affect human health if eaten

[10] http://www.gmsciencedebate.org.uk/meetings/pdf/110203-transcript.pdf.

inadvertently, should not be transformed into major food crops since unintended mixing of seed or cross-pollination events could contaminate varieties used as food or feed. The view is that it would be more appropriate to introduce such traits into non-food crops or that the production of pharmaceuticals should be confined to contained facilities and that field releases of such 'pharm crops' should not be allowed. An alternative view is that the most effective way of regulating such crops is on a case by case basis.

To what extent can we detect GM presence?

Our ability to detect and quantify unintended GM presence is fundamental in monitoring gene flow from GM crops and providing consumers with choice. The main analytical methods either target DNA (e.g. the polymerase chain reaction [PCR]) or the products of DNA expression (e.g. enzyme linked immunosorbant assays [ELISA] and immunochromatographic strip tests for proteins). The distinction between these two approaches is significant as transgenic DNA inserted into crop genomes is not necessarily expressed and translated into protein. Gene expression may be very low or non-existent in different organs of a plant or at different stages in its development. Therefore, tests for transgenic protein in a sample may be negative whereas DNA analysis demonstrates that transgenic DNA is present.

Currently, methods involving PCR predominate in detecting and quantifying DNA – these are very sensitive and can be used to detect DNA that is present at very low abundance (the threshold for detection is different for different crops). However, it is generally accepted that a minimum of 0.1% GM presence is required (i.e. 1 transgenic seed in 1 000 non transgenic seeds) for detection to be reliable. Detecting the presence of transgenic DNA in processed foods is potentially more difficult. This because the DNA may be damaged, or other constituents in the foodstuff may interfere with its detection. In the case of some highly refined products such as sugar and oil, a lack of DNA or protein means that it is impossible to determine whether the crop was GM or not. In these cases, authenticated audit trails would be necessary.

In order to enforce a threshold level of unintended GM presence, PCR techniques must also be able to accurately quantify transgenic DNA. The accuracy of measurements decreases at lower thresholds of transgenic DNA presence due to error associated with sampling and also with the PCR itself. Therefore increasing sample size (e.g. the number of seeds or tubers) reduces sampling error and increases the confidence in the accuracy of transgenic DNA measurements. However, to achieve accurate quantification at lower thresholds of GM presence the sample size must increase dramatically (Kay and Van den Eede, 2001). Sample sizes are likely to limit detection thresholds much below 0.1%.

The amount of pollen-mediated gene flow between different varieties, particularly those separated by bare ground or low vegetation, is likely to be significantly higher at the edge, than in the middle of the fields. Therefore, plants along the field edge may have higher than threshold levels of GM presence whereas the remainder of the field may have significantly lower levels.

Directive 2001/18/EC (for the release of GMOs in the EU) requires that information for the identification of individual GM varieties is made available for monitoring purposes i.e. DNA sequences specific to each transgenic crop variety (e.g. junction fragments that span the intersection of inserted transgenic DNA with native host DNA). Therefore, unintended presence of approved GM crop varieties that have gone through statutory regulatory

assessment in the EU may be identified. However, there are other classes of GM crops where this detailed molecular information is not readily available e.g.

- GM plants released for small-scale research and development trials (under part B regulatory approval).

- GM material that has not been through the EU regulatory process. Seed for use in the UK is frequently multiplied abroad because it makes it possible to obtain more than one generation of seeds per year. For example, some maize varieties grown on organic farms in the UK are multiplied in North America where gene flow from GM varieties that have not been approved in the EU could take place. In contributions to the review, concern was expressed about an instance where non-GM oilseed rape seed imported from Canada by Advanta Seeds UK Ltd was found to contain about 1% of a GM oilseed rape variety that was approved for food, feed and environmental release in Canada but not in Europe. There are international databases containing information on transformation events that have been approved around the world (e.g. the OECD's Biotrack online[11]), and links between them are being developed to give a more integrated resource. However, these do not contain the detailed molecular data that must be registered by applicants seeking approval to release GMOs commercially in Europe (see above). GMOs that have not received consent for release, or are not in the regulatory process, will generally be the most difficult to detect, and more particularly, to identify.

Screening for genetic elements commonly used in GM crops (e.g. the cauliflower mosaic virus [CaMV] 35S promoter) may identify unintended GM presence in some cases - however, this is not reliable since these elements are commonly found in nature (results in false positives because the DNA is derived from a source other than the crop), or the transgenic DNA present does not contain them (results in false negatives because different transgenic elements have been used).

To what extent can gene flow be contained by genetic isolation systems in crop plants?

There is an interest in developing mechanisms that could prevent or restrict gene flow from GM crops [so-called 'genetic use restriction technologies' (GURTs)]. There are a number of potential ways in which at least partial genetic isolation might be achieved. Some of these are established technologies whilst others require considerable research and development. There are also systems that have not necessarily been developed for this purpose but which also affect the transfer of genes from GM crops. Plastid transformation and so called 'terminator technologies' have been highlighted for the attention of this review:

- Insertion of transgenic DNA into chloroplasts rather than the nuclear genome has been proposed as a method for minimising gene flow (Daniell *et al* 1998). This proposition has stimulated considerable debate (e.g. Daniell and Varma, 1998; Chamberlain and Stewart, 1999). Firstly, it relies on the assumption that chloroplasts are always maternally inherited in crops. The mode of chloroplast inheritance is known for the majority of cultivated species but can be influenced by both genetic and environmental factors (Stewart and Prakash, 1998). In addition, a recent paper by Huang *et al.* (2003)

[11] http://www1.oecd.org/scripts/biotech/frameset.asp

indicated that gene flow from chloroplasts to the nucleus can occur at higher frequencies than previously supposed. However, this study used highly selective laboratory conditions that would not be present in the field. Nevertheless, plastid transformation could still significantly reduce the potential for gene flow by pollen-mediated hybridisation events, although it could also increase the probability of horizontal gene transfer (HGT) occurring due to increased copy number and similarity between plastid genomes and those of soil and gut microbes

- Several companies have developed strategies that make use of promoters, inducible by chemical stimulants, to regulate the expression of transgenic proteins that interfere with anther development or seed germination. This allows for seed multiplication but means that hybrid seed generated from the crop would be unviable or the resultant plants would be male sterile.

In addition to these, there are a number of other mechanisms that could prevent or reduce pollen-mediated gene flow from GM crops. These are based on systems that occur in nature and include: *Apomixis*: the production of seeds without fertilisation; *Cleistogamy*: flowers are produced that develop normally, but fail to open and the *exploitation of hybridisation barriers*. Alternatively the desired transgene can be coupled with genes that would render hybrid offspring or volunteers less able to compete with crops, weeds and wild species. Genes that prevent seed shatter or secondary dormancy, or that dwarf the recipient could all be useful for mitigation. Many such genes have been isolated in the past few years (Gressel, 1999). ACRE has issued guidance on the development of mechanisms in GM crops that could minimise transgene dispersal in its *Guidance on Principles of Best Practice in the Design of Genetically Modified Plants*[12].

The exploitation of differences in flowering time between varieties may restrict gene flow between them, but it is unlikely to be sufficiently reliable to prevent it. The use of crops with no sexually compatible semi-natural or weedy relatives in the UK, provides a simple and effective way of containing transgenes within crop plants.

In a contribution to the GM science review, the use of site-specific recombinases in constructs designed to prevent gene flow from GM crops was raised. The concern expressed is that these recombinases will cause DNA rearrangements (Mae-Wan Ho and Joe Cummins)[13]. Recombinase methods are not yet well developed, but refinements in technologies to excise transgenes or parts of inserted DNA are likely to become available in the future (Hare and Chua, 2002).

Is co-existence between different agricultural systems possible?

'*Farmers and consumers alike are concerned about the freedom of choice of different agricultural production systems. In my understanding, co-existence means that no form of agriculture, GMO or non-GMO, should be excluded in the EU in the future. Similarly, it is also linked to consumer choice. Only if farmers are able to produce the different types of crops in a sustainable way, will consumers have a real choice'* (Franz Fischler, member of the EU commission, 2003)[14].

[12] http://www.defra.gov.uk/environment/acre/bestprac/guidance/index.htm
[13] http://www.gmsciencedebate.org.uk/topics/forum/0046.htm
[14] http://europa.eu.int/comm/research/biosociety/pdf/rt_fischler.pdf

Strategies for co-existence of GM, conventional non-GM and organic crops are already in place in countries worldwide, even in Europe. For example, in Spain there has been successful cultivation of Bt maize over the past 5 years with the utilisation of cost-effective good agricultural practices. However, the experience of other countries may not be directly relevant and co-existence must be considered on a crop by crop basis in the UK. The European Union held a round table meeting with various stakeholders in April 2003 and are expected to deliver guidance on co-existence in July 2003. Gene Flow and co-existence in oilseed rape and maize was discussed at the open meeting held in Aberystwyth[15]. These crops were selected for discussion as both are open-pollinating crops with the ability to disperse genes quite widely from crop to crop and also because they are about to be commercialised, or are already commercialised as GM crops in Europe.

In May 2002, the European Commission published a study by their Joint Research Centre (JRC) entitled: Scenarios for co-existence of genetically modified, conventional and organic crops in European agriculture (Bock *et al.* 2002)[16]. This used computer modelling and expert scientific opinion to analyse the practicalities and costs of achieving co-existence for three crops (potatoes, grain maize and oilseed rape) in various hypothetical scenarios (thresholds for incidental GM presence of 0.1%, 0.3% or 1.0% with GM crops as 10% or 50% of the total cropping). The report concludes that co-existence around a 0.1% threshold would be very difficult, if not impossible for the crops considered. It suggests that in some cases, existing farming practices will be sufficient to achieve a 1% threshold. The report is generally considered to be a useful first step towards assessing the consequences of the introduction of GM crops on a commercial scale in Europe and in identifying appropriate measures at the farm level to minimise the unintended presence of GMOs below the legal thresholds laid down by the Commission but that it shouldn't be taken as an anticipation of future developments.

More recently a study by an expert working group in Denmark (Tolstrup *et al.* 2003) on '*the co-existence of GM crops with conventional and organic crops*' was published. The study concluded that if there was limited GM-production (10%) and a threshold of 1 % for unintended GM presence in non-GM crops, co-existence could be maintained for most crops in Denmark (i.e. beet, maize, potatoes, barley, wheat, oats, triticale, rye, lupine, broad beans and peas), although, for some of these crops current farming practices might need to be modified. For oilseed rape, as well as for seed production of certain crops, the working group suggested that reliably maintaining co-existence could be more problematic and suggested that further evaluation would be required, before guidelines could be developed.

The feasibility of establishing a separate supply and production chain for GM and non-GM crops is dependent on our understanding of the crops themselves and how the genes move. There is a substantive body of scientific evidence indicating that restricting GM presence (or non-GM presence in the case of GM crops) to very low levels is relatively straightforward for some crops, whereas for others, some alteration in farming practice is required. For example, it will be particularly difficult to achieve a very low threshold of GM presence in farm-saved oilseed rape seed on farms where both non-GM and GM oilseed rape varieties are grown. Ultimately however, the threshold levels that are set will determine whether co-existence is practical in the EU. The AEBC will shortly be publishing a report entitled: '*GM crops, coexistence, choice and redress*', and this looks at how far it would be practicable for the

[15] http://www.gmsciencedebate.org.uk/meetings/pdf/170303-transcript.pdf
[16] http://www.jrc.es/projects/co_existence/Docs/coexreportipts.pdf

commercial production of GM crops to co-exist with conventional and organic systems of agricultural production.

7.2.4 Is there general scientific agreement?

There is general scientific agreement that gene flow from GM crops will occur, although this will differ significantly depending on the crop and on the variety in question. The release of GM crops is regulated and the potential consequence of gene flow is a component of a detailed risk assessment. The vast majority of gene transfer occurs within a relatively short distance of its source. The use of separation distances and agricultural practices that limit gene flow will enable the unintended presence of transgenic DNA to be maintained at low levels for most crop varieties. There are many additional political and economic issues that will determine whether co-existence is ultimately possible – this includes what the maximum legal thresholds of GM presence in non-GM crops and their products will be.

The consensus view is that the minimum GM presence that can be achieved is dependent on the variety not just on the crop. For most major crops gene flow can be restricted to at least 1% and for a number it can be far less. However, as the probability of rare cross-pollination events can stay more or less constant for several kilometres for some crops, separation distances will not ensure genetic isolation.

There is some disagreement about whether the separation distances that are currently used to restrict gene flow from GM crops in research and development trials are sufficient. These thresholds have been extrapolated from a substantial range of available data that has been applied to whole field situations.

The potential error associated with quantifying transgenic DNA when it is present at very low levels limits the threshold levels of GM presence that can be accurately quantified and therefore regulated – as opposed to the sensitivity of the technology itself. The international adoption of validated sampling as well as analytical methods will be important in monitoring for unintended GM presence.

The registration of DNA sequences that are unique to particular GM crop varieties with approval for commercial release in the EU will facilitate their detection and identification. However, GM presence arising from commercial varieties that do not have EU approval will be more difficult to identify.

7.2.5 Are the issues unique to GM?

Gene flow between different varieties of the same crop is almost as old as agriculture itself. The desire to restrict gene flow is not unique to GM agriculture either. If GM crops are commercialised, restricting gene flow between non-GM and GM varieties will potentially be on a much larger scale than anything that has preceded it. Gene flow would be restricted because of legal thresholds and consumer demand rather than necessarily risk management. Gene stacking is not unique to GM, but it is possible that it could result in combinations of traits that would not occur in non-GM crops.

7.2.6 Are there gaps in our knowledge or scientific uncertainties and are these important?

We know a great deal less about long distance gene flow than we do about gene flow over distances of a few hundred metres or less. The consequences of this are more significant for some crops than others (e.g. those that have a greater tendency for cross-pollination such as oilseed rape varieties that contain male sterile plants). Such data has been difficult to obtain before the advent of GM crops except in unique situations such as that in Australia when non-GM herbicide resistant oilseed rape was introduced for the first time recently (Reiger *et al*. 2002). However, it is debatable whether more research on long distance gene flow will provide us with more useful information apart from reinforcing the conclusion that it occurs at very low frequencies and is variable.

In the main, gene flow between crop varieties has not been studied on a farm or regional scale (however, there are exceptions: e.g. Squire *et al.* 1999) - models have been developed but these are largely based on studies carried out on a much smaller scale. If GM crops are grown commercially in the UK, monitoring gene flow as the scale of their introduction increases will be important in refining our predictions.

A large area of uncertainty is the way in which the different factors in determining co-existence will combine at a commercial scale – i.e. the real-life consequences of the combination of adventitious presence in seed, cross-pollination, and the contribution of volunteers.

It important that farmers and others involved in supplying GM crops are provided with accurate guidance on management practices that restrict seed-mediated gene flow.

Advanced diagnostic and sampling methodologies for determining the extent of gene flow early in the production/ supply chain will be important in facilitating co-existence.

7.2.7 Likely future developments

It is unlikely that further studies on gene flow from crop varieties will allow us to reduce thresholds of maximum GM presence any further. However, in the longer term it is possible that gene containment systems will be developed that will significantly, if not totally reduce gene flow. If the complete genetic isolation of a GM crop variety is to be achieved, it is likely that it will need to contain a combination of systems that prevent seed, as well as pollen-mediated gene flow. However, these mechanisms are likely to introduce new complications for those developing such varieties and producing seed on a commercial scale.

Several strategies have, and are being developed to eliminate selectable marker genes from plant genomes after transformation, or to control fertility (e.g. site specific recombination, homologous recombination, transposition and co-transformation – Hare and Chua, 2002). Marker removal may be desirable for functional, economic, regulatory, or perhaps safety reasons. Fertility control might be used to produce hybrid seed, prevent pollen-mediated gene flow, stop pollen production for allergy or energy reasons, or prevent seed production for regulatory or commercial reasons. Recombinase methods are not yet well developed, but refinements in technologies to excise transgenes or parts of inserted DNA are likely to become available in the future.

Plant scientists working on the development of RNA interference technology (affects levels of gene expression) consider that this, along with our increasing understanding of the function of different plant genes will provide an opportunity to develop a range of GM crop varieties containing gene constructs based on native plant genes as opposed to transgenes from other organisms.

However, future generations of GM crops may also be used as 'biorefineries' for making novel products such as biofuels and pharmaceuticals.The existing practice of assessing each new GMO on a case by case basis is appropriate for regulating these new types of GM crops.

Transgene stacking will become more likely if a number of different GM crop varieties are grown on a commercial scale. This will provide challenges to the regulatory system in assessing the implications as it will involve the unintentional combination of genes in the countryside that will not have been combined deliberately during the development of the GM variety. The more GM varieties of the same crop that are grown commercially, the larger the potential combinations of transgenes combinations to be considered. The stacking of GM traits is not the only issue that regulators consider – the possibility that transgene products might interact at a biochemical level is also assessed.

Advanced diagnostic and sampling methodologies for detecting GM presence in non-GM crops are being developed. Approved methodologies for detecting, quantifying and identifying GM presence at different stages in the supply chain will be important in maintaining co-existence.

