

GM Science Review

Second 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

(January 2004)

Section 4

FEEDBACK ON THE FIRST REPORT

4.1 INTRODUCTION

An important element in the GM Science Review has been the opportunity presented to all to contribute, drawing attention to developments in the science and giving views on their significance. The Panel considered peoples' contributions at open meetings, and to the Science Review website during the first phase of the Review. This helped document a 'range of views' on the issues from which we sought to produce a balanced review. There has been a great deal of interest in our First Report. Website data indicate that over 20,000 copies have been downloaded so far.

As part of the second phase of the Review, we invited comments on our First Report. These can be viewed on the Science Review website¹ and a list of those individuals and organisations who commented is given in Annex I. Despite the large interest in the Report, relatively few individual members of the public took up the invitation to send us comments. In total, there were 54 electronic and 12 written responses from individuals and organisations. Broadly, the Report was regarded as a valuable document by a wide cross-section of respondents (including leading scientific research institutions, leading non-Governmental organisations (NGOs), conservation bodies, and industry). It was seen as consensus building, recognising public concerns about the technology and providing an important new initiative in an otherwise increasingly polarised debate. Against this favourable response, some continuing concerns were expressed. This section addresses comments on the First Report on which we thought a response would be helpful to deliver our remit of evaluating the current state of scientific knowledge on issues of public concern.

To help us identify these particular comments we looked for:

- concerns raised over the framework or what the commentator saw as presumptions on which parts of the discussion in our First Report are based;
- concerns raised over the completeness or interpretation of evidence in our First Report; and
- peer reviewed scientific literature cited, but not referred to in our First Report.

We have not responded to every specific comment that has been made. For example, some comments were opinions not supported with peer reviewed scientific evidence and others offered interpretations, which we had already considered as part of the range of views in the First Report.

¹ <http://www.gmsciencedebate.org.uk/report/comment/default.htm>

For each of the ‘science’ chapters contained in our First Report (i.e. Chapters 4, 5, 6 and 7), Sections 4.2-4.5 below we:

- (1) summarise the main conclusions in our First Report, as contained in the relevant parts of the executive summary;
- (2) summarise all the main issues raised by commentators; and
- (3) provide the Panel’s response, where appropriate.

4.2 CHAPTER 4: HOW RELIABLE IS GM PLANT BREEDING?

4.2.1 Summary conclusions from First Report

‘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.’

4.2.2 Main points made by commentators

Some commentators considered that Chapter 4 of our First Report was balanced and rigorous and thought that placing the GM issue in the context of existing plant breeding methods was useful and appropriate^{2 3 4 5 6}. However, other commentators^{7 8 9 10} stated that because GM

² Agriculture and Biotechnology Council. See Annex 1.

³ British Society of Plant Breeders Ltd. See Annex 1.

⁴ John Innes Centre. See Annex 1.

plant breeding starts with a molecular genetic manipulation process that has (in their view) no resemblance to other breeding technologies, comparing it with other breeding techniques placed it in the wrong context detracting attention from fundamental, generic technical and conceptual differences between GM and non-GM methods (especially natural reproductive processes), and failed to place GM within the context of our latest understanding of gene organisation and function. Some commentators⁷ stated that features of the transformation process such as the breaking and joining of DNA of the host genome at many unspecified locations ‘illegitimate recombination’ and substantial scrambling of both foreign and host DNA at the sites of integration were unique features not mentioned in our First Report. Moreover, it was stated that the ‘successes’ in GM are unstable, are prone to further changes, including the silencing of foreign genes, further rearrangements of DNA and loss of genetic material, all of which is unique to the GM process. The selection of transgenes in active regions of the genome also meant there was maximum possibility for gene disruption leading to cascading effects on a whole variety of genes. The removal of genes out of their normal context and randomly inserting them in a totally new genomic environment, would first lead to ‘position effects’ that result in a highly variable level, spatial and temporal pattern of transgene function and secondly a disruption of host gene function. It was argued that these are unique and generic properties of GM technology and therefore apply to all new varieties of crop plants produced in this manner. It was further stated that as a result of these generic properties associated with the use of GM in agriculture, the case-by-case assessment of GM crops is not applicable at the early stage of their development.

It was stated that current models of gene organisation no longer view genes as independent isolated units of information but depict genes to be organised into ‘functional domains’ and show that genes exist, are regulated, and work within complex interconnected networks. It is argued that this contemporary view of genome structure and function explains why GM is inefficient and imprecise. On this basis, other views expressed by this group⁸ included the notion that natural sexual reproduction methods used in plant breeding preserve the complex gene organisation and regulatory networks that have evolved over vast periods of time and which are vital for whole plant functional integrity. Other breeding methods for producing new plant varieties which disrupt the preserved natural genetic order, including GM, had to be looked at very carefully because these methods would result in major unintended effects with unpredictable consequences. A related criticism was that the use of agronomic criteria to assess GM as presented in this chapter continued to hold onto the now outmoded view that genes are isolated units of information, which as a result can be moved between organisms with totally predictable outcomes^{11 12}. The whole report was predicated on the reductionism view of the science of genetics, and the role that this theory states that DNA plays.

Some stated that because of these effects (as well as the chromosomal and point mutations associated with the tissue culture steps that accompany transformation processes), the sum total of unintended effects might not be seen until the crop was approved and grown for a

⁵ Syngenta. See Annex 1.

⁶ The Scientific Alliance. See Annex 1.

⁷ ISIS. See Annex 1.

⁸ Combined comments from: Econexus, The Five Year Freeze, Friends of the Earth, GeneWatch, Greenpeace, The Soil Association and Dr M Antoniou. See Annex 1.

⁹ J Clarke. See Annex 1.

¹⁰ N Mullan. See Annex 1.