7.2.8 Where there is important scientific uncertainty, what is the potential way forward?

Research

Research into gene flow on a larger scale is being undertaken. This involves a project to measure outcrossing from fields in Scotland that are sown with the GM herbicide-tolerant crops of oilseed rape used in the farm-scale evaluations. The easily detectable markers in these crops should allow more accurate estimates of cross-pollination at low frequency than had been possible before. For wider applications, a consortium led by SCRI is developing advanced, high throughput diagnostic techniques for measuring gene flow at low frequency among non-GM fields. Together these studies will also quantify the pollination efficiency of insects, such as bumble bees, hive bees and pollen beetles that contribute to crossing, quantify the spatial patterns of crossing in fields, and develop the sampling protocols necessary to estimate whole-field crossing accurately.

If GM crops are to be grown on a commercial scale in the UK, monitoring gene flow as the introductory process develops will be important in ensuring that measures to maintain co-existence are working and that further steps do not need to be taken. Assessing the relationship between crop-to-crop gene flow and the legal thresholds for GM presence in non-GM food chains is a key aspect of gene flow research.

Technological/ agronomic approaches

It is important that there are accurate guidelines on management practices that restrict seed-mediated gene flow and that farmers and others involved in producing and supplying seed implement them.

Current sampling and detecting methodologies must be capable of supporting legislation on maximum GM presence thresholds. To this end there must be internationally approved monitoring, sampling and detection methods for all crops (and products derived from them) that are capable of facilitating the detection and quantification of GM presence at, or below any threshold levels that are set. These are currently being developed in a collaborative effort involving a number of European laboratories.

Regulatory approach

The registration of DNA sequences that are unique to particular GM transformation events used to develop GM crop varieties, approved for commercial release in the EU, will facilitate their detection and identification. However, GM presence from varieties that do not have EU approval will be more difficult, if not impossible to detect. Information about transformation events that have been approved outside of the EU is widely available but this does not include the detailed molecular data available for EU approved events.

Currently, continuing assessments of the consequences of gene flow from GM crops are made on a case by case basis. This is the most effective way of dealing with GM crops with novel traits. Regulators will have to continue to be mindful of the possible consequences of transgene stacking.

7.3 GENE FLOW FROM GM CROPS TO AGRICULTURAL WEEDS AND WILD RELATIVES

Can the extent and consequences of gene flow from GM crops to agricultural weeds and wild relatives be predicted and controlled? Could gene flow from GM crops generate superweeds or eliminate wild plant populations?

7.3.1 Summary

Gene flow – the transfer of genes, in pollen or seed, from one population to another - is commonplace among closely related adjacent plant populations. Gene flow by cross-pollination involves both hybridisation and the incorporation of the gene into the new population (introgression). This last process varies greatly from one situation to another and provides most of the uncertainty in predicting actual amounts of gene flow.

Most modern crops have been bred from wild plants. Nearly all hybridise with one or more wild relatives somewhere in the world, but modern agriculture has moved many crops outside the range of sexually compatible wild relatives. Crop-to-wild relative gene flow varies between different crops and different regions. For example in the UK gene flow to the wild is not an issue for crops such as wheat, maize, potatoes and tomatoes but must be considered for those such as ryegrass, clover, sugar beet and oilseed rape.

The exchange of genes between crops and their wild relatives that has occurred during the long period of crop domestication continues today, often aided by farmers in small-scale agriculture. This and the movement of seed around the world has made it difficult to measure accurately in specific cases the rates of contemporary gene flow. Recent studies using molecular methods are providing new insights into these rates.

If hybridisation and introgression occur, the subsequent spread of the gene could be increased by continuing high rates of gene flow, the gene's accidental fixation in small populations or the overall greater fitness of wild plants with the gene than those without it. An increase in the gene in the wild relative does not necessarily mean it will become more persistent or invasive – other ecological criteria discussed in section 6.2 apply to invasiveness.

Modern studies (particularly on beet and oilseed rape) have confirmed that gene flow to wild relatives occurring as weeds in arable fields and disturbed agricultural habitats is higher than to wild relatives occurring in semi-natural environments. Gene flow rates also very considerably from place to place depending on a range of conditions. As expected gene flow mediated by seed transfer to semi-natural situations has been demonstrated (in sugar beet) but gene flow by cross pollination and subsequent introgression appears generally lower in these environments. This is believed to be due to selection during domestication of traits which are disadvantageous in the wild.

More than two decades of experience with the technology indicates that in the context of gene flow transgenes behave exactly as resident naturally-occurring genes. The issue of gene flow from crops to wild relatives is not unique to GM, and there is no evidence that current transgenes are more likely to transfer or persist in the wild than other genes. However, each crop/gene combination is, and must continue to be, considered on a case-by-case basis.

There is broad consensus that, in particular cases, gene flow to wild relatives is inevitable and that gene flow itself is not intrinsically harmful. It is the consequences of gene flow that are important. For example genes conferring herbicide tolerance have the potential to create an agricultural weed management problem, especially if weed tolerance of more than one herbicide occurs by gene stacking. On the other hand, herbicide tolerance has been shown to be at best neutral, and sometimes disadvantageous in wild plants and situations where herbicides are not applied. Genes conferring resistance to insect pests or pathogens have the potential to increase the fitness of a wild relative. Again, however, this possibility must be examined on a case-by-case basis.

Most of the gaps in our knowledge of gene flow relate to its consequences. Whilst genes for pest and disease resistance introduced into crops by conventional breeding have not produced invasions of wild relatives in semi-natural environments, current regulatory oversight of GM crops deals with this possibility on a case-by-case basis. In those cases where gene flow is possible, however rare or improbable, the consequences are assessed. Consent to release a GM crop would not be given were any harm to human health or the environment envisaged from the transfer of a transgene by gene flow to wild relatives.

7.3.2 Background

Gene transfer from GM crops to agricultural weeds and wild relatives has been addressed in two open meetings associated with the GM Science Review: the Royal Society of Edinburgh Meeting in January 2003 and the scientific discussion meeting at the Royal Society in February 2003. Abstracts and transcripts of these meetings are available on the GM Science Review website[17]. The GM Science Review has also received a number of contributions to its website about gene flow from crop plants to agricultural weeds and wild relatives. These have presented evidence (e.g. Chris Lamb)[18], concerns (e.g. Michael Cates)[19] and questions (e.g. GeneWatch)[20].

Gene flow is the transfer of genes, in pollen or seed, from one population to another and the incorporation of those genes into the gene pool of the recipient population (Futuyama, 1998). In the case of pollen transfer, it is essentially a two-stage process: hybridisation and introgression. For hybridisation to occur the plants must be sexually compatible and flower at the same time, viable pollen must be delivered to the stigma and successful fertilisation of the embryo must be followed by zygote and seed formation. Introgression requires the hybrid seed to germinate and the (F_1) plant to establish and flower in order to further hybridise with members of the recipient population.

Such gene flow is commonplace among closely adjacent populations of the same species, although in many species it can be reduced by self-fertilisation or various inherited incompatibility systems. The amount of gene flow reduces with increasing physical distance of populations of the same species and with increasing evolutionary distance of different species, i.e. decreasing relatedness. Although to some extent all plants are related, the

[17] http://www.gmsciencedebate.org.uk/meetings/default.htm
[18] http://www.gmsciencedebate.org.uk/topics/forum/0064.htm
[19] http://www.gmsciencedebate.org.uk/topics/forum/0015.htm
[20] http://www.gmsciencedebate.org.uk/topics/forum/0007.htm

presence and strength of the various barriers to hybridisation outlined above is determined by how closely related the species are. Whilst modern plant breeding uses a range of mechanisms to overcome these barriers (e.g. embryo rescue, GM), in the field the range of related species with which a crop may hybridise is specific for each crop species and is known for (almost) all of them. The extent to which introgression can occur however, varies greatly between species (and situations), and, by contrast, is less well known. This element of gene flow, introgression, is therefore responsible for most of the uncertainty in determining or predicting actual rates of gene flow. It is also influenced by the effect of the transferred gene(s) on the plant (specifically plant fitness). Gene flow and its consequences are thus intimately confounded.

Most crops have been bred from wild plants. On a global scale it is therefore not surprising that nearly all crops may hybridise with a wild relative in some part of their distribution range (Small 1984, Ellstrand *et al* 1999). However only a tiny fraction of the world's flora has been domesticated and in modern agriculture many crops are grown outside the range of the wild relatives, often their antecedents, with which they might hybridise. The potential for gene flow from crops to wild relatives will therefore vary from region to region. In the UK, for example, gene flow is not an issue for crops such as wheat, maize, potatoes and tomatoes (because they have no sexually compatible wild relatives in the UK) but is a major issue for, among others, ryegrass, clover, sugar beet and oilseed rape (Raybould & Gray 1993). (In Europe an analysis of possible gene flow has been made for the flora of the Netherlands (de Vries *et al* 1992), Switzerland (Jacot & Ammann 1999) and the UK (Raybould & Gray 1993)). The two crops for which applications for commercial release have been made where gene flow to wild relatives must be considered are oilseed rape (*Brassica napus*) and sugar/fodder beet (*Beta vulgaris*). These are discussed in more detail later.

In the context of gene flow three types of 'wild' plant populations can be distinguished. These are:

(i) **Feral Populations** – crop plants which have, perhaps temporarily, escaped from cultivation and are growing in the wild, often in habitats which are frequently disturbed. Oilseed rape on road verges is perhaps the most familiar current example but several crop species have established feral populations in the British countryside [e.g. lucerne, chicory, carrot (Stace 1991)].

(ii) **Weedy relatives** – species related to crops which may grow within the crop, sometimes becoming weeds ('plants in the wrong place'), or in peri-agricultural environments (tracks, verges, headlands etc). Examples include wild turnip, charlock and weed beet.

(iii) **Relatives growing in 'natural' environments** – plants which occur outside arable agriculture in the UK's semi-natural habitat-types such as chalk grassland, heathland, saltmarsh or woodland. These include clover, ryegrass, wild cabbage and sea beet.

Some species may occur in more than one category. For example both wild turnip (*Brassica rapa*) and wild radish (*Raphanus raphinistrum*) have populations that occur in agricultural environments and other populations (possibly subspecies) which are found in semi-natural habitats (*B.rapa* on riversides, *R.raphinistrum* in sand dunes). Other species, such as wild cabbage (*Brassica oleracea*) on coastal cliffs, may have

originated as feral populations many years ago, but are not weedy and are now regarded as naturalised (Preston *et al* 2002).

For any particular crop, on a case-by-case basis, it is necessary to assess the likelihood and consequences of gene flow to all wild relatives in all of the above three categories (and also to plants 'escaping' from crops e.g. 'volunteers' in oilseed rape, 'groundkeepers' in potatoes, 'bolters' in sugar beet). However in practice concerns about gene flow have usually made a distinction between gene flow to agricultural weeds and gene flow to wild relatives in semi-natural environments. In the first case concern has been largely centred on the possibility of creating agricultural problems such as more herbicide-tolerant weeds (so-called 'superweeds') (Hall et al 2000, Orson 2002). In the second case concerns have included the possibility of the wild plants becoming more persistent or invasive following transfer of a gene which increases their 'fitness', the potential impact on other plant and animal species, and the genetic 'pollution' of natural populations with genes derived originally from sources such as bacteria or viruses (Genewatch 1998, Hill 1999, Daniels & Sheail 1999).

During their long period of domestication many crops have hybridised with wild relatives, and *vice versa* (DeWet & Harlan 1975, Pickersgill 1981). Farmers have often selected these hybrids for cultivation, and in some crops, especially those under small-scale agriculture (which equals 40% of world agriculture) continue to do so (Jarvis & Hodgkin 1999). These crops include maize, rice, chillies, potato, sorghum, squash and pearl millet; in the last of these cultivated and wild forms are known to have exchanged genes for at least 3,000 years (Renno *et al* 1997). Many plant species can be found both as a crop and a weed (e.g. oilseed rape) and close relatives may 'mimic' crops under certain forms of agriculture (e.g. rice (Barrett 1983). Furthermore, past agriculture and the wide exchange and movement of seeds has transferred plants, and their genes, to many parts of the globe. For example thousands of tons of white clover seed were imported into Britain from many European countries in the eighteenth and nineteenth centuries, from North America as long ago as the beginning of the nineteenth century and from New Zealand in more recent times (making it difficult to define a 'native' genotype) (Gray *et al* 2003).

All these factors, the common evolutionary lineage, the link in various combinations between crops, weeds and wild relatives, the (frequently unknown) movement of seeds, mean that modern crops and their wild relatives often have many genes in common (technically they are said to share genes by descent). This has made it very difficult, at least until recently and the advent of molecular methods, to quantify the amounts of contemporary gene flow. In other words we know that gene flow has, or could have, happened but we cannot usually say with any accuracy how frequent it is today. However some studies, described below, are beginning to provide estimates of gene flow rates for specific crops.

Among the factors that are known to affect the amount of gene flow (relatedness/hybridisation barriers, degree of self-pollination, and so on), there is a great deal of information on the effect of distance. Cross-pollination falls off rapidly with distance but the distance at which it is zero is impossible to determine with accuracy. Curves describing the frequency of cross-pollination at various distances from a pollen source have been derived from experiments and used particularly to calculate the separation distances required between GM and non-GM crops in order to achieve minimal levels of crop-to-crop gene flow (Ingram 2000, Champolivier *et al* 1999). The relative size of the donor and recipient populations is also known to be an important factor (Squire *et al* 1999). In general the large amounts of crop pollen compared to that produced by the (normally) smaller feral or wild populations will tend

to increase gene flow to these.

Providing hybridisation and introgression are possible, genes from crops may theoretically increase in frequency in local wild populations under three conditions. These are –

(iv) Very high levels of cross-pollination giving a constant immigration of crop genes to the population (swamping),

(v) The 'accidental' fixation of the crop gene in a small wild population (genetic drift) and/or,

(vi) Where the gene confers greater lifetime fitness on the individuals with the gene than those without it (selection).

Genes may spread in a population under these conditions, including where the plants containing them are fitter, but this does not mean that the plant populations will become more persistent or invasive – the criteria for this to happen are discussed in section 6.2. It could mean that the wild species becomes genetically more uniform, or depauperate (genetic erosion). This is unlikely unless wild populations are exposed to gene flow from the crop across most of the geographical and ecological range of the species.

In assessing the risks from gene flow to wild relatives the ACRE consider both exposure (the probability of gene flow) and hazard (the harm that might result from gene flow). If it is known that a wild or weedy species is sufficiently sexually compatible for gene flow to occur, however rarely, it is assumed that it will happen (i.e. probability = 1) and the consequences are assessed. Were any harm envisaged, ACRE would advise against issuing consent (Gray 2002a). In cases where partially compatible wild relatives only occasionally co-occur with the crop, information on hybridisation rates may contribute to the risk evaluation; but, again, a transgene which was thought to have potentially harmful effects in that wild relative would not be released.

7.3.3 Range of views and quality of evidence

There is a range of views on the importance and consequences of gene flow from GM crops to agricultural weeds and wild relatives. These include – views about gene flow itself, views about the likelihood and rate of gene flow, and views about the impact and consequences of gene flow, both on agricultural weeds and wild relatives in semi-natural environments.

In addition to the fundamental view (mentioned earlier) that DNA originally derived from bacteria or viruses should not be transferred to wild plant populations, there is a view that their method of insertion in the plant (whether by bacterial plasmid vector or biolistics) makes the behaviour of transgenes unpredictable when inserted in the genomes of wild relatives (the issue of transgene stability is covered in Chapter 4). There is a further view that transgenes differentially interact with native genes in wild relatives under different environmental conditions and that this is not sufficiently understood (N. Rajanaidu – contribution to Review website)[21]. There is a general concern that genes from domesticated plants (including crop

[21] http://www.gmsciencedebate.org.uk/topics/forum/0003.htm

plants and those bred for amenity use) threaten the genetic integrity of local adapted populations and the patterns of genetic diversity within wild species (see Gray 2002b for a review). Whether gene flow from GM crops has additional implications for the genetic identity of these populations is an issue that has been raised during this review (GeneWatch)[22]. Another contributor to the review website expressed a more specific concern about the possible consequences of gene flow from GM crops containing transgenes that confer male sterility (Mae-Wan Ho and Joe Cummins)[23]. Another viewpoint is that the transgenes so far inserted in crops can be viewed and evaluated in essentially the same way as any novel genes and their transfer to weeds and wild relatives is not fundamentally different from the process of gene exchange between crops and relatives which has been occurring for thousands of years (see above). This last view, which is probably the majority of scientific opinion, argues that gene flow to wild relatives is not intrinsically harmful but that every transformation (each crop/gene combination) should be examined on a case-by-case basis to assess whether gene flow may have harmful consequences to human health or the environment.

Evidence that transgenes are inherited and transferred between individuals in a similar way to resident genes may be derived from more than a decade's experience with the technology. Genes inserted by recombinant DNA technology and selected for plant breeding programmes demonstrate Mendelian segregation and recombination and 'flow' from plant to plant exactly as resident genes. Experiments in which crosses have been made between transgenic crops and wild relatives show segregation patterns consistent with the expectation that transgenes are inherited in the same way as naturally occurring genes (Snow *et* al 1999, 2003, Halfhill *et al* 2002 - in these examples for herbicide tolerance and Bt in *Brassica* and Bt in sunflowers). Evidence that past gene flow has had an impact on the population biology and survival of wild species in the UK is difficult to find, although the potential impact globally of modern crops on local land races of several species is a widely acknowledged problem and the presence of genes derived from crops has been established in a number of wild species (e.g. sunflower (Linder *et al* 1998). An example of hybridisation threatening the genetic integrity of a native species is given by Al Mazyad & Amman (1999) who describe gene flow from Lucerne (*Medicago sativa*) to tetraploid populations of sickle medic (*M.falcata*) in regions of Switzerland. Indirect evidence of past gene flow may also be inferred from current patterns of population differentiation, as in rye grass (Warren *et al* 1998).