¹¹ R Patterson. See Annex 1.

¹² Munlochy Vigil. See Annex 1.

number of years. Of relevance to this argument were another two points raised by these commentators who disagreed: (1) that mutagenesis, tissue culture and wide crosses are widely used in conventional plant breeding; and (2) that much of the risk arising from random genetic mutations and other procedures produced by these methods is removed by backcrossing. However, some GM crops varieties released commercially appear to have never been backcrossed, including all transgenic varieties of potato and papaya.

Marker assisted breeding (MAB) was suggested¹³ as a more generally acceptable way forward for the use of biotechnology in agriculture.

4.2.3 The Panel's overall response

Genes, and gene products, do not act in isolation. They interact with each other and the environment in complex and incompletely understood ways. It is clear that if you introduce novel genes (by conventional as well as GM means) into a plant, as a consequence, interactions with other plant genes or their products may occur in novel and unpredictable ways. This raises the possibility that such novel interactions may include the production of substances that could potentially be harmful to human health or the environment.

Are there unexpected effects that arise from the production of GM plants? The answer is sometimes 'yes'. Examples exist in the literature, and anecdotally, that when transgenes are put into plants it is not uncommon to observe changes that you don't expect. Such effects may arise at least in part as a consequence of the fact that genes introduced into GM plants insert at random locations in the genome, and may disrupt the function of other genes. It may activate or inhibit expression of one or more of the plant's own genes. Similar disruptions may also occur during other steps when producing GM plants, including extensive tissue culture or plant regeneration methods. The widely accepted view in biological research and plant breeding is that there are some parallels in the properties of GM and non-GM plant breeding methodologies but important differences too. *De facto*, GM is regulated because a special feature of GM breeding is that it allows the transfer into crop plants of one or a few genes from radically different organisms opening up new possibilities for application as well as uncertainties, which we need to look at on a case-by-case basis. In Europe, both the process and the product are regulated, giving several points at which extra requirements and scrutiny are invoked in recognition of the special attributes of this technology.

An important question is whether unpredicted effects are more likely to arise with GM methods than with classical plant breeding methods? This depends very much on the type of conventional plant breeding that is carried out and, the nature of the genetic modification and the method used to achieve it. There is evidence that both the genetic modification and the tissue culture process used to achieve it, both introduce genetic variation, some of which is expected, and some of which is not. Conversely conventional methods of breeding using tissue culture can introduce considerable genetic and chromosomal variation, and this is influenced by the precise method of tissue culture used and the time spent in the tissue culture phase. There are also other examples of (non-GM) conventional crosses that generate significant amounts of genetic variation, so it depends very much on the particular comparisons that are made. The key point is that all conventional methods we have used to

¹³ Combined comments from: Econexus, The Five Year Freeze, Friends of the Earth, GeneWatch, Greenpeace, The Soil Association and Dr M Antoniou. See Annex 1.

generate food crops since plant breeding and selection began 10,000 years ago will have produced effects which we cannot necessarily predict because of the extraordinary complexity of the systems we are dealing with. What conventional breeding has shown is that it is possible to disrupt or scramble the genome in such a way as to cause random and major genomic damage and yet recover plants that are valuable improvements and regarded as having a safe history of use. So ‘genetic damage’ leading to unintended effects is not unique to GM but the consequences may be, depending on the properties of the new genetic material that is being incorporated.

Does GM offer enhanced opportunities for delayed unintended effects and what are the prospects for detecting adverse effects before commercialization? The answer to the first part of the question is possibly, in some cases. The answer to the second part concerns the assessment practices in place before commercialization and how effective they are. In this respect, it is important to appreciate that plant breeders do not just introduce a novel gene, pick a line and place it on the market. There is selection during the breeding program, as in conventional breeding programs, and most plants are rejected. In addition, GM crop varieties need to satisfy the requirements of variety registration as well as the GM legislation system, predicated on safety, and to answer the question: are any of these effects harmful? However, it is not unusual for conventional crops that are placed on the market to show unanticipated effects after commercialization. The same logic therefore applies to GM crops and this presents similar challenges, although the GM regulatory system is special in requiring post market monitoring.

So the question arises, are the current regulatory systems in Europe adequate to address these effects? The degree of uncertainty is related to our ability to detect and interpret changes, at the molecular and agronomic level. The current, widely accepted view among biological researchers and plant breeders is that the methods for evaluation of the current generation of GM crops for food and feed in the European regulatory framework are robust when applied consistently. However, a minority holds the contrary view and this point is picked up in more detail in the main body of our First Report. Regulatory evaluation needs to keep pace with the challenges posed by new developments in technology and also recognise and respond to progress in our understanding and knowledge. It is important that further research to ensure effective risk assessment is supported. It is also important in the UK that regulatory oversight is proportionate to the degree of risk and uncertainty, which recognises the distinct attributes of GM as well as its parallels with conventional crop breeding.

4.2.4 The Panel’s response to specific points

The focus of the chapter

Chapter 4 of our First Report considered the question ‘How reliable is GM plant breeding?’. This was prompted by one of the questions raised by the public, which was: Does GM work? A concern was expressed that ‘The process is haphazard, 90% of all attempts to modify organisms fail, and some experiments and trials have been halted because side effects emerge’¹⁴. As a primary focus of the Science Review was to address issues raised by the public, it was considered important to include this in our First Report. Comparisons with the

¹⁴ See: ‘GM Nation?’: The Public Debate. The handbook prepared from the Corr Willbourn Foundation Workshops, based on questions raised by the general public.

many methods used in non-GM breeding over the past 50–100 years provides an important context to illustrate the point that there is imprecision associated with all breeding approaches and that it is not a unique feature of GM plant breeding, and secondly, to demonstrate that crops with a safe history of use have resulted from plant breeding programmes that have involved massive structural genetic rearrangements.