There is general agreement that in specific cases gene flow to sexually compatible wild relatives will occur. Disagreements are principally quantitative in nature – how much? And how far in specific situations? The evidence from studies of cross-pollination experiments and from crop-to-crop gene flow using marker genes indicates variation in specific cases but the generality of the pollination curve has been established. This shows a rapid decline in cross-pollination after the first 10 to 20 (-50) metres from a pollen source with a low level of cross pollination continuing often over considerable distances (in excess of 500m) (Champolivier *et al* 1999, Rieger *et al* 2002). Such curves vary in detail from species to species depending on their breeding system and their mode of pollination (wind versus insect) but their general form (technically described as leptokurtic) confirms that (a) most plants mate with near neighbours or themselves and (b) rare cross-pollination events occur at long distances. These data suggest that for most crops with wild relatives, as well as those whose relatives co-occur as weeds of agriculture, it will not be possible to prevent cross-pollination. (There are exceptions, e.g. gene flow from lettuce in the UK could be prevented by growing the GM

[22] http://www.gmsciencedebate.org.uk/topics/forum/0007.htm
[23] http://www.gmsciencedebate.org.uk/topics/forum/0046.htm

crop outside the rather restricted range of its sexually compatible wild relative – Raybould & Gray 1993). Empirical evidence that levels of hybridisation are extremely low except where the crop and wild relative occur together (i.e. are separated by less than 2-5m) has been provided for oilseed rape hybridising with wild turnip (*B.rapa*) on river banks (where it is known as Bargeman's cabbage). Using a combination of remote sensing and genetic analysis, Wilkinson *et al* (2000) detected only a single hybrid in *B.rapa* populations in a 15,000 km^2 area of S.E. England. This low number was largely because few wild turnip populations occur next to (within one or two metres) oilseed rape fields. The work is continuing (funded by a BBSRC/NERC gene flow initiative) and has been extended to the rest of the UK, to provide an estimate of annual hybrid production. For *B.rapa* as a crop weed, work in Denmark (Jorgensen & Andersen 1994) has confirmed earlier studies (in 1962 – Palmer) that the highest number of hybrids (80%+) are produced when small numbers of the (self-incompatible, diploid) *B.rapa* are placed in oilseed rape fields (i.e. there is a large excess of oilseed rape pollen).

The likelihood of stable introgression of transgenes into wild populations depends critically on the survival of subsequent generations. Here there is evidence of differences between gene flow to relatives which are arable weeds and gene flow to relatives growing in semi-natural environments. For example, in Denmark, where both oilseed rape (*B.napus*) and wild turnip (*B.rapa*) occur together as weeds in set aside land or organic farmers' fields, substantial introgression beyond the F_1 stage with back-crossing involving both species has occurred. This is supported by clear molecular evidence (Jorgensen *et al* 2003). This contrasts with the potential rates of gene flow to *B.rapa* in semi-natural habitats (i.e. beyond the very low numbers of F_1 hybrids) described above. Similarly, studies in northern France on gene flow between sugar beet (*Beta vulgaris ssp vulgaris*), weed beet (*B.vulgaris ssp. Vulgaris*) and sea beet (*B.vulgaris ssp maritima*) have demonstrated high levels of gene flow between the crop and the (annual) weed beet populations in heavily infested sugar beet fields (Desplanque *et al* 2002) but detected little or no gene flow between sugar beet and nearby sea beet populations (Cuguen 2003). In particular a gene removing a requirement for vernalisation (leading to an annual life cycle) does not appear to have been transferred to sea beet populations in N.France and the UK despite a long exposure of the wild plant to crops and weeds containing the gene Cuguen 2003, Van Dijk *et al* 1997). A recent paper from the Lille group has confirmed that gene flow to habitats where sea beet occurs can be seed mediated (in this case by the transfer of soil) (Arnaud *et al* 2003).

The contrast between rates of gene flow to arable crop weeds and to wild relatives supports the general view that genes transferred from domestic to wild species produce hybrids with poor survivorship in semi-natural environments. Genes transferred with the transgene will include some which code for traits adapted to agricultural environments but inappropriate in the wild (e.g. pod shattering, low or inappropriately cued dormancy). This phenomenon (known as linkage drag) may explain why no crop-wild relative hybrid has become invasive in the UK. The few seriously invasive species have come from the 1 274 naturalised exotic species introduced in the UK usually for horticulture or by accident [see section 6.2, box 6.1 and a recent Nature report (Adam, 2003) mentioned by R. J. Berry on the Review website[24]]. Transgene stacking in sexually compatible wild relatives of GM crops, although possible, is likely to be rare for the reasons discussed above. However, if it did happen, it is theoretically

[24] http://www.gmsciencedebate.org.uk/topics/forum/0058.htm.

possible for the benefits of some combinations of transgenes to outweigh the disadvantages of linkage drag and result in wild plants with increased competitive ability. Therefore, risk assessments should, and do consider which transgenes are present in different GM varieties of the same crop and what the consequences of their combination in the same plant might be (for crop plants and any sexually compatible wild relatives). Assessing the consequences of some stacked traits on wild populations, whether they are from GM or non-GM varieties, is potentially difficult, although this may not be the case for other trait combinations (e.g. tolerance to different herbicides). The most direct way of assessing the fitness of any wild relative with stacked transgenes would be to create such plants and test their fitness under field conditions. However, given the current nature of the regulatory system in the UK, studies involving deliberately modified wild plants outside of contained conditions are very unlikely. There are few studies that have looked at the performance of hybrids of GM crops and wild relatives in semi-wild conditions (containing single traits) in the USA. Most of these data comes from agricultural weeds that have been deliberately crossed with transgenic crops (Snow *et al.* 2003; Spencer and Snow, 2001).

It is widely agreed that the spread of the transgene, and hence the consequences of gene flow, is likely to vary on a case-by-case basis. Genes conferring herbicide tolerance have the potential to create an agricultural weed problem if transferred to arable weeds (or volunteers). This can be seen from studies of oilseed rape in Canada where complete freedom among farmers to grow varieties tolerant to one of three herbicides (two of which were transgenic) has led to gene stacking and to multiple tolerance (Senior & Dale 1999, Hall *et al* 2000, Orson 2000, Beckie *et al* 2002 and Warwick *et al* 2003). Senior & Dale (2002) point out that careful management of herbicide tolerant crops can delay, or even prevent, the emergence of a herbicide-tolerant weed problem.

Plants containing herbicide tolerant genes are likely to survive and spread in conditions where the herbicide is being applied (in the last 40 years more than 120 plant species worldwide have developed herbicide resistant individuals under modern agricultural conditions). Elsewhere, and particularly in semi-natural environments such plants may be at a disadvantage compared to individuals without the herbicide-tolerance gene (Crawley *et al* 2001) and there is experimental evidence that herbicide-tolerance actually confers a cost on its possessor (Bergelsen *et al* 1996, Snow *et al* 1999). In other cases (e.g. resistance to insects conferred by the possession of the *Bt* gene) the transgene will not necessarily confer a cost under greenhouse conditions and will actually lead to increased fitness under insect pressure (Stewart *et al* 1997). It may also lead to increased seed production in semi-natural environments (Snow *et al* 2003). This reproductive advantage in the wild, an increase in fecundity, does not necessarily mean that the gene would increase the biological fitness of the plant (Bergelsen 1994, Snow *et al* 2003). In environments where the specific herbivores are absent (enemy-free environments) plants defended genetically against them may be out-competed by undefended plants (Agrawal *et al* 1999, Redman *et al* 2001). It is clear from studies of viruses in wild *Brassica* species in the UK that the complex interaction between different pathogens and different host species prevents generic assessments or predictions of the likely outcome of the transfer of a particular pathogen or herbivore resistance gene to a wild relative (e.g. Maskell *et al* 1999, Raybould *et al* 1999, Thurston *et al* 2001, Pallett *et al* 2002, Raybould *et al* 2003).

A contribution to the review has raised concern about crops genetically modified for male sterility and in particular the use of the *barnase* gene (Mariani *et al.* 1990 and 1992) in case it transfers to wild plant populations and causes their extinction (Mae-Wan Ho and Joe

Cummins)[25]. In transgenic plants the *barnase* gene is controlled by a promoter that restricts its expression to tapetal cells of the anther associated with the production of pollen. Plants containing the *barnase* gene will be male sterile and will need pollen from male fertile plants to reproduce - they cannot therefore transfer the gene to other plants (wild relatives or crop plants). The expression of a second gene, *barstar*, also controlled by a tapetum specific promoter in this same cell layer, stops the activity of the barnase gene product and restores male fertility. Consequently, plants containing *barnase* and *barstar* genes can produce pollen, which could potentially pollinate sexually compatible wild relatives as well as other crop plants (the likelihood of this occurring is discussed above and in section 6.2 respectively). However, in this case, approximately a quarter of the progeny of any crop/wild relative hybrid that does result will not be able produce pollen and therefore transfer the *barnase* gene to other plants. This is because *barnase* and *barstar* genes are not genetically linked in the GM crop (i.e. they are not in close proximity to each other on the same chromosome) and so will become separated in the progeny of future generations. Even if both *barnase* and *barstar* genes were transferred to a non-GM crop plant, or to a wild relative there would be no immediate consequences because seeds would be produced and oil harvested. The male sterile progeny from these plants would be greatly diluted by progeny from seeds from non-GM plants or wild-relatives that did not contain the *barnase* gene. The likelihood of *barnase* gene transfer is therefore extremely low, much lower than for most other genes. Many plant species have male sterility and this does not result in their extinction (reviewed by Williams, 1995). On the contrary, male sterility has been exploited in conventional plant breeding because it necessitates out-crossing and therefore generates genetic variation and hybrid vigour. However, in some crops male sterility may be associated with other characteristics that are not wanted, or it may not be stable. In such cases the use of genetic modification to confer male sterility may be useful. With respect to concerns about the characteristics of *barnase* gene expression, there is a body of quality evidence that shows that the barnase enzyme is restricting to specific cell layers and its activity is very effectively prevented when barstar is present (Mariani *et al*.1990 and 1992). If this were not the case, the GM crops in which this system is used would either not survive, or would perform poorly compared to their non-GM counterparts and there is no evidence of this.

Although the likelihood and consequences of gene flow must be assessed on a case-by-case basis and will differ in weedy and wild relatives, the evidence discussed above supports the view that some broad generalisations can be made based on our current understanding of population biology and genetics (and underpinned by the paradigms of evolutionary biology). Genes likely to confer fitness (e.g. virus resistance) have greater potential to lead to 'ecological release' (the expansion of a population locally following the removal or disablement of a regulatory mechanism such as herbivory or a pathogen) than genes which are neutral or disadvantageous (e.g. herbicide tolerance). However other constraints on population expansion such as density dependent competition could prevent an increase in population growth rates (discussed in Chapter 6.2). Overall, the fact that genes for pest and disease resistance inserted into crops by conventional breeding have not produced invasions of wild relatives in semi-natural habitats, coupled with the evidence that transgenes behave as naturally-occurring genes, suggest that predictions based on the tenets of invasion biology are supported by genetic evidence.

[25] http://www.gmsciencedebate.org.uk/topics/forum/pdf/0046.pdf

7.3.4 Is there general scientific agreement?

There is agreement that cross-pollination with wild relatives, where the latter are sexually compatible with the crop species, is likely to occur and the extent of hybridisation will vary from species to species and under different conditions. However as described above, the likelihood of gene flow depends not only on the range of factors influencing hybridisation but also on factors affecting the survival, growth and reproduction of the hybrid (introgression). The production of a crop/wild relative hybrid is but the first step in genetic exchange between populations.

The majority of scientific opinion argues that gene flow to wild relatives is not intrinsically harmful but that every transformation (each crop/gene combination), and every potential transgene/transgene conbination which could arise through gene stacking, should be examined on a case-by-case basis to assess whether gene flow may have harmful consequences to human health or the environment.

7.3.5 Are the issues unique to GM?

The phenomonen of gene flow from crops to weeds and from crops to wild relatives has been a part of agriculture for many hundreds of years, and remains a possibility in modern agriculture (see above). What is new is the possibility of introducing genes that code for entirely novel traits such as the production of novel enzymes or pharmaceutical products. This possibility provides the imperative for regulating GM technology on a case-by-case basis. There is currently no evidence to indicate that transgenes are more likely to transfer and persist in the wild than naturally-occurring genes.

7.3.6 Are there gaps in our knowledge or scientific uncertainties and are these important?

Although there are uncertainties about the scale and variability of crop to wild relative (and indeed crop to crop) gene flow, the major gaps are in understanding the potential consequences of gene flow. The effect of particular traits on the fitness of the weed or wild relative which may receive them is an important target of ongoing research (e.g. the studies of virus resistance in *Brassica* referred to above are a series of research projects funded under the BBSRC/NERC Initiative 'Gene Flow in plants and micro-organisms').

Ways are being sought of assessing the potential impact of transgenes on fitness that provide less expensive alternatives to the PROSAMO type experiments described in Chapter 6. These include targeted experiments, modelling and the development of protocols using a tiered approach (Linder 1999, Linder & Schmitt 1994, Bullock, Raybould *et al* 1999a, Hails 2000, Wilkinson *et al* 2003). All these approaches aim to assess the relative fitness of wild relative with and without the trait of interest (see section 6.2). If a range of GM varieties of the same crop are grown extensively in the UK, it is possible that transgene stacking will occur in sexually compatible wild relatives. Predicting the possible effects of potential transgene combinations in wild relatives (as well as in crop plants, see section 7.2) will become increasingly complex if a range of different GM crop varieties are grown on a commercial scale. This is unlikely to be a significant issue in terms the near future because of the number

and type of GM varieties that could be approved for commercial release in the UK. However, regulators will have to continue to be mindful of the consequences of gene stacking.

7.3.7 Likely future developments

Several technological solutions to containing or reducing gene flow from GM crops have been proposed (discussed in more detail in section 6.2). These can variously be labelled 'gene containment systems' and include insertion of the gene into the chloroplast rather than the nuclear genome (Daniell *et al* 1998), the use of chemical stimulants to express traits, through the action of an inducible promoter which prevent anther development or seed germination, and various mechanisms for preventing pollen production and dispersal (apomixis, cleistogamy). The risk assessment prior to growing crops expressing pharmaceutical or industrial proteins will need to have addressed the risk management issues around containment. Gene containment systems might be one genetic route amongst the considerations of physical, biological and genetic containment approaches.

7.3.8 Where there is important scientific uncertainty, what is the potential way forward?

The major uncertainties relate to the consequences of gene flow and must be dealt with on a case-by-case basis.

The way forward is the continuing assessment on a case-by-case basis of the consequences of gene flow to weedy relatives and those in semi-natural environments. As indicated earlier some of this research has been carried out or is underway. However ecological questions tend to lie along the critical path of any environmental risk assessment and some demand long-term research and/or monitoring.

7.4 CAN DNA FROM GM CROPS TRANSFER TO SOIL MICROBES?

In nature, how important and prevalent is horizontal gene transfer from plants to microbes in the soil, and does the presence of transgenic DNA make this more likely to occur? To what extent are the ecological effects of horizontal gene transfer from plants to soil microbes predictable?

7.4.1 Summary

Soil microbes are exposed to plant DNA from the normal processes of decay of plant material in soil. Most DNA is degraded, but there is a small but not zero possibility that genes in plant DNA will be acquired and expressed by soil microbes. However, the probability may be higher for transgenes in current use than for average plant genomic DNA because they contain DNA derived from bacteria. The chance of acquisition and expression by bacteria would be reduced by avoiding sequences of DNA that have similarity to bacterial DNA or that resemble bacterial insertion sequences or expression signals, and by using genes containing introns. Genes in chloroplasts may have an increased probability of being acquired and expressed because they are present in higher copy number and have bacterial-type signals. Ultimately, only acquisitions that are advantageous to the microbe have the potential to have ecological impact. Constructs that can rationally be predicted to cause harm if expressed in microbes must be avoided, but many constructs will be harmless because they will confer no advantage on microbes. In some cases, experimental tests may be required to confirm this.

7.4.2 Background

Horizontal gene transfer (HGT) means the transfer of genetic material between organisms with distant genetic relationships in such a way that the genes become heritable in the recipient. HGT is undoubtedly very infrequent, so it is hard to observe directly. Most evidence comes from events that happened long ago (detected by searching genomes for sequences that are shared between distantly-related organisms), or when the acquired genes are strongly beneficial to the recipient (as in the case of antibiotic resistance genes in disease-causing bacteria). While HGT undoubtedly occurs between bacterial species, the existence of HGT from higher organisms to bacteria is less well established. The issues raised here are parallel to those for the potential transfer to gut microbes (section 5.4).

HGT from plants to soil microbes was discussed at a GM science Review open meeting at the Royal Society of Edinburgh in January 2003. In addition, a number of contributions to the Review website are concerned with this issue. The points and questions raised fall into three broad categories (i) Whether and to what extent HGT occurs between GM plants and soil microbes (e.g. ISIS)[26] (ii) what the possible consequences might be (e.g. Brian Stratton[27]; Penny Hirsch[28]) and (iii) what the major uncertainties are (e.g. Greenpeace)[29]. The evidence, concerns and questions presented during the review have framed the writing of this paper.