It was not the intention of Chapter 4 of our First Report to underplay the distinctive attributes of genetic modification. On page 49 we state in the summary that ‘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’. Nor was it our intention to underplay the potential novel sources of uncertainty; and in the Executive Summary of our First Report we stated, ‘Inevitably this raises the possibility that some new consequences of GM plant breeding may be unexpected’. We described some of the mechanisms that could give rise to novel sources of variation associated with GM crops. For example, on page 53, we pointed out that genes introduced into plants by genetic modification can cause insertion mutations in resident genes (‘illegitimate recombination’), that variation in the border sequences inserted into different transgenic plants can occur and that where tissue culture steps are included in the transformation procedures these can be associated with a whole range of changes. Other molecular events associated with gene technologies discussed were gene silencing, variation and position effects (page 55). The joint NGO comments (page 6) describe the molecular mechanisms by which a transgene might activate or inhibit expression of one or more of the plant’s own genes which could potentially lead to a whole range of unanticipated effects. Many of these mechanisms are plausible.

Gene order

It is clear that genetic linkages can have adaptive significance. Ultimately natural selection and evolution selects adapted organisms at many levels, including at the level of genomic organisation and function. This will include spatial and temporal control of genes, but also genetic recombination at meiosis, sexual fertility and breeding system. The common evolutionary origin of species, along with the biological and adaptive significance, is the reason why gene order can be preserved between evolutionary diverse organisms. Evolutionary biology and comparative genomics tell us that gene orders arising from natural sexual reproduction change constantly throughout evolution (a comparison of conservation and rearrangement between rice and *Arabidopsis* gives some quantitative data (Mayer *et al.* 2001) or *Arabidopsis* and Brassicas (Lukens *et al.* 2003)).

Breaking gene order

It is also clear that rearranging these linkages happens not only during plant speciation but is a valuable source of genetic variation that has been used to considerable success in plant breeding. For example, some wheat varieties in commercial use carry a fragment from a rye chromosome. Tissue culture destabilises genetic systems, wide crosses and mutations do the same, and yet crop plants that have been regarded as safe have been recovered from such programmes. Naturally occurring transposons have been implicated in generating some of this variation in all these systems.

Plant breeders have induced ('illegitimate') recombination events and scrambled the genome for many decades by wide hybridisation and mutagenesis. Chemical or radiation breeding causes point mutations, breaks in chromosomes, chromosome translocations (chromosome inversions and interchanges), loss and duplication of genetic material (aneuploidy, nullisomy, disomy, trisomy, tetrasomy) and fragmentation of chromosomes. Often the level of mutagen treatment is set to cause sufficient mutation (or 'genetic damage') to give about 50% plant mortality (LD50). A recent study by Kashkush *et al.* (2002) reports that gene loss, silencing and activation accompany the development of wheat allotetraploids widely used in conventional wheat breeding and variety development programmes.

A wide array of 'illegitimate' genetic changes is also a feature of somaclonal variation occurring in plant tissue culture. In the 1980s the somaclonal method of inducing genetic variation was used by many breeders for seed and vegetatively propagated crops. Many millions of breeding lines were evaluated under field conditions at that time. In the case of sexually propagated crops it was possible to backcross to eliminate undesirable genetic characters. For potatoes (for example) backcrossing is not possible in some cultivars which are sterile and vegetatively propagated, so lines had to be produced on a large scale to provide a large number of genetic variants from which to select superior lines which maintained all of the quality and agronomic characteristics of the original line.

With respect to sexual hybridisation, although the vast majority of the genome must be stable between generations (to account for the observed Mendelian inheritance patterns in plants and other organisms), recent experimental work shows that gene content can be hugely different in different inbred lines of maize and wholesale deletions can occur in lines that have been developed by sexual reproduction (Fu & Dooner 2002; Bennetzen *et al.* 2003). Although the applicability of these observations in maize to other crop plants remains to be determined, these data indicate that generalisations about the functional significance of gene order need to be approached with caution when looking at specific crops, and in some cases this may have more to do with other variables that influence the order of genes in plants (described above) rather than functionality. Although the functional significance of gene order has been established in some animal model systems as illustrated by the *hox* (Mann, 1997; Lufkin, 1997; Veraska, 2000) and globin (Fraser & Grosveld, 1998) gene families, there is no evidence for such gene control systems in plants. Indeed, there is considerable evidence that single individual genes originally located in gene clusters are able to function properly when integrated elsewhere into separate locations in plant genomes. However, the availability of genome sequencing information (Goff *et al.* 2002; Yu *et al.* 2002) will assist research on genomic structure/function relationships for agriculturally significant species.

Genetic variation, from a wide range of sources, provides the fundamental raw material for developing superior plant varieties. The whole of plant breeding has been based on this premise, and has been remarkably successful. Breeding has succeeded in accessing genetic variation from wild or unimproved lines as well as naturally occurring and induced mutations. The breeders have then combined the best genes to produce elite new varieties. What these conventional breeding examples indicate is that it is possible to disrupt or scramble the genome in such a way as to cause random and major genomic damage and yet recover plants that are considered to have a safe history of use. Therefore, the process of breaking and joining of DNA of the host genome at many unspecified locations 'illegitimate recombination' and substantial scrambling of host DNA are not features unique to GM, but associated with all these breeding systems mentioned above. The breaking and rejoining of DNA and changing its juxtaposing to other regulatory sequences are tantamount to 'position

effects' where such rearrangements place genes in different genome environments albeit with reference to host genes rather than the cross-species gene transfers that are usually involved in GM.

Views expressed by the NGO group included the notion that natural sexual reproduction methods used in plant breeding would result in fewer major unintended effects with unpredictable consequences. They cite papers comparing the variation measured in GM and non-GM lines that have passed through tissue culture with parent material (Wang *et al.* (1996); Labra *et al.* (2001)). This evidence is welcome, but other recent experimental work shows that gene content is significantly different in different varieties of maize and large and extensive deletions can occur in varieties that have been developed by sexual reproduction. (Fu & Dooner 2002; Bennetzen *et al.* 2003). So, the linkage between the two is not clear-cut. Marker assisted breeding (MAB) was suggested as a more generally acceptable way forward for the use of biotechnology in agriculture¹⁵. While molecular marker assisted plant breeding is a valuable breeding tool that will increase in utility in coming years, it is not a substitute for certain other plant breeding methods including genetic modification.

Gene stability

Some commentators stated that the 'successes' in GM crops are unstable, are prone to further changes including the silencing of foreign genes, further rearrangements of DNA and loss of genetic material, all of which is unique to the GM process. We discussed the silencing of transgenes in our first report and the need to take account of this in risk assessments. To test for structural instability, the inserted transgene would need to be monitored through successive generations. Such tests form part of the evidence that is submitted to regulatory authorities in dossiers prior to commercialisation. New varieties also have to satisfy other 'seeds legislation' (which applies to varieties bred by non-GM methods) to show that the material shows distinctness, uniformity and stability (DUS tests). We are unaware of any published research that has monitored transgene stability and shown that further rearrangements of DNA and loss of genetic material occurs in commercial lines. The key issue is whether this occurs more often in transgenes rather than elsewhere in the genome. Such monitoring experiments are technically feasible and could be conducted to establish whether such structural rearrangements do occur. Failure of product performance is another check on stability of the introduced trait that would be seen by farmers and notified to the seed supplier.

Prospects for identifying unintended harmful effects before commercialisation

Some commentators stated that because of the unique features associated with genetic transformation (as well as the chromosomal and point mutations associated with the tissue culture steps that accompany transformation processes), the sum total of unintended effects might not be seen until the crop was approved and grown for a number of years.

¹⁵ Combined comments from: Econexus, The Five Year Freeze, Friends of the Earth, GeneWatch, Greenpeace, The Soil Association and Dr M Antoniou. See Annex 1.

Plant selection and regulation

In coming to a judgement on whether GM poses a greater likelihood of adverse unintended effects, it is important to realise that, although a population of transgenic plants generated using a gene cassette can show a unanticipated and unexpected effects, it is from this primary population that plant breeders select individual plants for further analysis. Breeders do not simply select a primary transformant and place it on the market. On the way to commercialisation, plants are selected using molecular genetic, biochemical, and agronomic criteria favouring plants that are stable, predictable and do not cause adverse effects. So from a variable starting point, lines that are stable and predictable are selected.

A related criticism from some commentators was that the use of agronomic criteria to assess GM, as presented in Chapter 4 of our First Report, continued to hold to the now outmoded view that genes are isolated units of information, which as a result can be moved between organisms with totally predictable outcomes; and that the report was predicated on the reductionism view of the science of genetics, and the role that this theory states that DNA plays^{16 17}. It was stated: ‘in genetic engineering it is assumed, without adequate experimental proof, that a bacterial gene for an insecticide protein, for example, transferred to a maize plant, will produce precisely that protein and nothing else. Yet in the alien genetic environment, alternative splicing of the bacterial gene might give variants of the intended protein, or even proteins bearing little structural relationship to the original one, with unpredictable effects on ecosystems and human health’. This statement misrepresents the current thinking and practices amongst the plant sciences, plant breeders and the regulatory community. First, a century of scientific plant breeding and plant genetics research has established that genes interact with one another and with the environment. This is widely recognised. This is why all plant breeding involves several years of evaluation under different environments and in different genetic backgrounds. Interactions between genes, and between genes and the environment have been studied since the birth of genetics and are detailed in the earliest genetics textbooks. More recent advances in molecular genetics are now explaining the sources of genetic variation that plant breeders have worked with successfully for almost a century. Second, and specific to GM plant breeding; the regulatory framework does not assume that what is used in a gene manipulation experiment is what is actually inserted. The legislation recognises that genes can be disrupted so that coding sequences can come under the influence of host promoters or transgene promoters can activate host genes. Applicants are required to submit detailed dossiers that characterise the structure of the DNA actually inserted. Therefore, safety assessments made on GM crops are not only based on agronomic criteria but molecular characterisation forms an essential part of the process of risk assessment.

Risk management

One commentator stated ‘Much of the risk arising from random genetic mutations and other procedures is removed by backcrossing. In comparison, some GM crops varieties released commercially appear to have never been backcrossed. These include all transgenic varieties of potato and papaya.’

¹⁶ R Patterson. See Annex I.

¹⁷ Munlochy Vigil. See Annex I.

Sexually compatible (seed producing) transgenic crops are almost invariably backcrossed because the inheritance pattern is valuable information in assessing a breeding line and is often required in risk assessment. Plant breeders also very frequently need to backcross a transgenic plant into locally adapted and new elite crop varieties. To breed a crop for a particular agricultural purpose it is important to test for many characters (disease resistance, pest resistance, flavour, cooking quality, earliness, height). The transgenic crop character is usually only one of many characters that breeders must evaluate and combine. In the case of vegetatively propagated crops such as potatoes, it can be still be possible to backcross. But if breeders do not wish to backcross they would normally make many transgenic lines and select the ones with superior characters after a range of field and quality tests are applied. This is the same procedure for mutation breeding in vegetative propagated crops where backcrossing may not be feasible or desirable. We agree that such backcross approaches may be helpful and that where this is not available, selection from a wider range of primary transgenic plants to find those with desired characteristics may compensate for this. However, of greater importance is the need to accurately characterise the inserted genetic material as a basis to develop sound risk assessments. This is discussed in detail in an ACRE publication¹⁸.