[26] http://www.gmsciencedebate.org.uk/topics/forum/0030.htm
[27] http://www.gmsciencedebate.org.uk/topics/forum/0021.htm
[28] http://www.gmsciencedebate.org.uk/topics/forum/0085.htm

7.4.3 Range of views and quality of evidence

The frequency at which HGT occurs between plants and soil microbes.

Is the plant DNA available?

Yes. Plant roots slough off some dead cells, and all plant parts eventually die and decay in the soil. Most of the DNA is broken down in the dying cell, or digested by extracellular enzymes, or eaten by animals. However, some persists in the soil for months. There is no reason to suppose that the longevity of transgenic DNA is different from that of other plant DNA, but most studies have in fact looked at transgenic DNA because the issue has been raised in relation to GM plants. Studies have shown that transgenic DNA can be detected for at least four months (Widmer *et al.*1997, Hay *et al.* 2002) or up to two years (Gebhard and Smalla 1999). DNA was detected using the polymerase chain reaction (PCR), which is extremely sensitive and can detect just a few molecules of a gene, though it should be noted that soil often contains compounds that reduce the sensitivity of this assay.

In these experiments, and more generally, DNA may be protected from degradation by cell debris or by binding to clay in the soil, and this may affect its availability to bacteria (reviewed by Dröge et al. 1999). DNA adsorbed to sand or clay can transform competent bacteria (Lorenz and Wackernagel 1990; Chamier *et al.* 1993; Romanowski *et al.* 1993) and Lorenz *et al.* (1988) even showed increased transformation efficiency for *B. subtilis* as compared to transformation in solution. Others found lowered availability (Demanèche *et al* 2001a), especially for bound plasmid DNA (Chamier *et al.* 1993).

There are indications that pollen can be an accessible source of DNA in soil: a study by Meier and Wackernagel (2003) found the transgene in soil up to 50m from pollen-producing transgenic sugar beet plants, detected by both PCR and the transformation of *Pseudomonas stutzeri*.

If the transgene is located in the chloroplast genome, rather than the plant nuclear genome, then availability may be enhanced because, in green tissue, chloroplast genes may be thousands of times more abundant than nuclear genes. The relative availability of chloroplast and nuclear DNA has not been compared directly, but persistence of chloroplast DNA in soil has also been demonstrated (Ceccherini *et al.* 2003).

Can microbes take up plant DNA?

Yes. A significant number of bacteria have the ability take up DNA from the environment (they are "competent for transformation"). This competence is often induced temporarily and is sometimes confined to DNA from the same species, but uptake of foreign DNA is definitely possible in a number of species (Lorenz and Wackernagel 1994). Species that are not normally transformable can be forced to take up DNA in the laboratory by electric shock treatment (electroporation). It has been suggested that this might occur naturally through lightning, and this process has been simulated in the laboratory (Demanèche *et al.* 2001b), though probabilities may be low in the field.

The situation in fungi is less well studied than in bacteria, but laboratory methods for transformation have been developed in many species (e.g. Gietz and Woods 2001). Hoffmann

[29] http://www.gmsciencedebate.org.uk/topics/forum/0023.htm. This contains a link to Crops of Uncertain Nature, Controversies and Knowledge Gaps Concerning Genetically Modified Crops, An Inventory (A.J.C. de Visser, E.H. Nijhuis, J.D. van Elsas and T.A. Dueck).

et al. (1994) demonstrated transient expression of hygromycin resistance in the plant-pathogenic fungus *Aspergillus niger* infecting *Brassica* transgenic for the resistance gene, but obtained only a single stably resistant clone of the fungus. The mechanism of acquisition was unknown.

Can microbes incorporate transgenic plant DNA?

Acquired DNA may persist in a bacterial cell for some time and may even be transcribed and translated to make protein, but it will eventually be lost unless it is able to replicate. If it includes a plasmid origin of replication and can circularise, it may become established as an autonomous plasmid. Although transgenic constructs containing a plasmid origin have been made (Schlüter *et al.* 1995), this is not generally true. Normally, the DNA has to integrate into the genome, and the chances of this depend strongly on its sequence. Transposon terminal repeats would provide an obvious mechanism, but there is evidence that any stretch of DNA that has sequence homology to the bacterial genome can greatly enhance the rate of incorporation by homologous recombination (de Vries *et al.* 2001). Details vary between bacterial species, a perfect match between 26 base pairs in a row at one end of the incoming DNA is enough to allow recombination in *Escherichia coli*, whereas *Bacillus subtilis* requires a match at both ends, each of about 20 base pairs (Majewski and Cohan 1999). The stringency of this requirement may vary even within a bacterial species if mutations occur in the DNA repair systems (Vulic *et al.* 1997). Many transgenes are of bacterial origin, which would in principle increase the probability of finding homology in a recipient bacterial genome. However, it is common practice to modify the codon usage[30] of the gene in order to increase expression in the plant. It is likely that this will introduce more than one or two changes in each run of twenty bases, which will be enough to prevent homologous recombination in bacteria that possess the original gene sequence.

Can microbes express transgenic plant DNA?

The regulation of gene expression is different in the plant nucleus from that in bacteria, and the promoters that drive expression in plants may not work in bacteria, although some do. In fact, promoters vary among bacterial species, so it important to consider the likely recipients and a demonstration that a promoter is inactive in certain species should not be extrapolated to all bacteria. For example, a promoter that works well in *Rhizobium* may not function in *Escherichia coli* (Spaink *et al.* 1987). In any case, it is possible (with a correspondingly reduced probability) for the DNA to be inserted behind an existing promoter in the bacterial genome. Most plant nuclear genes contain introns that would not be correctly spliced out in bacteria, so no complete protein product would be produced. However, most transgenes in current use originate in bacteria and do not have introns, so effective expression in bacteria is more likely than for native plant genes. Genes from the chloroplast, whether native or transgene, are much more likely to be expressed in bacteria than are nuclear genes, because expression in chloroplasts is similar to that in bacteria, and most chloroplast genes lack introns. Alterations in codon usage made to improve expression of the gene in plants would be likely to reduce the expression in the original bacterial host, but bacteria vary greatly in their codon usage so some potential recipients might still be able to express the gene efficiently.

[30] this means altering the DNA code without changing the protein that it represents.

What direct evidence is there that bacteria can acquire DNA from plants?

There are no reports that natural soil bacteria have acquired genes in the field from crop plants, whether transgenic or not. However, there has been no systematic large-scale search for evidence.

There have been a number of laboratory studies seeking to determine whether it is feasible for bacteria to incorporate DNA from plant tissues. These studies have used conditions designed to maximise the chance of HGT occurring and of the result being detected. They have been reviewed in detail by Nielsen *et al.* (1998) and Bertolla and Simonet (1999). Citing this work, Nap *et al.* (2003) conclude that 'Several experimental studies have been published that all failed in demonstrating HGT from transgenic plants to bacteria'. However, the following evidence shows that such transfer is possible, albeit demonstrated in more or less artificial circumstances.

Bacteria can incorporate DNA from transgenic plants. Schlüter *et al.* (1995) studied the acquisition by the plant pathogen *Erwinia chrysanthemi* of a bacterial plasmid replication origin and marker gene that had been inserted into the genome of potato. They detected acquisition from purified plant DNA but not from plant tissue, and from a consideration of the various factors that they demonstrated to affect the rate they concluded that under field conditions HGT "is so rare as to be essentially irrelevant to any realistic assessment of the risk involved in release experiments involving transgenic plants".

Plant tissue can be a source of DNA for bacterial transformation. Gebhard and Smalla (1998) showed that a highly-transformable strain of *Acinetobacter* could acquire a kanamycin-resistance gene from homogenised leaves of transgenic sugar beet. However, the rate was low (10^{-10}) even though the potential for incorporation was strongly enhanced because the recipient bacteria had sequences that exactly matched part of the transgene (a "marker rescue" experiment).

Bacteria can acquire genes from plant tissue that has not been artificially prepared. Kay *et al.* (2002) repeatedly detected transfer of chloroplast-encoded sequences from tobacco to *Acinetobacter* in plants damaged by infection with the plant-pathogenic bacterium *Ralstonia*. Again, there was sequence identity between the incoming DNA and the recipient genome, which would strongly enhance the rate.

Evidence for HGT under field conditions has been sought but not found. Gebhard and Smalla (1999) showed that transgenes from sugar beet litter were detectable for up to two years in field soil. The transgenic construct included a bacterial kanamycin-resistance gene, but although kanamycin-resistant bacteria were abundant in the soil, this resistance was not caused by HGT from the beet because none of the 4000 resistant strains tested carried the transgenic DNA. The authors did detect the transgene by PCR in some samples of total DNA from mixed soil bacteria, but there was no evidence that this was derived from the bacteria rather than from unincorporated DNA.

There is one scenario that does not strictly involve gene transfer from plants to bacteria, but has given rise to some concern. The bacterium *Agrobacterium* is commonly used to transfer genes into plant cells because it possesses a natural mechanism for this. After transfer, antibiotics are applied to remove the residual donor bacteria. However, Barrett *et al.* (1997) showed that the commonly-used levels of antibiotics were insufficient to kill all the bacterial cells, so that bacterial contamination persisted over several months. Since the transgenes are

normally carried on a transmissible plasmid in the *Agrobacterium*, there would be a substantial probability that they would be transferred to soil bacteria if such an infected plant were planted out. This could be an issue during the early stages of GM crop breeding, though it would not be likely to affect commercial seed since this would be several plant generations removed from the initial transformation, and *Agrobacterium* is not seed-transmitted. For vegetative crops (e.g. potatoes), this situation is considered in risk assessments.

What do genome sequences reveal about HGT?

Now that the genomes of many bacteria and quite a few higher organisms have been completely sequenced, it is possible to examine them directly for genes that show a pattern of evolutionary relationships which is clearly different from that supported by the majority of genes. Many such examples have been identified, including genes that appear to have transferred between higher organisms and bacteria, but the transfers would have happened long ago, often hundreds of millions years ago (Koonin *et al.* 2001, Brown 2003). Such ancient events are not relevant to the issue of HGT in relation to GM crops.

There is, so far, no evidence for recent successful establishment of plant genes in bacterial genomes. Examination of the complete genomes of an *Agrobacterium* and three rhizobia, all soil bacteria that are very closely associated with plants, provides no evidence of any genes that are very similar to plant genes (Kaneko *et al.* 2000, Galibert *et al.* 2001, Wood *et al.* 2001, Kaneko *et al.* 2002).

What these bacterial genomes do reveal is abundant evidence that some genes have been transferred between bacterial species. While transformation is a possible mechanism here, many bacteria carry conjugative plasmids or transposons that provide a more robust means for genes to spread within and between species. The significance of these processes in the environment has been reviewed many times (e.g. Dröge *et al.* 1999, Davison 1999, van Elsas *et al.* 2003). The surface of roots provides a favourable environment for such transfer because of the availability of nutrients, high bacterial densities which trigger conjugation through quorum sensing[31] (e.g. Oger and Farrand 2002), and possible activating compounds in plant exudates (Zhang *et al.* 1993). Transformation can also be enhanced by compounds exuded by plant roots (Nielsen and van Elsas, 2001). Experimental evidence for the spread of genes between bacteria in the soil environment has been reviewed by Bailey *et al.* (2001)[32]. The relevance of this for the issue of HGT from plants is that, if plant-derived genes were to get into some component of the bacterial community that may be particularly prone to transformation, there is a likelihood that they could be transferred to other bacteria that are not themselves readily transformed.

Predicting the consequences of HGT from plants to bacteria

Any potential consequences of HGT depend on the fate of the recipient microbe. A single microbial cell is too small to have any detectable environmental impact, even if it carries a potent transgene, so the critical question is whether this cell will multiply into a sizable population. It will only do this if it confers some advantage, or at the very least, does not confer any disadvantage over the other microbes that it is competing with.

[31] Quorum sensing is a phenomenon by which some bacteria measure their own abundance - it involves the release and detection of signalling molecules.
[32] http://www.defra.gov.uk/environment/gm/research/pdf/gm_research_17.pdf

To address the potential environmental impact of HGT it is therefore important to ask two questions. Firstly whether expression of the gene would benefit the recipient, because if it is disadvantageous then the number of cells will remain too low to have a detectable effect. Secondly whether the spread of microbes carrying transgenes would alter the functioning of the ecosystem in any significant way. It is obvious from these considerations that the potential consequences of HGT depend on the exact nature of the transgenic DNA, and there can be no general answer.

Many of the relevant issues are addressed in some detail in a report for Defra authored by Bailey *et al.* (2001). This report is concerned with the possibility and potential consequences of HGT from GM bacteria, but of course the subsequent fate of the recipient will be subject to similar considerations regardless of the source of the transgenic DNA.

There is a considerable body of theory concerning the conditions under which a type of organism that is initially rare will establish and spread. A selective advantage is important, but in the early stages there is a high probability that the new type will die out through chance events even if it has an advantage. Although these generalisations are undoubtedly true of the products of HGT, the theory can offer few quantitative predictions without specific knowledge of the relevant parameters in a particular case.

There have also been many experimental studies on the spread of plasmids, etc., through bacterial populations, and the results are concordant with theoretical predictions. However, these have been simple laboratory systems such as well-mixed liquid cultures or plain surfaces (e.g. Simonsen, 1990), which are not representative of the spatial complexity and heterogeneity of the soil and plant environment. There is no immediate prospect of a quantitative predictive theory for the population dynamics of individual microbes in soil, but the same is true for many other complex systems that nevertheless have predictable average properties.

In a similar vein, comparative genomics has revealed that all natural genomes are full of 'accidental' features, such as transposable elements and genetic rearrangements that differ from one species, and even individual, to another (for examples of soil bacterial genomes, see Kaneko *et al.* 2000, Galibert *et al.* 2001, Wood *et al.* 2001, Kaneko *et al.* 2002). Bacteria, in particular, have a large fraction of 'accessory DNA' that varies in content from strain to strain and is often subject to HGT. In this context, there is clearly no longer any basis for the view that the genome is a finely-tuned machine that might be disrupted by the introduction of a 'foreign' gene, causing the organism to 'run amok' in some unpredictable way. Bacterial genomes are, in general, resilient to the acquisition of new genes, so the focus has to be on the specific effect of the particular gene.

Even if the transgene confers an advantage that allows its recipient to increase in frequency, a key issue is whether overall soil functioning is affected – this is the case when assessing any change e.g. as a result of pesticide usage or altered crop rotation. Soils are dynamic systems that are in constant state of flux, for example they are affected by the weather, agrochemicals, what crop and even what variety is grown (reviewed by ACRE's soil ecology sub-group)[33]. Against this background, the significance (if there is any), of most change is not apparent. The scientific evidence shows that change is often reversible and soil functioning is robust. For

[33] http://www.defra.gov.uk/environment/acre/soilecology/acre_soilecology_interim.pdf

example, Griffiths *et al*. (2001) created soil communities with biodiversity reduced to a half, but found no change in measures of overall functioning.

One issue that has attracted a good deal of attention is the potential transfer of antibiotic resistance genes from GM crops to bacteria, and the fear that this may lead to increased resistance in bacteria of clinical importance. Antibiotics are a normal feature of the soil ecosystem. Most natural antibiotics were isolated from soil microbes (bacteria or fungi), and antibiotic resistance genes originate from these same organisms. Of course, it was the spread of these genes into clinical bacteria that first alerted microbiologists to the potential of HGT between different bacterial species. This spread has been driven by strong selection imposed by the clinical use of antibiotics and their widespread use as growth promoters in animal husbandry. Antibiotic resistance genes have been used in the creation of GM plants because they provide an effective means to select the transformed cells. They are not a necessary component of the final product and, as effective alternative methods are developed, it is likely that future GM plants will not carry antibiotic resistance genes. Nevertheless, it is improbable that GM plants would significantly affect the incidence of clinical antibiotic resistance through transfer in the soil, for two reasons. Firstly, the resistance genes in question are already widespread in bacterial populations, including those in clinical settings, so it is much more likely that a bacterium will acquire them from another bacterium (HGT between bacteria being relatively common) than from a plant (HGT being extremely rare). Secondly, the concentration of man-made antibiotics in soils is very low compared with clinical usage, so there will not be a similar level of selection favouring a bacterium that receives the resistance gene in the soil. Set against the first argument, it must be acknowledged that the commercial growing of a GM crop would provide an enormous multiplication in the number of copies of the gene that might offset the very low HGT rate, but this is irrelevant because the dynamics of antibiotic resistance spread are driven by the strong selection pressures rather than by the rate of HGT (which is relatively rare even in clinical settings).

This emphasis on selection pressure is key to assessing the potential fate of a transgene if it were to transfer to a microbe. The question that needs to be asked is whether a particular gene could confer a benefit on its recipient. There are some plausible cases in which the answer might be positive. To take a hypothetical example, the herbicide glyphosate is also toxic to some fungi (Morjan *et al.* 2002), so glyphosate resistance would confer an advantage on a fungus in the presence of the herbicide. If the fungus were able to acquire the resistance gene from a GM crop, then there is a possibility that the fungus with the transgene would spread within the herbicide-treated environment at the expense of other fungi. Whether this was of any ecological or agronomic significance would depend on the nature of the fungus. If the only phenotypic effect of the gene was to confer herbicide resistance, then the transgenic fungus would not be distinguishable from its non transgenic relatives except in the presence of the herbicide, so it should have no impact outside the crop. This hypothetical scenario illustrates the kind of questions that need to be asked when assessing the potential consequences of gene flow from GM crops, and more generally in assessing the impact of organisms with new traits (in particular, see sections 6.2 and 7.3).