This raises a wider issue of risk management and the intelligent design of GM plants that minimise the opportunities for unintended adverse effects. For example, targeting transgene insertions (First Report, Section 4.7, paragraph 1) is one future development that addresses some of the major concerns arising from the random integration events. This is an emerging area of research and is discussed in an ACRE publication¹⁹. Other advances highlighted in our First Report (Section 4.8) are molecular (genomics, transcriptomics, proteomics) and metabolic (metabonomics) profiling as well as the role of epigenetic states (DNA methylation patterns and the histone code). This should provide insight into basic biological processes and the effects of genetic alterations through GM and other procedures.

4.3 CHAPTER 5: THE SAFETY OF FOOD AND ANIMAL FEED DERIVED FROM GM CROPS

4.3.1 Possible nutritional and toxicological differences in GM food

Summary conclusions from First Report

‘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 people reason that the absence of evidence of harm should not be treated as evidence of

¹⁸ <http://www.defra.gov.uk/environment/acre/molecdata/index.htm>

¹⁹ Guidance on best practice in the design of genetically modified crops.
<http://www.defra.gov.uk/environment/acre/bestprac/index.htm>

the absence of harm. This argues for greater reliance on scientific research and epidemiological monitoring. Other people 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 specifically 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 and allergens); increase levels of health promoting factors (e.g. antioxidants); and modify levels of macro or micronutrients (e.g. proteins, lipids and vitamins).'

The adequacy of safety assessment: best practice and reality

Main points made by commentators

One group of commentators²⁰ stated that our First Report gave an over-optimistic picture of the safety of GM foods in the food chain by focusing on best practice, or what is theoretically possible, in GM food safety assessment rather than what is actually done, which they stated was inadequate. They cited a study of EU GM product dossiers by Spok *et al.* (2002) in evidence. Another commentator²¹ stated that we should have given more references to specific papers on safety testing rather than quoting the review by Kuiper *et al.* (2001), which only mentions 10 refereed publications.

A common concern amongst commentators was the adequacy of safety evaluation information, such as nutritional and toxicological studies and feeding studies in general. It was repeatedly stated that these needed to be of higher quality, peer-reviewed, and more publicly accessible^{20 21 22 23 24 25 26 27 28 29}. References were made to reports by the Royal Society, in relation to the possible toxicity of GM potatoes³⁰ and the Scottish Parliament's Health Committee enquiry³¹ in support of the need for adequate safety evaluation information. It was also indicated by some that there was a need for feeding trials on human

²⁰ Combined comment from: Econexus, The Five Year Freeze, Friends of the Earth, GeneWatch UK, Greenpeace, The Soil Association & Dr M Antoniou. See Annex I.

²¹ ISP. See Annex I.

²² P Elliot. See Annex I.

²³ J Dutton. See Annex I.

²⁴ R Patterson. See Annex I.

²⁵ Munlochy Vigil. See Annex I.

²⁶ H Gatt. See Annex I.

²⁷ Swindon FoE. See Annex I.

²⁸ ISIS. See Annex I.

²⁹ D Gledhill. See Annex I.

³⁰ Royal Society report 11/99, June 1999.

³¹ The Scottish Parliament's Health and Community Care Committee. Report on Inquiry into GM Crops, 1st Report 2003, SP Paper 743.

volunteers, as the results of tests on animals could not necessarily be extrapolated to other species^{32 33 34 35}.

The Panel's response

Chapter 3 of our First Report considered 'Science in the Regulatory Process'. There are also frequent references to the regulatory approach and safety assessment in Chapter 5 on 'GM Derived Food and Animal Feed Safety'. We do acknowledge on page 65 that 'if the new gene product or endogenous plant metabolites were not as intended they could potentially lead to toxic, allergic or antinutritional effects'. And we go on to discuss that this is no different in extent than for other novel foods or indeed new varieties of plants including variation from various sources. We further state on page 71 that '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'. The comments in our First Report considered current evidence and what is scientifically achievable today, recognizing areas of uncertainty, and not some hypothetical or theoretical ideal. We also considered future developments (e.g. expression profiling in Section 5.2.3, page 69).

We welcome the identification of the Austrian report, which criticizes the adequacy of the information relating to toxicity, allergenicity and substantial equivalence in 11 EU GM application dossiers (which complied with the regulations which existed at the time of their scientific review). The European Commission Guidance Document for 'The risk assessment of genetically modified plants and derived foods and feed', issued in March 2003 and cited in our First Report (EC, 2003), provides guidance to notifiers and risk assessors on the preparation and information needed for regulatory dossiers. It notes that while 'It is not the purpose of this guidance document to prescribe specific protocols for the execution of experiments...Data provided in support of an application should be of at least the quality expected of data submitted to a high-ranking peer-review journal. Particular attention should be paid to the sensitivity and specificity of methods employed and to the adequacy and appropriateness of the controls'. Industry has itself issued guidance on a range of technical information required for scientific assessments.³⁶

Science can underpin advice on robust and achievable regulatory mechanisms. However, the enforcement and consistent application of the regulatory framework and what constitutes an acceptable regulatory burden are outside the scope of our Review. As we say in our First Report, science provides the evidence base for decisions on safety to human health and the environment. But it is not the basis for how effectively (scientifically achievable) regulatory requirements are enforced. If there is an identifiable deficiency in scientifically achievable aspects of a regulatory system, then it is for regulatory bodies and Government to consider the implications for health and safety. More evidence, through further research on the extent to which regulatory practice does actually comply with aims and aspirations, would be desirable.

In this regard, we should clarify that certain provisions referred to elsewhere in our First Report, such as the composition testing of entire foods (page 65) or the practice of sub-chronic 90 day rat feeding studies (page 69) are only routinely applied in a proportion of

³² J Clarke. See Annex I.