7.4.4 Is there general scientific agreement?

There is good evidence and general agreement on the following points:

- DNA from crop residues remains available in the soil for months.

- The chloroplast genome is present in higher copy number than the nuclear genome in plant material.
- Some bacteria can acquire DNA by transformation.
- The probability that acquired DNA will be incorporated into a genome is greatly enhanced if it includes sequences closely similar to sequences in the recipient's genome.
- The probability that acquired genes will be expressed is enhanced if they resemble bacterial genes in their control elements, their codon usage, and in lacking introns.
- The transfer of genes from GM plants to soil bacteria under field conditions has not yet been observed.

From the available evidence, some authors have concluded that the rate of HGT from plants to microbes is so low (perhaps zero) that it can be neglected for the purposes of risk assessment. However, all the necessary stages of the process have been demonstrated individually, so it would be prudent to assume that they can occur, albeit at a rate that is too low to have been detected yet. Enormous areas are covered by crops (maize is currently grown on 140 million hectares worldwide, and there may be 10^{16} bacteria per hectare of soil), so even very low rates might not be negligible.

The critical question is whether the transgene would confer a selective advantage on a microbial recipient in the particular environment in which it is living, because if it does then even a very rare HGT event could lead to a significant effect. This has to be assessed on a case-by-case basis for each transgene and the context in which it will be used, considering all plausible classes of recipient.

7.4.5. Is the issue unique to GM?

As all plant roots slough off dead cells and plant parts eventually die and decay, soil microbes are exposed to significant amounts of native plant DNA. The consequences if gene flow were to occur, are no more predictable for native plant DNA given the complex, diverse and fluctuating nature of soil ecosystems. There is no evidence from genome analysis for the acquisition of normal plant genes by soil bacteria. However, one might predict that HGT from transgenic crops will be more likely if the DNA contains sequence that is homologous to genetic material in the soil microbes.

Agricultural practices such as ploughing, fertilisation, irrigation and the growing of monocultures have large effects on the size and composition of soil microbial populations. The fact that soil processes continue under these circumstances, although rates may be altered, is evidence of the robustness of soil functional communities. Any additional perturbation caused by the introduction of a gene has to be considered against this background.

7.4.6 Are there gaps in our knowledge or scientific uncertainties and are these important?

Little is known about the proportion of bacteria in a given community that are naturally transformable, i.e. competent to take up DNA (Dröge *et al*. 1999). The competence of bacteria to take up DNA in natural environments is poorly understood. However, if the assumption is that the potential rate of HGT from plants to certain bacteria is not zero, and we know that

genes can spread between bacteria, then policy is not dependent on a more precise estimate of the rates.

HGT to other microbes, e.g. fungi and protists, has not been as well researched as for bacteria. Again, there is some indication that the rate may not be zero. Since these are eukaryotes, some further consideration should be given to the likelihood of incorporation and expression of the transgenic DNA used in GM plants, as the work directed at bacteria will not be applicable.

In many cases the obvious effect of a transgene in a potential microbial (or, at least, bacterial) recipient is easily predicted. An antibiotic resistance gene will confer antibiotic resistance, and so on. However, a gene may also affect other cellular processes, which could be revealed by transcriptomics, proteomics or metabolomics. Some representative studies of this kind would establish whether this is an issue that needs further attention.

Even if we know how a genetic change affects an individual cell, our understanding of soil ecosystems is insufficient to predict whether a gene will afford an advantage and, if so, what environmental impact(s) it will have. Over the last decades, microbial ecology has taught us that microbial populations can vary rapidly over time and space, so that only really major effects will be distinguishable against the natural fluctuations. However, ecosystem functioning appears to be quite resilient to changes in individual microbial components. A better understanding of microbial ecology is clearly desirable for all kinds of reasons, and will increase our confidence in assessing the potential consequences of all kinds of perturbations. What determines the establishment and spread of new genotypes is particularly relevant. At a more specific level, the experimental introduction of potential transgene constructs into representative bacteria may be necessary in some cases where the possible effects on the fitness of the recipient cannot be predicted with reasonable certainty.

7.4.7 Likely future developments

GM plants that have transgenes in plastids rather than the nuclear genome are being developed. This may be useful to reduce gene transfer through pollen or for achieving higher expression levels. However, transformation of plastid genomes (i.e. chloroplasts) may facilitate HGT to bacteria because of the increased copy number and closer relationship to prokaryotic gene structure.

Conversely, antibiotic resistance genes are being phased out and should eventually cease to be an issue.

Most current transgene constructs are based on ‘natural’ components, particularly bacterial genes, and it can be argued that they are unlikely to confer significant benefits on bacteria that have been exposed to them already by HGT from other bacteria. If constructs become increasingly ‘novel’, with substantial synthetic sequences, then this argument will have less force and the effect of the constructs may need to be explicitly tested in representative bacteria. On the other hand, the potential for homologous recombination will be reduced.

7.4.8 Where there is important scientific uncertainty what is the potential way forward?

Research

We need more knowledge and understanding of soil ecosystems. As a start, it would be useful to define methods to measure a set of meaningful parameters of ecosystem state and function and to collect baseline measurements against which the effects of treatments can be assessed. ACRE have established a soil ecology sub group to consider the potential generic effects that GM plants and the agronomic practices associated with them might have on soil ecosystems and how these might be measured. This requires an understanding of the changes
that occur in soil ecosystems associated with the cultivation of non-GM crops in order that changes associated with the release of GM crops can be put into context and parameters that it would be meaningful to monitor can be identified. In March 2003, the sub group produced an interim report that reviews the current state of knowledge of soil ecosystems relevant to the potential impacts of GM plants[34].

In the case of transgene constructs whose effect in bacteria is not readily predicted, there may be a need to test directly in representative potential recipients.

Technological approaches

The potential for transfer and expression of transgenes from GM plants to soil bacteria might be minimised by removing unnecessary vector DNA that provides homology with soil microbes (in particular origins of replication and sites for transposition) and introducing introns where possible (e.g. Libiakova *et al.* 2001). This precautionary approach is in line with ACRE's guidance on best practice for designing future GM plants.[35]

Regulatory approach

Given that we cannot guarantee that the probability of gene transfer will ever be truly zero, careful consideration should be given to the likely consequences of transgene expression in any plausible microbial recipient, and transgenes should be avoided if there is a reasonable expectation of harm if they were to get into the wrong organism.

When ACRE assesses the safety of the deliberate release of GMOs into the environment, this includes their potential impact on soil ecosystems. These risk assessments are conducted on a case-by-case basis and take into consideration direct, indirect, immediate and delayed effects principally associated with expression of the transgene(s) inserted to create the GMO. ACRE takes a precautionary approach and assumes that HGT from plants to soil microbes will occur and considers the potential consequences on a case by case basis. It is the view of this panel that this is the most effective way of considering HGT from GM crops to soil microbes.

[34] http://www.defra.gov.uk/environment/acre/soilecology/acre_soilecology_interim.pdf.
[35] http://www.defra.gov.uk/environment/acre/bestprac/guidance/index.htm

7.5 CAN GENETIC MATERIAL IN GM PLANTS TRANSFER TO VIRUSES?

Can plant-virus-derived transgenes recombine with, and be transferred to viruses? If horizontal gene transfer is possible between GM plants and viruses could this result in new viruses that could cause irrecoverable damage to the ecosystem or to crops?

7.5.1 Summary

Since 1986, many thousands of transgenic plant lines expressing one or more functional or dysfunctional viral sequence(s) have been shown to render the GM plant resistant or even immune to subsequent virus inoculation. The technical facility, durability, efficacy and heritability of this approach are now well-established.

In the past, any weakness in, or complete absence of 'natural' resistance genes in the breeding stock of many virus-susceptible non-GM crops required the liberal use of pesticides to control the insect, fungus or nematode which naturally transmitted the devastating virus. A specific virus-targeted resistance transgene in a GM crop variety thus can offer a selective, traceable and environmentally sustainable route to protect crop yield and quality.

In addition to the many thousands of contained laboratory, glasshouse and small-scale field trials, several GM crops (e.g. yellow crookneck squash, sweet potato and papaya) that express viral sequences, which confer functional field resistance to devastating wild-type viruses have been grown commercially on large-scales, over the past 7 years. No new types of virus have been reported in association with the development, or commercialisation of these crops. One large-scale field study has been published that looked specifically for evidence of altered properties in infecting viruses and recombination (HGT) between infecting viruses and GM plants containing virus-derived transgenes over a six year period, none was found (Thomas *et al.* 1998).

Many artificial, laboratory-based recombination-selection systems can and have been established between two or more debilitated (mutated) viruses in A non-GM host plant, or between a more-or-less defective virus in a GM plant containing a transgene sequence capable of restoring wild-type virus. In most cases, usually depending on the strength of the evolutionary selection pressure applied, wild-type virus can be recovered in some plants (from <1% to approx. 30%) through homologous recombination (template strand switching) and selection during RNA-RNA replication (as in most plant viruses). Similar laboratory results have been reported during RNA-DNA reverse transcription (in plant Pararetroviruses) or through DNA-DNA replication (in Geminiviruses). In contrast, in nature, infecting viruses will be fully viable wild-type strains and mixed infections are common, providing far greater opportunities for virus-to-virus genetic reassortments, recombination, etc.

The seminal paper on this topic (Greene and Allison, 1994) decribes how a deletion mutant of cowpea chlorotic mottle virus (CCMV) was restored to wild-type in 3% of defective CCMV-inoculated GM plants. Homologous recombination had occurred between the debilitated virus and a transgene transcript that had a perfect sequence overlap of 338 nucleotides and a fully functional 3'-replication origin. When the replication origin was later removed the frequency of recombination fell dramatically (Greene and Allison, 1996).

Recombination is well-documented and plays a key role in natural virus evolution (see Tepfer, 2002). As yet, however, there is no evidence of such recombination (i.e. horizontal viral transgene transfer; HVTT) occurring in the field where single transgene resistance has been engineered against a wild-type virus. The possibility that HVTT might occur and its possible virological, biological and/or ecological consequences have been broadly discussed and speculated on in virtually every review article on this subject since the earliest days of the technology. The nature of any hazard, the probability of its occurrence and any possible consequences, or lack thereof, remain real but manageable issues for those who design, produce and test virus-resistant GM crops.

It is theoretically possible, but without precedent, that any tested and approved viral transgene sequence could, or would render any invading wild-type virus more pathogenic, affect its transmissibility, pathogenicity or other characteristics. On the contrary, high mutation frequencies, genome reassortments and recombination events in natural (often mixed) virus infected plants are common. Hence, any new genetic trait which is beneficial to a virus is presumed to have been selected already, through millenia of evolution, especially in the highly mutable pool of genomes which comprise the "quasi-species" of each RNA virus (>90% of all plant viruses). Indeed, since the 1970s, the accepted and approved practice of intentionally infecting many valuable crops (e.g. glasshouse tomatoes) with a mild strain of a virus to "cross-protect" them from infection by severe, devastating strains of the same or a related virus poses far greater (and documented) opportunities for recombination to create new virus strains (e.g. citrus tristeza virus in Brazilian oranges – see section 7.5.5).

The recommendations contained in an earlier (1999) DETR Research Report remain relevant today, although with our improved knowledge and understanding of RNA-interference (RNAi) and gene silencing/plant defence pathways, many of the risk issues that were proposed last century can now be avoided when designing new viral resistance transgene strategies.

Containment of any newly emerging plant virus is achieved through standard current and widely accepted phytosanitary control measures including replanting with healthy stock, spraying with pesticides or heat or chemical soil sterilisation to limit virus spread by killing its insect, fungus or nematode vector.

7.5.2 Introduction

Most first-generation GM plants (post 1983), whether made and used for research or commerce, contained short DNA sequences derived from plant viruses. These included non-coding elements used to regulate expression of any novel, functional transgene. Popular early examples were the so-called 35S and 19S promoter and terminator signals from the common Caulimoviruses (e.g. cauliflower mosaic virus, CaMV; or figwort mosaic virus, FMV – see later discussion on: *what effects could interactions between viruses and transgenes have*?). These sequences respectively start or stop transcription, the process by which the natural viral or transgene double-stranded DNA template is copied into a single-stranded messenger RNA (mRNA) for subsequent translation into a protein (the length/size of the viral RNA made dictates the naming of the promoter and terminator). The 35S promoters also generate the long viral RNA template used for viral reverse transcription (copying back) into daughter DNA molecules – a process peculiar to Caulimoviruses and other so-called plant Pararetroviruses. Still other plant virus-derived sequences have been used, generically, to

increase expression of a novel protein from a transgene. These include several short, non-coding viral mRNA leader sequences (typically only 10-100 nucleotides long) that recruit the protein synthesising machinery of the cell extremely efficiently.

In other cases, commencing in 1986, GM plants were created that contained a DNA copy of a whole plant viral gene or a non-functional fragment of a viral gene. Such plants were found (somewhat serendipitously at first) to be resistant to subsequent challenge infection by the same or a closely related plant virus. Some of the first field trials with GM plants (1986-1990) included those that expressed a functional viral coat protein gene that conferred resistance to challenge virus infection. RNA plant viruses represent the vast majority of plant viruses and hence have been targets for almost all virus-derived pathogen resistance transgene strategies in GM plants over 17 years. Indeed, reports of successful transgene-mediated resistance against DNA plant viruses are relatively rare. Thus while recombination-selection and rescue events through RNA-DNA reverse transcription (Pararetroviruses), or DNA-DNA replication (Geminiviruses) are less dependent on the presence of a viral replication origin (cf RNA viruses), any GM-based field resistance strategy may offer limited success with these virus types anyway. This may be because RNA viruses are more susceptible to transgene-derived RNAi-mediated cellular silencing at an early stage of infection (discussed later).

The issue of whether or not viral transgene DNA (or RNA copies thereof) could recombine with naturally occurring viruses in GM crops, and the possible consequences of such events were raised in the GM Science Review by Econexus (JR Latham & RA Steinbrecher, Royal Society of Edinburgh Meeting 27 January 2003[36]).

The same issues have been speculated-on and reviewed extensively over many years revealing a broad diversity of opinion (see De Zoeten 1991; Gibbs 1994; Falk and Bruening 1994; Allison *et al*. 1996; Miller *et al.* 1997; DETR, 1999; Hammond *et al*. 1999; Rubio *et al.* 1999; Tepfer 2002 and many references contained therein).

More than 1000 plant viruses have been described and studied in greater or lesser detail over the last 100 years. Collectively, they are ubiquitous in nature and affect all plants, including all food crops, and even trees. Individually, however, their host-range may include only one or a few species, or up to 400 different species of plant. As omnivores or vegetarians, we consume plant viruses constantly without any ill-effects. They multiply (replicate) efficiently inside living susceptible plant cells, sometimes only in specific cell types, and usually accumulate to very high copy numbers (over 100,000 virus particles per cell, is typical). Almost all plant viruses consist of a geometrically assembled shell of coat protein subunits (the capsid) that protects the delicate (esp. RNA) and relatively small genome of the virus. All plant viruses encode three or more proteins. Most plant viruses (92%) use single-stranded RNA as their genetic material. Of these, over three-quarters use positive-sense RNA [i.e. as with cellular mRNA, the packaged ("encapsidated") viral RNA uses the cellular machinery to code directly for one or more proteins]. The first complete viral genome sequence (tobacco mosaic virus) comprising 6395 nucleotides of single-stranded RNA was published in 1982. Minor taxa (groups) of plant viruses have double-stranded RNA, or single- or double-stranded DNA as their genetic material inside virus particles of various shapes. Plant viral satellite RNAs, viroids and virusoids also exist in nature, but are not considered further here.

[36] Abstract: http://www.gmsciencedebate.org.uk/meetings/pdf/270103-speaker-2.pdf
Transcript: http://www.gmsciencedebate.org.uk/meetings/pdf/270103-transcript.pdf

A complex series of spatial and temporal interactions between virus-encoded proteins and RNA or DNA genome sequences, and host proteins and sub-cellular structures is required for successful viral multiplication (replication). GM plants that express a functional or dysfunctional plant viral sequence at the wrong time, wrong place or in the wrong amount, can interfere with one or more of these delicate stages in the normal virus infection cycle and thus render the plant phenotypically 'resistant'. Resistance may be complete or partial, and directly attributable to the functional or dysfunctional viral protein being expressed, or to activation and virus-targeting of an intrinsic plant cell pathway responsible for post-transcriptional gene silencing (PTGS) through highly sequence-specific RNA degradation.

Global estimates of crop losses due to all viruses range from 5-20% but can be 100%, locally, especially in sub-tropical and tropical regions in developing countries where there are high numbers of insects that transmit viruses to commercial and subsistence crops; and where resource-poor farmers cannot afford effective pesticides. As well as insects, fungi and nematodes transmit specific viruses between plants through feeding and/or wounding, which allows plant viruses to enter and infect susceptible plant cells. Most agriculture in industrial countries relies on traditional (enhanced) breeding and selection to deploy natural resistance to viruses (where available). This is backed-up by strict phytosanitary (plant quarantine) controls, high health status planting material, and/or extensive use of agrochemicals and soil-sterilisation techniques to control the insect, fungal or nematode vectors which spread viruses in nature. It is neither technically nor economically feasible to spray antiviral chemicals against plant viruses. Mechanical transmission (through handling, pruning etc) or vegetative transmission (through tubers, cuttings etc.) are alternative means of spread. Very few plant viruses are seed transmitted, and even fewer are pollen-transmitted. Plant viruses are ubiquitous and a natural part of our diet. They cause no disease or harm to herbivores. Some plant virus particles are so robust they pass through the gastro-intestinal tract intact. There is no evidence of any HGT between plant viruses and humans – despite eons of co-existence with our raw food crops.

As with all pathogens (and pests), any large-scale deployment of a new crop exhibiting single dominant genetic resistance (GM or non-GM) creates a selection pressure that will favour the emergence of resistance-breaking strains of the virus, fungus, bacterium, or pest. The rapidity with which this occurs will vary, case-by-case. With their short replication cycle and high copy numbers, viruses naturally evolve and recombine rapidly. Viruses that multiply (replicate) only by copying RNA-into-RNA have no molecular mechanism to repair spontaneous genetic errors (point mutations) that occur about once in every 1000 nucleotides. Hence, a typical plant virus with a genome of 6000 nucleotides will carry 6 random point mutations. This led to the "quasi-species" hypothesis for RNA virus populations, in which the best adapted "type" strain predominates; but infinite permutations arise naturally and continuously, to be selected under altered conditions. In practice it is remarkable how stable the "type sequence" remains (e.g. when the original TMV isolate made in 1935 was compared with the modern type strain of TMV that has gone through infinite replication cycles).

Many hundreds of examples of enhanced resistance against one or more viruses in GM plants using a wide range of virus-derived transgene sequences in a range of crop and model species have been published and reviewed (see 392 references cited in DETR, 1999; Wilson 1993). Much positive interest has been driven by the promise of a limitless supply of single dominant resistance genes which can be introduced simply, rapidly and durably into existing elite germplasm without loss of desirable agronomic or quality traits and without prolonged back-crossing programmes. Moreover, for many viruses and crops, native single dominant

resistance genes either do not exist or have not been identified in sexually compatible germplasm for breeding.

There are several examples of exploitation of virus-derived transgenic resistance to viruses. Although the number of crop varieties that are already in commercial cultivation is not high, there are many more reports of small-scale experimental tests of different crops with transgenic resistance to viruses (see Box 7.1).

Box 7.1

An example of successful field control of a devastating virus is given by commercial-scale cultivation of genetically modified **papayas** (transformed with the coat protein gene of ***papaya ringspot virus***) under high disease pressure conditions in Hawaii (Souza *et al.* 1999; Ferreira. *et al*. 2002: Gonsalves *et al.* 2002)

Trials of **wheat** varieties transformed with the coat protein gene of ***wheat streak mosaic virus,*** which showed some resistance to WSMV in glasshouse experiments (Sivamani *et al*. 2000 and 2002), showed that incorporation of the replicase or coat protein gene from WSMV did not provide field resistance to viral infection. In general, transgenic lines yielded less than their parent cultivar, 'Hi-Line', although resistance to WSMV was shown in glasshouse tests (Sharp *et al.* 2002).

Stable, heritable resistance to **rice yellow mottle virus** (RYMV) was reported in rice varieties transformed with a transgene encoding the *RYMV replicase* gene. In the most extreme cases, there was complete suppression of virus replication (Pinto *et al.* 1999).

Transformation of commercial **potato** cultivars with replicase (Thomas *et al*. 2000) and coat protein (Murray *et al.* 2002) genes of ***potato leaf roll virus*** (PLRV) resulted in a high degree of resistance to PLRV in some lines, although the results of field trials were less impressive than the outcome of glasshouse tests. Also, transgenic potatoes have been made with some resistance to the devastating, severe ***potato virus Y***–NTN isolate (transformed with the coat protein gene of PV-NTN) (Racman *et al.* 2001) and to Potato virus X (transformed with PVX coat protein) (Doreste *et al.*2002).

Field trials of **chilli pepper** transformed with ***cucumber mosaic virus*** and ***tobacco mosaic virus*** sequences showed milder disease symptoms and increased yield (Cai *et al.* 2003).

Tomato plants transformed with a fragment of the replicase gene of ***cucumber mosaic virus*** (CMV) showed resistance to CMV, and the selected lines are being used as breeding material for CMV resistance (Nunome *et al.* 2002). Transgenic **soybean** plants resistant to ***soybean mosaic virus*** **(**SMV) were obtained by transforming with the coat protein gene and 3'-UTR. Lines highly resistant to SMV were selected (Wang *et al*. 2002).

Resistance to ***carnation mottle virus*** (CarMV) was observed in **carnation** plants transformed with the CarMV coat protein gene (Yu *et al.* 2002). Heritable transgenic resistance of **sweet potato** plants to ***sweet potato feathery mottle virus*** (SPFMV-S) was conferred by transformation with SPFMV-S coat protein gene (Okada *et al.*2002).

Transgenic **Mexican lime** trees resistant to ***citrus tristeza virus*** (CTV) were generated by transformation with the CTV coat protein gene. Protection was also efficient against non-homologous CTV strains and was generally accompanied by high accumulation of CP in the protected lines, which suggest a protein-mediated CP-mediated protection mechanism (Dominguez *et al.* 2002).

The list of transgenic plants with functional resistance to viruses is likely to be extended as work continues in many laboratories (several hundred) worldwide. For further examples, see materials selected from just one recent meeting; the June ***2002 Congress on In Vitro Biology, Orlando, USA*** (**see Box 7.2**).

Box 7.2: Transgenic resistance to viruses in a variety of crops was reported recently: 2002 Congress on In Vitro Biology, Orlando, FL, USA, June 25- -29.

Kamo K., Gera A., Cohen J. and Hammond, J. Transformation of **Gladiolus** for resistance to **bean yellow mosaic virus**.

Hily,J.M., Malinowki, T., Ravelonandro, M.and Scorza R. Post-transcriptional gene silencing (PTGS) results in **PPV** resistance of transgenic **plum** trees after four seasons of growth in the field.

Hanbing An., Sarita E.V, Verchot-Lubicz, J. Transgenic resistance in **wheat** containing **soilborne wheat mosaic virus** (SBWMV) genes.

Oropeza M., Abouzid A. M., Miller J. D., Comstock J. C., Gilbert R. A. and Gallo-Meagher M. Analysis of transgenic **sugarcane** plants containing an untranslatable **sugarcane mosaic virus strain E** coat protein gene.

Scorza R., Ravelonandro M., Callahan A. M., Malinowski T., Damsteegt V. D., Levy L. and Briard P. Studies of **plum pox virus** resistance in transgenic **plum** C5 and its progeny.

Reustle G. M., Wetzel T., Jardak R., Ebel R., Worl R., Meunier L., Becker M. and Krczal G. Genetic engineering of **grapevin**e rootstocks to induce **nepovirus** resistance.

Raquel H., Lourenco T., Batista, R. and Oliveira M. M. **Almond** (Prunus dulcis Mill.): Preparing and testing of constructs for resistance to **prune dwarf virus** (PDV).

7.5.3 Range of views and quality of evidence

A survey of 23 UK and 49 overseas public and private sector organisations actively involved in GM crop science and virology in 1995-96 provided an analysis of the gene sequences, viruses and target plants being used (DETR, 1999). Most respondents cited RNA-RNA recombination or transcapsidation (i.e. the packaging of the genome of an invading virus in the coat protein of another virus expressed as a transgene) as potential hazards, although many saw no additional hazard beyond what would happen in nature during mixed infections by endemic viruses [up to 11 different viruses have been reported in a single plant (Falk and Bruening, 1994)]. Interestingly, those who had carried out field tests and had real data perceived fewer, if any, hazards than those preparing to do so or speculating on possible events.

However, one minority viewpoint expressed at the open meeting at the Royal Society of Edinburgh[37] contends that:

(i) genetic material from GM crops containing DNA derived from viruses will inevitably recombine with and transfer to naturally occurring viruses that infect them and that this could result in new virus strains which, in the worst case scenario, could cause irrecoverable ecosystem or crop damage.

(ii) the probability of HGT of transgenes is greater than for mixed infections because transgenic crops may result in new or enhanced opportunities for virus recombination (De Zoeten 1991; Gibbs 1994; Allison *et al.* 1996) – even though there is no evidence for this.

[37] Abstract: http://www.gmsciencedebate.org.uk/meetings/pdf/270103-speaker-2.pdf

(iii) our understanding of virulence determinants and ecological fitness is not sufficient to predict which viruses that could theoretically acquire genetic material from GM crop plants would have altered pathogenicity. (Although the same argument applies to naturally evolving strains, mutant and recombinant viruses).

The view that the transfer of virus-derived transgenic DNA or RNA to viruses is inevitable is based on laboratory experiments in which defective viruses were artificially inoculated onto GM plants that contained restorer viral transgene sequence which then regenerated viable (wild-type) virus (Lommel and Xiong 1991; Greene and Allison 1994; Greene and Allison 1996; Borja *et al.* 1999; Adair and Kearney 2000; Varrelmann *et al.* 2000; Schoelz and Wintermantel 1993; Wintermantel and Schoelz 1996; Gal *et al*. 1992; Frischmuth and Stanley 1998).

In fact all published examples of stable recombination between a defective (non-viable) virus and a homologous viral transgene contained in a GM plant have been achieved using experimentally designed, laboratory-based systems. In every successful case, a selection pressure was engineered into the test system in order to restore viability to wild-type or near-wild-type levels in any recovered virus through recombination rather than simple genetic reversion.

It is important to note that, although laboratory experiments demonstrate that viral transgene transcripts can be available for recombination, there is no evidence to suggest that such recombination events take place in the absence of suitable selection pressure. For example, a large-scale 6-year field trial with potato plants expressing the CP or replicase genes of potato leafroll virus (PLRV) provided no evidence for any modifications in transmission, transcapsidation, or synergism with any virus that could infect the potatoes (Thomas *et al*. 1998). Similarly, large-scale field introductions of coat-protein transgenic virus-resistant papayas in Hawaii have had no negative pathological or ecological impacts such as HGT creating new or devastating viruses (Ferreira *et al.* 2002).

There are no reports from laboratory or glasshouse trials, or in commercial use, of any infectious wild-type virus picking-up any transgene of viral (or any other) origin, or indeed picking-up any host RNA. On an evolutionary time-scale, a small proportion of a few atypical strains of plant viruses do appear to have incorporated small fragments (c. 70-119 nucleotides) of various cellular RNAs such as a chloroplast tRNA (Matsuta *et al*. 1992), or an exon from a chloroplast mRNA (ORF196; Mayo and Jolly, 1991), and possibly even a homologue of a nuclear heat-shock protein gene (hsp70) in a closterovirus (Karasev 2000). These examples appear to be rare and possibly unique. Modular sequence evolution in the generation of plant viruses became apparent in the 1980s and 1990s as complete sequence data accumulated.

Clearly, since the commercial purpose of a virus-derived transgene is to render the GM crop resistant (ideally immune) to infection by the target virus, any opportunity for homologous RNA-RNA or DNA-DNA recombination is greatly reduced (ideally to near zero). And, by definition, any unrelated virus to which the GM plant remains susceptible, will be unable to recombine through homologous template switching/copy choice mechanisms with an RNA copy of the original viral transgene. Non-homologous recombination is even less efficient.

There have been no published or anecdotal reports of recombination between viral transgene(s) and natural (wild-type) viruses during large-scale GM crop field trials (many

thousands since 1987), or in commercial-scale cultivation [e.g. all Hawaian papayas since 1997 (Gonsalves *et al*. 2002); US yellow crookneck squash (Asgrow/UpJohn Co.) since 1995; potato leafroll virus-resistant "New Leaf" potatoes (Thomas *et al.* 1998) since the early 1990s].

Any viral transgene that produces a functional or dysfunctional protein which failed to generate field-level resistance [for example, through spontaneous mutation (loss of function)], or somehow exacerbated the severity of an infection by an otherwise mild strain of the same virus (or an unrelated virus), poses an economic risk to the farmer, the seed producer and the biotechnology company - but not to the environment or to consumer health or safety. If they occurred, such events would render a particular GM crop variety commercially useless. They do not however result in any stable genetic change to the primary target virus or its progeny. As with transcapsidation, such events represent a genetic and epidemiological 'dead-end'. In fact, one genus of plant viruses, the Umbraviruses, relies on transcapsidation of their RNA-only genome in the coat protein of a co-infecting Luteovirus in order to move from plant-to-plant. In short, any loss of crop protection, or deleterious agronomic trait arising from a viral transgene, or its RNA or protein product acting in *trans* may pose an economic problem for commercialisation of that particular GM crop line, but it is not an HGT issue. As described later, under 'Likely future developments' and 'Technological and regulatory approaches" (and in Tepfer, 2002), detailed studies of the mode of action of viral (trans)genes has made it possible to eliminate proposed potential sources of potential risk such as transcapsidation or altered transmission by mutating the coat protein transgene.

However, most examples of viral transgene-mediated plant resistance operate through signalling rapid degradation of the incoming viral genome by a natural plant defence pathway (analogous to post-transcriptional gene silencing/cellular mRNA degradation). If a second virus infected a GM plant and encoded a suppressor of PTGS of the primary target virus from which the transgene was derived, then the efficacy of the original transgene may be compromised. Although not relevant to HGT, such an event could create an economic risk during commercialisation. Obviously, those who develop viral GM crops should test each transgenic line with viruses that may co-infect the plant in the field, especially those known to act synergistically with the primary target virus in nature (i.e. to suppress PTGS). Evidence for such an interaction was published by Barker *et al*. (2001).

The horizontal transfer of genetic material from a GM plant to a super-infecting virus can be achieved in the laboratory using model recombination-selection systems, but remains a hypothetical situation in the field. Given that the detail and origin of any viral transgene sequence will be known, predicted or even speculative risks can be avoided. Thorough screening, experimentation and analysis prior to any experimental or commercial field release would be required to test the efficacy, durability and stability of the new transgene and detect any predicted or unpredicted consequences.

Our understanding of the potential for virus-derived transgenes to recombine with and transfer to viruses is based on a substantial evidence base. The general questions asked by scientists working in this area and recent peer reviewed publications addressing them are described below:

Does RNA recombination between virus-infected transgenic plants transformed with portions of viral genomes occur?

Whether RNA recombination in virus-infected transgenic plants transformed with portions of viral genomes could potentially generate novel viruses with biological properties distinct from those of parent strains has been considered over 15 years. The strategy widely used to investigate this possibility is to apply a strong selection pressure to ensure that any virus generated as a result of a recombination event between a (defective) virus and a transgene has an advantage over the virus used for inoculation. The experimental procedure used by several authors has involved inoculation with a movement-deficient mutant (non-viable) virus (usually with a deleted or non-functional coat protein gene) onto transgenic plants (in most cases *Nicotiana benthamiana*) that expressed a gene encoding the corresponding part of the viral genome (including the coat protein gene) in a functional form. Systemic movement of symptoms then indicates recombination in that particular plant. Recent examples of this type of experiment are described in Box 7.3.

Box 7.3.

When *N. benthamiana* plants transformed with a non-translatable 3'-half of the tobacco mosaic virus (TMV)-GFP genome (from part of the RNA polymerase to the 3'-untranslated region) were inoculated with a TMV coat protein mutant, which could not move efficiently through the host, recombinant RNA was detected in 32% of inoculated plants. Nevertheless, the resulting recombinants were less fit than wild-type and no encapsidation of the recombinant viral RNA was detected (Adair and Kearney, 2000).

Wild-type plum pox virus (PPV) was restored in transgenic *N. benthamiana* plants that expressed the PPV coat protein with a complete 3'-non-translated region when inoculated with either a CP-deficient PPV, or a chimaeric PPV with CPs derived from other potyviruses (Varrelmann *et al.* 2000).

Recombination between an infecting virus and a transgene derived from a different viral species or strain cannot be ruled out. It was shown that the viral RNA-dependent RNA polymerase of several potyviruses and tomato aspermy virus have an ability to recognize heterologous 3'-untranslated regions (UTRs) included in transgene mRNAs (Teycheney *et al.* 2000).

The 3'-UTR adjacent to the capsid protein gene is frequently included in the construction of coat protein-mediated virus-resistant transgenic plants. Recombination frequencies between transgenic RNA and viral RNA can be reduced significantly by omitting or disrupting the 3'-UTR. This was shown using transgenic *N. benthamiana* plants transformed with the cowpea chlorotic mottle virus coat protein gene with or without its 3'-UTR (Greene and Allison 1994, 1996) which is the natural replication signal.

One report describes a rare double recombination event (i.e. not needing the 3'-UTR signal) leading to restoration of a wild-type viral RNA genome. Wild-type tomato bushy stunt virus (TBSV) was regenerated by a double recombination event in *N. benthamiana* plants transformed with the wild-type TBSV coat protein gene and infected with a mild-symptom TBSV mutant containing a defective coat protein gene. Similarly the TBSV-CP was restored when TBSV-CP transgenic plants were inoculated with a chimeric cucumber necrosis virus (CNV) containing the defective TBSV coat protein gene (Borja *et al.* 1999).

Does DNA recombination between virus-infected transgenic plants transformed with portions of viral genomes occur?

Similar to RNA recombination, there are examples of DNA recombination between a plant DNA virus and a viral transgene. Successful recovery of nearly wild-type geminivirus African cassava mosaic virus (ACMV) as a result of a recombination event between a CP-deletion mutant of ACMV and an ACMV CP transgene was reported in *N. benthamiana* (Frischmuth and Stanley 1998).

What effects could interactions between viruses and transgenes have?