³³ ISP. See Annex I.

³⁴ R Patterson. See Annex I.

³⁵ Munlochy Vigil. See Annex I.

³⁶ http://www.europabio.org/pages/workgroups_detail.asp?wo_id=14

cases. For example, 90 day studies are not always conducted for reasons of palatability or dietary balance. Such studies are typical but not mandatory and a case-by-case approach is applied. We welcome this opportunity to clarify any misunderstandings that might unwittingly have occurred concerning the scope and extent of existing regulatory provisions as routinely implemented in practice.

As we said on page 43 of our First Report, any regulatory system is dynamic, continuous (i.e. always under review to take account of advances in science and technology and prevailing knowledge), subject to critical challenge and continuously subject to improvement. Any regulatory system will in practice be less than 100% perfect. It is important not to exaggerate the efficacy or completeness of regulatory appraisal but also to take all reasonable opportunities to ensure that actual practice makes full use of feasible and appropriate techniques. If applied as intended, the approval system for GM foods is certainly no worse, and in this respect more detailed, than that for conventional novel foods. We explained on pages 68 and 69 of our First Report, in relation to the evaluation of toxicity and allergenicity, how the nature of the substance determines the testing methodology. For instance, pharmaceuticals are chemically well-defined substances used in relatively small amounts which enables a somewhat different approach to be applied compared to that for GM foods.

Regulatory dossiers, drawn up for the Competent Authority for different GM products, by different notifiers, at different times, will by definition vary in style, format and content. A wide range of bioinformatic, *in vitro*, *in vivo*, *in planta* and field trials work is contained within these dossiers and it is unusual for this all to be published. Often the work is 'new product' development as opposed to basic research and as such does not add significantly to the science base. As such it is less suited as well as less attractive for publication in peer reviewed scientific literature. In many cases relatively standard protocols are used and the work is performed to Good Laboratory Practice (GLP), possibly with some of it contracted out. While a number of individual studies have been published by various authors this is not done routinely, which accounts for a relative shortage of such work in the peer reviewed literature. The same is true for pharmaceutical (as well as pesticide) registrations. Apart from the pharmacology and mode of action, which may be novel, there is insufficient material of original interest. However, detailed summaries will be provided within the new European Food Safety Authority (EFSA) system. Some companies publish detailed summaries on their company websites. This being said, several hundred studies designed to demonstrate toxicological, antinutritional, growth performance, mode of action, bioavailability or allergy potential have been reported quite widely over the last few years. Other studies have been reported in associated safety areas, such as: viral DNA; DNA/protein absence from milk meat and eggs; assessment of animal models; antibiotic resistance markers (ARMs); compositional analysis; and the overall safety assessment procedure. All of this information is considered holistically in the process of safety evaluation described in our First Report.

Low-level human health effects and long term monitoring

Main points made by commentators

Several commentators^{37 38 39} highlighted statements on pages 10 and 73 of our First Report, indicating a lack of substantiated adverse human health effects from the extensive cultivation

³⁷ Swindon FoE. See Annex I.

³⁸ D Gledhill. See Annex I.

and consumption of GM foods over the last seven years, as not proving GM foods are safe. Some commentators called for more research leading to the introduction of systematic long-term health monitoring^{40 41 42 43 44 45}, whilst others acknowledged the difficulty of linking cause with low-level human health effect^{46 47 48}. Two commentators made a link between the consumption of GM food and what they saw as an increase in food-borne illnesses in the US (Mead *et al.* 1999)^{40 49}.

The Panel's response

We addressed this issue in Section 5.2 of our First Report (pages 62 and 73). And on page 10 we indicated that the long-term assessment of health effects for whole foods or feeds, containing thousands of individual macro and micro-nutrients as well as other substances, is considerably more difficult than the post-marketing surveillance of a single substance such as a new pharmaceutical active ingredient. We also stated on page 73⁵⁰ that the Food Standards Agency (FSA) had commissioned further research in this area. The results of this research were published during the summer of 2003⁵¹.

The argument is put that 'absence of evidence of harm is not evidence of absence of harm'. In essence this is correct, over the timescale of testing conventionally-bred novel crops of any origin, or indeed for any human activity. In our First Report, we did not claim that GM foods were absolutely safe. However, in the longer term, if all the available evidence fails to indicate that adverse effects have occurred, then we become more confident that harm is unlikely and our risk-benefit analysis changes. Most traditional foods have never been tested for safety because extended safe use does ultimately provide reassurance of safety. (Also see Section 4.4.8 on the statistical aspects of environmental monitoring.)

The current view of post-marketing surveillance methodology indicates that if the effect is immediate and severe then it probably is possible to establish a causal link between a new food or food ingredient and the effect. If it is not severe and only manifests itself in the longer term, it may be possible retrospectively to identify a specific food or feed as a causal factor. However, it is difficult to prove a causal link between a food substance and some as yet unspecified long-term or minor effect with a background of changing diets and lifestyles. This is due to the following factors that would confound any attempt to establish such a link and make it difficult to design a rigorously controlled experiment:

- (1) Data would be required about the food consumption by individuals over a long period, and a broader range of risk factors. Any survey would need to include food bought and consumed outside the household (currently more than 30% and likely to rise to 50%

³⁹ Green Party. See Annex I.

⁴⁰ ISIS. See Annex I.

⁴¹ P Elliot. See Annex I.

⁴² J Clarke. See Annex I.

⁴³ R Scullion. See Annex I.

⁴⁴ Combined comment from: Econexus, The Five Year Freeze, Friends of the Earth, GeneWatch UK, Greenpeace, The Soil Association & Dr M Antoniou. See Annex I.

⁴⁵ D Gledhill. See Annex I.