It is theoretically possible that interaction between a virus and a transgenically expressed heterologous proteins could increase symptom severity as a result of synergism**,** as occurs in some natural pairwise mixed infections in non-GM plants. Cases where this happens, and the protein responsible have been elucidated experimentally over the past 15 years.

It is also possible that an otherwise non-transmissible virus could become packaged in the coat protein of another virus expressed at low levels in a transgenic plant (transcapsidation) and then be able to be moved by insects etc. to another plant. This will not result in heritable genetic change and is believed to be an epidemiological 'dead-end'.

It is possible that a transgene which signals 'silencing' of an incoming target viral RNA may cease to function if the plant is infected by a virus that can suppress (overcome) RNA-mediated resistance (i.e. suppress gene silencing). It was shown that when *N. benthamiana* plants were transformed with potato leaf roll virus (PLRV) full-length cDNA, only a minority of mesophyll cells accumulated virus. When these plants were then inoculated with potato virus Y or a tobacco mosaic virus-vector that expressed the potyviral PTGS suppressor protein P1-HCPro, the proportion of cells that showed PLRV replication increased dramatically (Barker *et al.* 2001).

In the debate on the safety of GM plants that express viral sequences it has been claimed, that the CaMV 35S promoter poses some alarming risks. It was proposed (Ho *et al.* 1999) that this promoter could recombine to activate dormant viruses, create new viruses, and cause cancer by the overexpression of normal genes. A full and measured response to these claims has been published (Hull *et al.* 1999) and there has been further discussion on the Review website (ISIS[38,39]; Roger Morton[40,41]). Ho *et al*. cite a paper by Kohli *et al.* (1999) in support of their theory – in this study several genes under the control of the CaMV 35S promoter were bombarded into rice and became integrated into the genome by DNA repair-mediated recombination. Although only a small number of transformed rice lines were studied, the authors showed that when integration occurred by recombination within the 35S promoter, one site was frequently involved. However, the 35S promoter itself was not any more likely to recombine than any other part of the DNA construct. Nevetheless, Ho *et al.* (1999) claimed that the 35S promoter would be promiscuous and mobile in the plant genome (like a transposon). They also proposed that such mobility would permit the CaMV promoter to insert into the genome of any organism that consumed the transgenic plant's DNA with adverse consequences. The scientific evidence does not support this reasoning:

[38] http://www.gmsciencedebate.org.uk/topics/forum/0030.htm
[39] http://www.gmsciencedebate.org.uk/topics/forum/0067.htm
[40] http://www.gmsciencedebate.org.uk/topics/forum/0062.htm
[41] http://www.gmsciencedebate.org.uk/topics/forum/0079.htm

(a) there is no evidence that the CaMV 35S promoter is mobile, unlike natural and widespread plant transposable elements. Quist and Chapela (2001) reported the fragmentation of the CaMV 35S promoter in maize landraces when transgenes were transferred from GM maize. However, the design of the experiments on which this particular conclusion was drawn was deeply flawed (Kaplinsky *et al*., 2002; Metz M. and Fütterer, 2002).

(b) we eat large amounts of CaMV-infected crucifers (a 1980's study showed 10% of UK cauliflowers and cabbages were infected. Organically grown crops are likely to have even higher levels). Each plant cell typically produces 100,000 virus particles, and hence 100,000 copies of the 35S (and 19S) CaMV promoter. Thus throughout evolution, humans have consumed plant viruses, or have eaten animals which have themselves consumed plant viruses. There is no evidence for integration or recombination with our genomic DNA. Evidence supporting the view that CaMV DNA does not pose any novel cancer risk is discussed further in a contribution to the website (Professor D. Murphy[42]). Please also refer to Chapter 5.4: *the fate of transgenic DNA in GM plants*.

(c) in addition to CaMV-infected vegetables and transgenic crops containing CaMV 35S, all banana varieties that have been studied contain multiple copies of another Pararetrovirus - banana streak badnavirus – naturally integrated into their genomes. Despite exposure of humans to these Pararetrovirus DNA sequences and promoters there is no evidence for any ill-effects from newly emerging viruses or cancer genes, even in Uganda where bananas are the staple diet and HIV (a Retrovirus) is rife.

7.5.4 Is there general scientific agreement?

There was consensus among scientists at a USDA Workshop 'Assessing the Risk of Plant Viral Transgenes' (1995). It was concluded that there was no rational or conceptual reason to assume that any particular plant viral insert has an increased potential for viral RNA recombination events. Expression of any fully functional viral protein such as a coat protein, cell-to-cell movement protein, replicase enzyme, suppressor of gene silencing or protein involved in the transmission of a particular plant viruses by its associated insect, fungal, mite or nematode vector obviously has the potential to act *in trans* to complement a defective strain of the primary virus, or even a secondary virus – as can happen in any natural mixed field infection (e.g. synergy). Such phenomena do not, however, lead to HGT.

7.5.5 Is the issue unique to GM?

With the exception of the Badnaviruses mentioned above, plant viral sequences do not occur naturally in plant genomes. Thus, even the hypothetical risk of recombination and gene flow from a natural viral insert in a plant genome to another (un)related invading virus is zero. However, gene transfer, recombination, genetic reassortments, complementation, synergy and transcapsidation can and do happen in natural mixed virus infections in a wide range of plants. Moreover, opportunities for such events have been increased over the last 30 years, since the phenomenon of cross-protection (first reported in 1929 by McKinney) has been

[42] http://www.gmsciencedebate.org.uk/topics/forum/pdf/0011.pdf

widely deployed in several major glasshouse and field crops. Here, a natural or artificially generated mutant mild strain of an otherwise virulent natural virus is applied to the crop to 'protect' each plant against subsequent natural infection and hence devastating symptoms. Occasionally, the mild (protecting) virus strain mutates or reverts spontaneously to a more devastating form, or recombines with another infecting virus with serious consequences. For example, Brazilian orange trees normally 'cross-protected' using a mild strain of citrus tristeza virus have recently become decimated by a new CTV strain. Similar risks are present in glasshouse tomato and cucurbit crops that are "cross-protected".

7.5.6 Are there gaps in our knowledge or scientific uncertainties and are these important?

No good data exist on how the strength (however this might be measured) of a particular genetic/fitness selection pressure could affect viral (or other) genome recombination rates. However while (difficult) experiments to study this may provide useful genetic and evolutionary data, better understanding and some reference points, they may be of academic interest compared with practical experiences and experimental data already accumulated during extensive GM crop trials and commercial plantings. For example, Monsanto's 'New Leaf' potato was the subject of an extensive 6-year study (Thomas *et al*. 1998). Over 25,000 plants in 442 lines transformed with 16 different coat protein gene constructs (with a Luteovirus replication origin), and 40,000 plants in 512 lines transformed with 7 different replicase gene constructs of potato leafroll virus were exposed to field infection over a 6-year period. Individual plants were inspected annually, and extensive molecular and biological studies done on any PLRV or heterologous viruses found to be infecting the crop. No changes in virus properties or evidence of recombination (HGT) were found.

The probability of occurrence of any plausible hazard occurring through homologous or heterologous recombination between a virus and viral transgene-derived genetic material may be influenced by the scale of any commercial release. However, as in nature and laboratory experiments, almost all recombinant viruses are unlikely to survive or to dominate the population against competition from the wild-type parent viruses.

As in the later stages of conventional breeding and selection of a new non-GM crop plant variety, the inheritance, stability and functional utility of any new trait (whether GM-derived or not) should be assessed under all probable environmental conditions and agronomic practices. Any loss of function poses economic and efficacy problems that would render the new variety non-profitable, but does not raise HGT issues.

7.5.7 Likely future developments

Recent progress in increasing our understanding of the mechanism of induction of PTGS has led to the development of new and more targeted strategies to induce RNA-mediated resistance to viruses (Vaucheret *et al.* 2001; Chicas and Macino 2001; Hannon 2002). Specific constructs designed to express double-stranded (ds)RNA corresponding to parts of the target virus genome has proved very efficient in inducing protection against the whole virus. For example, barley plants transformed with a single copy of a construct designed to produce a hairpin RNA from barley yellow dwarf virus-PAV (BYDV-PAV) showed strong

heritable resistance to BYDV-PAV. This protection was rated as immunity. The virus could not be detected by ELISA methods, even in those plant tissues challenged with virus inoculum, or be recovered by aphid feeding experiments (Wang *et al*. 2000).

It has been known for some time (Voinnet *et al.* 1999) that some virus-coded proteins can suppress PTGS and thereby overcome a plant's defence mechanism. When this happens *in trans*, between two viruses in one plant, it is called 'synergy'. The impact of natural viral synergy-type interactions operating against target viral transgene-mediated resistance in a field crop are largely predictable, yet the phenomenon has not yet been reported (Tepfer 2002). Nevertheless, although not directly relevant to heritable HGT, the area probably merits further careful analysis both for commercial reasons as well as to increase our knowledge. Clearly, care is required to avoid stacking in one plant of viral gene sequences with the ability to complement or act synergistically with one other or with common field viruses in the locality.

Another opportunity to design virus-resistant transgenic plants involves expression of mutant forms of viral proteins, which interfere with viral infection. For example, transgenic expression of a tobacco mosaic virus (TMV) coat protein mutant (CPT42W) resulted in very high levels of resistance to TMV. This was due to interference by the mutant CP with the normal movement protein production and subsequently with cell-to-cell movement of the virus (Bendahmane *et al*. 2002)

Transgenic resistance to viruses may also be induced by transforming plants with non-viral genes. Broad resistance to a variety of plant RNA viruses was reported in tobacco plants transformed with the gene for human dsRNA-dependent protein kinase (PKR), placed under the control of a plant wound-specific promoter. In human cells, PKR confers resistance to viruses by inhibiting their replication by inactivating the translation initiation factor, eIF-2α, following activation by dsRNA. Transgenic plants expressing the PKR gene showed significantly reduced viral symptoms, or no viral symptoms at all, when challenged by different plant RNA viruses, such as cucumber mosaic virus, tobacco etch virus, or potato virus Y (Lim *et al.* 2002).

Weeds frequently act as over-wintering reservoirs for viruses that can then re-infect a genetically unrelated crop the following year, provided they share some suitable vector to transfer the virus. *A priori*, there is no reason that the seasonal crop host should be sexually compatible with the virus-susceptible over-wintering weed species. Nor that weeds that may be able to acquire a viral transgene by cross-pollination with a GM crop are necessarily hosts for the same virus, or even that such an event would provide any evolutionary advantage (e.g. increased weediness). With the exception of very few pollen transmitted viruses, plant-to-plant gene flow and virus transmission are two completely unrelated and independent events. Indeed, if a weed was both a host for the target crop virus and sexually compatible with the crop, then hybridisation and transfer of the resistance trait would render the weed resistant to the virus. This would then reduce the viral inoculum pressure next year by lessening the viral reservoir. As described previously, crossing with the weaker crop genome would also reduce the overall competitiveness or persistence of the weed (see section 7. 3 where linkage drag is discussed in more detail).

7.5.8 Where there is important scientific uncertainty what is the potential way forward?

Research

If some suitably precise and sensitive experimental system could be devised, then it may be instructive to compare the frequency of recombination events in natural mixed virus infections with events in a single virus-infected GM plant.
If, or when, a viral transgene mediated resistance trait breaks down, or high field inoculum pressure overcomes GM protection, are spontaneous mutants in the ("quasi-species") population being preferentially selected and do they persist?

There is always a drive to further refine the parameters to design more effective RNAi transgenes to target PTGS at the incoming viral genome(s). It would also be valuable to achieve broader spectrum resistance against related viruses.

Just as different viruses could reduce their propensity to recombine or reassort genome segments with co-infecting viruses during mixed infections, we know relatively little about the relative compartmentalisation of transgene transcripts and their target viral RNAs. PTGS and dysfunctional protein-mediated strategies that generate functional field resistance in GM crop plants may therefore not provide effective opportunities for efficient template switching and recombination/HGT.

Technological and Regulatory Approaches

Several practical recommendations can be made to minimise any even the theoretical risk of adverse effects. In an earlier report (DETR 1999), key safety features in designing transgene constructs included:

(i) minimising the length of any virus-identical, homologous sequence and avoiding origins of RNA or DNA virus replication such as genomic or sub-genomic RNA promoters
(ii) including multiple dispersed point mutations (i.e. non-revertible and translationally silent if necessary) in any potential protein-coding sequences to render them dysfunctional or any possible recombinant genome defective
(iii) omitting insect/fungus/nematode transmission signal sequences on coat proteins
(iv) focussing on RNAi strategies and
(v) avoiding hyper-mutable molecules such as defective interfering (DI) or satellite RNAs.

For example, to eliminate any possible risks related to vector transmission of non-transmissible viruses as a result of transcapsidation, point mutations can be introduced into the coding sequence of the coat protein transgene to eliminate its ability to package RNA. Thus *N. benthamiana* plants were transformed with a plum pox virus coat protein gene with a deletion of the amino acid triplet (DAG) involved in aphid-transmission. Experiments demonstrated that the modified form of the PPV coat protein in transgenic plants provided good control, without any potential biological risk associated with transcapsidation and spread (Jacquet *et al*. 1998).

Knowledge-based agriculture, evidence-based regulations and open-minded decision-making depend on facts and weighing-up benefits and risks. GM crops that express viral sequences exploit a natural plant defence pathway to target many of the otherwise intractible viral pathogens. Viruses greatly reduce crop yields, blemish products and require farmers and growers to rely on pesticide sprays, chemical fumigants or steam/flame soil sterilisation methods to remove their insect, fungal or nematode worm vectors. We should judge GM crops against these options.

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LIST OF ABBREVIATIONS

ACAF	Advisory Committee on Animal Feedingstuffs (for the UK)
ACNFP	Advisory Committee on Novel Foods and Processes (for the UK)
ACRE	Advisory Committee on Releases to the Environment (for the UK)
AEBC	Agriculture and Environment Biotechnology Commission
ARM	Antibiotic resistance marker
BA	British Association for the Advancement of Science
bp	Base pairs
Bt	Bacillus thuringiensis
CEC	Commission for Environmental Cooperation
cry	Cryptochrome
cv	Cultivar
DNA	Deoxyribonucleic acid
EEA	European Environment Agency
ENTRANSFOOD	European Network for Safety Assessment of Genetically Modified Food Crops.
EU	European Union
EPA	Environmental Protection Agency (for the USA)
ESTO	Earth Science Technology Office
FAO	Food and Agriculture Organisation (of the United Nations)
FDA	Food and Drug Administration (for the USA)
FISH	Fluorescence in-situ hybridisation
FSA	Foods Standards Agency (for the UK)
FSE	Farm-scale evaluation
GM(O)	Genetically modified (organism)
GMHT	Genetically Modified Herbicide Tolerance
HGT	Horizontal gene transfer
ICSU	International Council of Scientific Unions
IFPRI	International Food Policy Research Institute
IgE	Immunoglobulin E
ILGRA	Interdepartmental Liaison Group on Risk Assessment (for the UK)
ILSI	International Life Sciences Institute
JRC	Joint Regulatory Commission (for the European Union)
mRNA	Messenger ribonucleic acid
OECD	Organisation for Economic Cooperation and Development
ORP	Open reading frame
PCR	Polymerase chain reaction
RT- PCR	Reverse transcriptase - polymerase chain reaction
SSSI	Site of Special Scientific Interest
UK	United Kingdom
UNCED	United Nations Conference on Environment and Development
USDA	US Department of Agriculture
WHO	World Health Organisation

A glossary of scientific and technical terms used in this report will be placed on the GM Science Review website (http://www.gmsciencedebate.org.uk) shortly after publication and a printed copy will be available on request.

Annex I

Questions about GM to be addressed by information (extract from Corr Willbourn report)

The foundation discussion workshops conducted by Corr Willbourn Research and Development as part of the GM public debate, allowed the general public to frame the issues for the programme of debate. The Corr Willbourn work has played a central role in setting the agenda of the Science Review process. The report that arose from this exercise can be viewed at: http://www.gmnation.org.uk/docs/corrwillbourn.pdf. It contains the following key questions about GM, framed by the public.

A Basic Information and Definitions

A1 What is GM? How is it done? Where is it done / Does it have to be done in a lab?

A2 What does it mean? How wide is its definition?
- can everything with genes be modified / can it be done on humans?
- is spraying crops with pesticides classed as genetic modification?
- is it a speeding up of a natural process like the survival of the fittest?

A3 Does it involve chemicals? Which ones and how?

A4 When and how did it begin? How long has it been going on?

A5 Does it work?

B Current Status of GM

B1 How much is on the market? What percentage of foods on the market are GM? What crops are already genetically modified?

B2 What new GM crops / foods are planned?

B3 Who produces GM food?

B4 Who eats GM food? Do the producers eat it?

B5 Are we being fed GM foods without knowing it? Do we get told what is GM and what isn't in supermarkets? Do you have to label GM food as GM? How can we tell if it is a GM product / if we've eaten GM?

C Rationale

C1 Why do it? Why change what we've got? Is there a need for it? What can it be used for? Who is demanding GM/who says there's a need for it? Is it principally driven by profit? Is it driven by scientists seeing what they can do by playing with nature?

C2 What are the real benefits? Who is benefiting and who will benefit?

C3 Will it benefit our lives and how? What's in it for me?

C4 Will it make life easier / give us better food / more nutritious or healthier food / food with a longer shelf life? Will it be cheaper (by how much and why?) or cost more?

C5 What is the biggest advantage GM crops can bring the world?

C6 Will it have medical benefits eg. a cure for diseases such as cancer?

C7 Will it benefit the world's population, especially the Third World eg. problems of food and water supply?

C8 What impact will GM crops have on alternative uses of crops eg. GM OSR for biofuels?

D Possible Risks to Health

D1 Is it good for me or dangerous? How will it affect us? Are there negative effects / side effects / drawbacks to balance against the benefits?

D2 Is it harmful? Could it be harmful in the future? What harm / damage could it do to the world? Do the people who do it know if it can harm us?

D3 Could it harm me and my family? Could it harm future generations? Will eating GM foods undermine my health?

D4 Could harm be caused by:

- the chemicals used
- cross-contamination
- additives
- mutations
- altering the basic structure of things?

D5 Could harm take the form of:

- allergic reactions
- new diseases
- general negative effect on health?

D6 Will they be able to cope with problems / treat any new diseases that arise?

E Other Possible Effects

E1 Could jobs be lost?

E2 What will happen to ordinary farmers?

E3 How will farming in the UK progress and compete?

E4 What could be the effects of the commercialisation of GM crops in the UK?

- on UK science?
- will it increase our dependence on industrialised farming methods?
- will it increase our dependence on lower diversity and chemical dependent farming?

E5 Could corporations end up controlling the food chain?

E6 Could world climate change be affected? What does the future hold re food, energy, environment etc?

E7 What effect might GM have on the environment? Is it destroying nature as we know it? What will the effect be on natural (non-GM?) crops / wildlife?

E8 What about pesticide harm?

F Regulation and Monitoring of Safety

F1 Is it safe and how do I know that it is safe? What proof is there that it is safe? What tests are in place? Are all foods fully tested?

F2 What research has been carried out into the effects on health of modified foods that are already available? What research is being carried out into the potential long term effects?

F3 What are the real experiences of US farmers and consumers?

F4 Who funds and carries out the research? How much corporate funding is there? Is the research independent? Should it be?

F5 What controls and regulations / legislation are in place?

F6 Who is the regulator and are they independent? Do we need one?

G Boundaries

G1 Will there be boundaries around what can be changed? How far will they go?

G2 Where will it stop? eg. Will we get lettuces the size of houses? Will it lead to the cloning of all animals?

G3 What are the long-term aims of all this research into GM?

H Trust and Confidence

H1 Why is there so much disagreement about the benefits and risks of GM?

H2 Is everything we hear about GM from the people developing the technology?

H3 Can we get unbiased and impartial information and from whom? Who can you believe or trust? Can scientists be neutral? What is the involvement and attitude of farmers, producers, environmentalists, supermarkets, Government?

H4 Why does the Government think that the commercialisation of GM crops should go ahead (in concrete terms)? Why did it feel it necessary to decide on sites for FSEs without local consultation?

H5 Will we be given the full picture? Do we know what happens behind the scenes?

H6 If problems arise, will we get honest answers from Government? Will Government present research findings properly and fairly?

H7 Who will be liable for contamination from the commercialisation of GM crops (or any other form of damage?)?

J Moral / Ethical Issues

J1 Is it right for man to be tampering with nature? Are we playing God?

J2 What legacy are we leaving future generations?

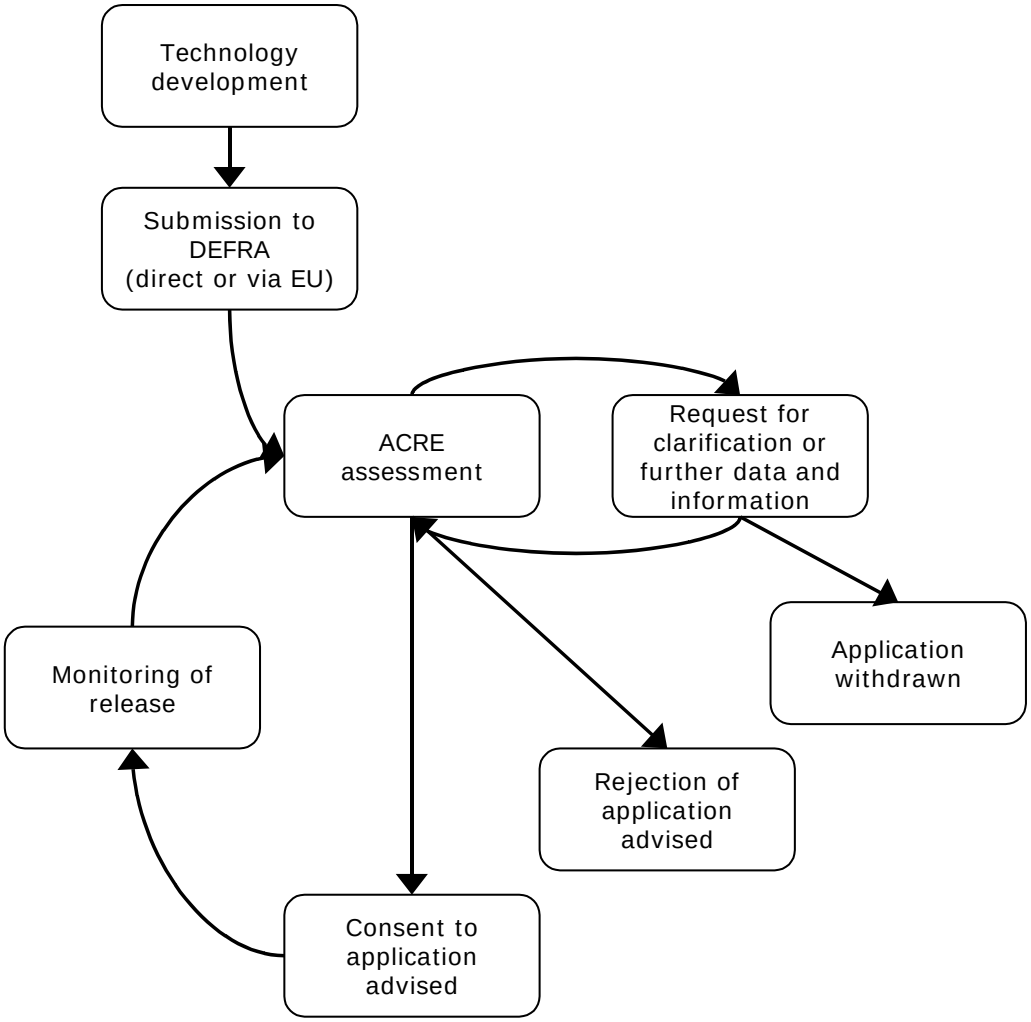
J3 The involvement of the Third World: Is Africa being used as a dumping ground? If the Third World needs GM, then why use it in the West? Will it really help the poor or is it about making the rich richer?

J4 Need to confront more basic problems: Why don't we acknowledge that we waste too much food rather than search for perfect food? Will GM distract us from looking at proven solutions to current farming problems?

J5 How democratic is it to patent genes?

Annex II

Review process undertaken by ACRE in assessing applications for the deliberate release of a GMO in England



Annex III

Description of the regulatory frameworks

The Deliberate Release Directive (2001/18/EC)

The release[1] and marketing[2] of genetically modified organisms (GMOs)[3] in the EU are controlled under a EU-wide regime. The essential point about this legal framework is that releases and marketing of GMOs can only take place in the EU with explicit consent of the regulatory authorities. The aim of the legislation is to protect human health and the environment across the EU from any adverse effects that may be caused by the deliberate release into the environment of GMOs. To achieve this objective the directive sets out a system by which GMOs have to be approved on safety grounds and, to this end, each GMO is subjected to a science-based risk assessment. The EU Directive covers both small-scale trials for research and development (so called part B consents) and consent to place on the market in Europe (part C consents). GM Products on the market can be withdrawn if there is information that indicates that a GMO will be harmful.

In the UK, all of this information is evaluated and weighed by the Advisory Committee on Releases to the Environment (ACRE), an independent, expert scientific committee. On this basis, the committee advises whether there are any significant risks associated with the GMO release. The committee operates in an open and transparent way and its work can be viewed on their website[4]. Annex I shows the review process undertaken by ACRE in assessing applications.

GM food and feed

Comparable legislation covers GM food and Feed Safety. The Novel Food Regulation (258/97) introduced a statutory pre-market approval system for novel foods throughout the EU which is directly applicable and legally binding in all Member States. These regulations cover a range of novel foodstuffs and by definition all foods and food ingredients containing, or consisting of, GMOs or produced from GMOs are novel.

The protocols for the safety assessment of GM foods are based upon a decision tree approach, which was developed by Advisory Committee on Novel Foods and Processes (ACNFP) prior to the current regulation and which has been endorsed by FAO and WHO. This assessment ensures an integrated, stepwise, case-by-case, evidence-based approach. The safety assessment uses the concept of substantial equivalence. This is not an end

[1] A GMO is 'released' if someone deliberately allows it to pass from their control into the environment. A GMO would 'escape' if it passed unintentionally from a person's control into the environment.

[2] GMOs of any description are 'marketed' when products consisting of or including such organisms are placed on the market.

[3] Techniques of genetic modification include recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules, produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation.

[4] www.defra.gov.uk/environment/acre

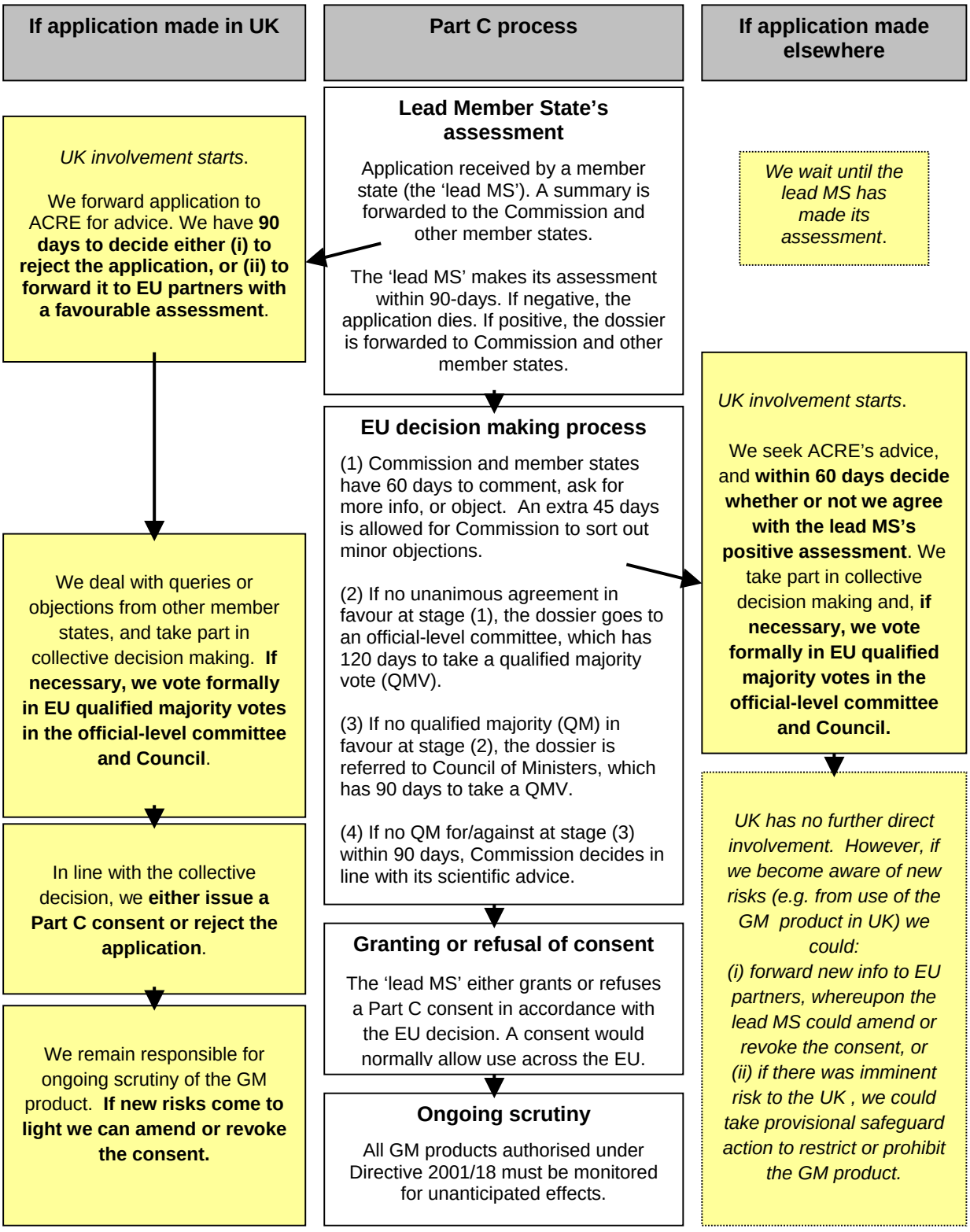
point but a comparative approach used to identify significant differences between the new food and its traditional counterpart, which are then the subject of further investigation. Arrangements are subject to review in light of developments in science and technology, for example new applications of GM and improved analytical methods.

In the UK two Advisory Committees are at the forefront of this activity – these are Advisory Committee on Novel Foods and Processes (ACNFP) and Advisory Committee on Animal Feeding stuffs (ACAF). Both Committees are made up of independent experts appointed solely for their particular expertise and experience. They do not represent any sector, organisation or government department. Likewise, both Committees are committed to a policy of openness and publish agenda, minutes, reports and dossiers on their respective websites.

Under the Novel Foods Regulation (258/97), companies wishing to market a novel food in the EU are required to submit an application to the Competent Authority in the Member State where they first tend to market the product. Since 1 April 2000 the Food Standards Agency has been the UK Competent Authority.

Annex IV

Key UK decisions/actions in the Directive 2001/18 Part C (marketing) procedure



Notes

(1) The centre boxes show key stages of the Part C procedure. The boxes on either side indicate key decisions/actions the UK must take, depending on whether or not we are the 'lead member state' (if we are, our involvement is greater and we must take decisions earlier).

(2) Currently, Defra leads on 2 Part C applications, and other member states lead on another 17.

(3) The timescales given below are maxima set by the Directive: in practice things could happen *faster*, but they could also happen *slower* because the clock can stop if there is a justified request for more information from a member state or the Commission.

(4) UK decisions are taken in consultation with devolved administrations (DAs).

(5) Defra and DAs get expert scientific advice principally from the Advisory Committee on Releases to the Environment (ACRE).

Annex V

European Commission proposals on GM food and feed

The European Commission published two proposals for new legislation concerning genetically modified organisms (GMOs) in July 2001, one covering Food and Feed and the other, on Traceability and Labelling of GMOs. These proposals were issued in response to the current impasse in the approval process for consents to release GMOs into the environment, to address the lack of specific legislative controls on GM animal feed, to revise the approval regime for GM food and feed and extend the current labelling requirements.

This proposed GM food and feed regulation will replace the existing approval procedures for GM foods, as contained in Regulation 258/97 and introduce for the first time rules for the approval of GM animal feed and a harmonised procedure for the scientific assessment and authorisation of GMOs and GM food and feed. It would be a uniform and transparent Community procedure for all marketing applications, whether they concern the GMO itself or the food and feed derivatives.

The proposal will place the European Food Safety Authority (EFSA), rather than individual Member States, at the centre of the approval process. EFSA will carry out the scientific risk assessment covering both the environmental risk and human and animal health safety assessment. On the basis of the opinion of EFSA, the Commission will draft a proposal for granting or refusing authorisation.

The proposal includes labelling provisions that will require labelling of all GM food and feed products derived from GMOs, regardless of the presence or absence of GM material in the final food or feed product. This is an extension to the existing labelling rules and means highly processed products such as oils and glucose syrup, alcoholic drinks, made using GM grain and foods sold in restaurants, which had been cooked in oil derived from GM crops would require labelling. Honey produced by bees foraging nectar from GM crops would also have to be labelled.

Foods produced using processing aids which have been obtained with the help of GM technology (e.g. the enzyme chymosin derived from a GM microorganism, which is used extensively to make hard cheeses) and products from animals fed GM animal feed will continue to be exempt from the labelling requirements.

The proposal agreed at Common Position includes threshold at levels of 0.9%, for GM material in food and feed that has an EU authorisation, and 0.5%, for material not yet authorised but that has a favourable EU risk evaluation (or safety assessment) for accidental present GM-derived material in non-GM supplies below which labelling is not required. The 0.5% threshold will last for three years.

Political agreement was reached on the proposal on 28 November 2002 at the EU Agriculture Council. The proposal was agreed by a qualified majority vote. Common position was adopted on 17 March 2003.

The proposal has now returned to the European Parliament for its second reading with the plenary session due in July 2003. Depending on the outcome of the plenary the proposal may be adopted in late 2003 with Member States implementing the new Regulations within six months of adoption. Alternatively the proposal may go through the conciliation process.

Annex VI

Further information available on the GM Science Review website

(http://www.gmsciencedebate.org.uk/default.htm)

GM Science Review Panel:
http://www.gmsciencedebate.org.uk/panel/default.htm

GM Science Review Panel meetings:
http://www.gmsciencedebate.org.uk/panel/default.htm#Meetings

GM Science Review open meetings:
http://www.gmsciencedebate.org.uk/meetings/default.htm

Contributions to the GM Science Review website:
http://www.gmsciencedebate.org.uk/topics/forum/default.htm

Printed in the UK on recycled paper with a minimum HMSO score of 75.
First published in July 2003. Department of Trade and Industry.