⁴⁶ J Bond. See Annex I.

⁴⁷ Bristol Area SERA Group. See Annex I.

⁴⁸ ABC. See Annex I.

⁴⁹ ISP. See Annex I.

⁵⁰ There is an error in the 7th line of the 3rd paragraph on page 73 of the First Report which should read:

'...delayed adverse effects can not be completely ruled out...'

⁵¹ (<http://www.foodstandards.gov.uk/multimedia/webpage/feasibility>).

over the next 20 years). There will often be strong correlations between products not obviously related (e.g. a particular brand of nappy with a particular food brand because the same shops stock both, or a particular bottled water with an activity because of advertising, although any could cause later illness). Household surveys do not distinguish which person in the household consumes how much of each of the components of the weekly shopping basket.

- (2) The exact composition of each product, many with multiple ingredients, would be required to be known. Manufacturers are reluctant to provide precise details of product ingredients, which are often a trade secret. It would be difficult to track ingredient changes. This is essential information for post-market surveillance in identifying the causative agent and establishing any dose-response relationship.
- (3) Systematic linking of causality to a particular food intake retrospectively after 10 or even 40 years, on a background of changing food consumption patterns and lifestyles, would need enormous sample sizes and complex databases.
- (4) The need for a relational database to connect health records to food consumed raises issues of patient confidentiality and informed consent.

Difficulties in demonstrating the validity of claims linking food to health effects are widely known and the subject of much regulatory discussion with respect to labelling and legal liability. Despite these considerable difficulties, as we said in our First Report (pages 73 and 77), there are ongoing epidemiological studies, and international interest in the feasibility of implementing some form of post-marketing surveillance of potential human food-related late health effects.

The study by Mead *et al.* (1999) on 'Food-related illness and death in the United States' involved the compilation and analysis of information from multiple surveillance systems and other sources. The commentators who quoted this source said that the study had 'found that between 1994, when GM food was first introduced, and 1999, foodborne illnesses in the United States have increased two to ten-fold'. This is an incorrect interpretation of the report's findings. Nowhere in the report was GM food or feed associated with changes in foodborne illness. Estimates of the overall rate of death from foodborne illness were found to be lower than those estimated for 1994, and the overall incidence of illness was within the range estimated in 1985. Estimates of overall disease were greater (by a factor of about 2) since the 1994 estimate but this was almost entirely accounted for by a different method used to estimate the impact of the Norwalk Virus. The authors concluded that:

'Methodologic differences between our analysis and previously published studies make it difficult to draw firm conclusions regarding overall trends in the incidence of foodborne illness. In general, the differences between our estimates and previously published figures appear to be due primarily to the availability of better information and new analyses rather than real changes in disease frequency over time.'

Toxicity testing

Main points made by commentators

A number of commentators stated that we had swept aside or misrepresented evidence of harm from GM food crops, particularly on toxicity and the work of Dr Pusztai and co-workers^{52 53 54 55}. These studies involved the administration to rats of various diets, some containing potatoes (both raw and cooked), either with or without the addition of non-potato lectin (from the snowdrop (*Galanthus nivalis*) or the jack bean (concanavalin A), used as a positive control) or potatoes which had been genetically modified to produce the snowdrop lectin. Adverse effects were reported on the rats' growth and their immune function (Ewen & Pusztai, 1999). The most extensive comments on both the interpretation of these data and this issue came from Dr Pusztai himself and from Professor Burke^{54 56}. One commentator stated that we could have given more references to acute toxicity testing in animal models⁵⁴. Another referred to recently published work by Chen *et al.*, which found no significant difference between rats and mice fed on GM and non-GM diets⁵⁷. Yet another gave an incomplete reference to a study that appears to support Dr Pusztai's findings⁵⁸.

The Panel's response

Dr Pusztai's work was mentioned in Section 5.2 of our First Report (page 66), which considered 'Possible Nutritional and Toxicological Differences in GM food', where we acknowledged that there are different opinions over whether GM foods present a problem for human health. We considered this in the subsequent sections of this chapter. Several commentators on the First Report also referred to a recent review of the potential human health effects of GM foods (Pusztai *et al.* 2003).

Since these studies first came to the attention of the media in 1998, they have been the subject of considerable scientific scrutiny. The data have been examined by an independent audit committee chaired by Professor John Bourne, the Royal Society (1999), the Biotechnology and Biological Sciences Research Council (BBSRC), the Advisory Committee on Novel Foods and Processes (ACNFP) and the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT). Fundamental flaws were found in the experimental design, its execution and the analysis of, and conclusions from, the data.

ACNFP and COT studied the work in great detail and concluded that the rat diet did not have appropriate nutritional adequacy or nutrient density and that consumption of raw non-GM potato starch has been shown to produce alterations to rat gut morphology. ACNFP noted that 'many of the adverse effects seen occurred in rats fed potatoes spiked with high levels of the con[canavalin] A lectin'. Finally the reliability of the lymphocyte proliferation assay used was called into question. Both Committees considered that no reliable conclusions could be reached (ACNFP 2000). Subsequent repetition of this and similar studies, performed more rigorously and systematically, have not confirmed the conclusions of Dr Pusztai and his colleagues. (See for example Chen *et al.* (2003) on the safety assessment for GM sweet

⁵² ISIS. See Annex I.

⁵³ G Humphreys. See Annex I.

⁵⁴ ISP. See Annex I.

⁵⁵ Bristol Area SERA Group. See Annex I.

⁵⁶ D Burke. See Annex I.

⁵⁷ *Ibid.* The reference was to a publication in the Plant Journal, which could not be traced, but see Chen *et al.* (2003).

⁵⁸ Munloch Vigil. See Annex I. 'work of the Russian Academy of Sciences, Institute of Nutrition'.

pepper and tomato.) The study could however be used to help indicate the lines of investigation and procedures that might be followed in any future work in this area.

See also Sections 4.3.3 and 4.5.4.

Insecticidal endotoxins

Main points made by commentators

Two commentators^{59 60} highlighted the possible dangers of including insecticidal endotoxins, particularly *Bt*, in our diet and stated that our Report had either misrepresented or not highlighted this sufficiently (Pusztai *et al.* 2003).

The Panel's response

In response to these comments we provide the following review of the literature related to *Bt* proteins (Cry proteins) with insecticidal or other effects.

In susceptible insects (e.g. lepidoptera), the toxicity of a particular *Bt* protein is correlated with the number of high-affinity binding sites on the microvilli of gut epithelial cells (English & Slatin, 1992; Schnepf *et al.* 1998; Ballester *et al.* 1999; Van Mellaert *et al.* 1999). The lack of toxicity of Cry proteins to non-target invertebrate species (IPCS, 1999) is due to the absence of high-affinity Cry protein binding receptors on gut epithelial cells (Ballester *et al.* 1999). Several studies with vertebrate species (mice, rats, monkeys, humans) have also failed to find high affinity Cry protein binding sites on gut epithelial cell membranes (Noteborn *et al.* 1993; Noteborn *et al.* 1995; Hofmann *et al.* 1998). This is the basis of the selectivity of Cry proteins. They are acutely toxic to susceptible insects with high-affinity binding sites (LD50's of approximately a few µg/kg body weight), but are non-toxic to vertebrate species even when dosed at greater than 1 g/kg body weight (EPA, 1998; McClintock *et al.* 1995).

Other considerations that support the dietary safety of Cry proteins for humans and animals are: (English & Slatin, 1992) the acidic environment of the mammalian stomach that does not favour dissolution and activation of the Cry proteins (susceptible insects have alkaline gastrointestinal tracts); (Schnepf *et al.* 1998) the rapid degradation of the Cry proteins by pepsin in the stomach and (Ballester *et al.* 1999) the lack of high-affinity Cry-specific binding receptors on mammalian gut epithelial cells. There are no reports of microscopic changes in the gastrointestinal tract of rodents administered very high, acute dosages of microbial *Bt* pesticide formulations by the enteral route (McClintock *et al.* 1995). There was also no evidence of toxicity in sub-chronic and chronic feeding studies where *Bt* microbial formulations were administered at dosages up to 8400 mg/kg/day (McClintock *et al.* 1995).

Findings from these studies on exogenously applied (e.g. organically sprayed) *Bt* are relevant to the safety assessment of GM *Bt*-protected plants because the microbial preparations contain the same classes of Cry proteins (Cry1, Cry2 and Cry3) introduced into GM food and feed crops (Betz *et al.* 2000). More recently, rats have been fed grain from *Bt* corn over 13 weeks at dietary levels far higher than human exposure levels and showed no macroscopic or microscopic changes in gastrointestinal tract tissues (Dudek *et al.* 2002). Moreover, there was little evidence of toxicity during incubation of mammalian cells *in vitro* with Cry proteins (Shimada *et al.* 2003; Thomas & Ellar 1983).

⁵⁹ P Elliott. See Annex I.

⁶⁰ ISP. See Annex I.

There are reports in the literature of binding of Cry proteins to mammalian gastrointestinal cells *in vitro*, changes in the ileum of mice dosed with a *Bt* microbial formulation (unspecified strain) and immunogenicity of Cry1Ac protein given by parenteral or enteral administration (Fares *et al.* 1998; Vazquez-Padron *et al.* 1999; Vazquez-Padron *et al.* 2000; Pusztai *et al.* 2003). The relevance of these studies to human safety assessment of Cry proteins have been questioned due to deficiencies in the studies and the use of dosing techniques that bypass the protective role of the stomach in breaking down ingested proteins (Siegel, 2001; Federici, 2002). The weight of evidence of the many safety studies that have been completed indicates that the use of Cry proteins to protect conventional organic or GM crops against insect damage poses no meaningful food safety risks to humans. The highly selective mode of action of these insect control proteins forms the basis of their history of safe use in conventional and organic agriculture for over 40 years (IPCS, 1999; EPA, 1998; McClintock *et al.* 1995; Betz *et al.* 2000; Siegel, 2001; Federici, 2002).

GM foods and developing countries

Main points made by commentators

Some commentators^{61 62} stated that it was a misconception to suggest that GM foods would provide a solution to malnutrition in developing countries when its causes were largely social and economic. Several commentators^{62 63 64 65} stated that Golden Rice was a poor example to quote as a nutritionally enhanced GM food, when it would be necessary to eat some 9kg to obtain the daily Vitamin A requirement.

The Panel's response

In our First Report (page 8) we said that our Review focused on the potential use of GM crops in the UK, although issues about the use of GM crops elsewhere, particularly in developing countries, were raised and discussed and we hoped that our work might be of use in other countries in clarifying issues and generally informing debate.

We discussed the development of safer, nutritionally enhanced foods in Section 5.2.7 of our First Report. At the end of this Section we noted that nearly one sixth of the global population of some six billion people do not have adequate diets and that micronutrient deficiencies are common. We illustrated some ways (such as Golden Rice) in which GM technology was being used to enhance nutrient quality. In doing this we simply wished to illustrate a line of future development, which might be of particular benefit to developing countries. Whatever the significance of developments in GM food for developing countries, we acknowledge that science can only play a part in the complex issue of how worldwide malnutrition might be addressed. A general discussion of this is well beyond the scope of our Review.

The international impact of UK developments in GM food and crops were considered in the Prime Minister's Strategy Unit study into the costs and benefits associated with growing GM

⁶¹ Combined comment from: Econexus, The Five Year Freeze, Friends of the Earth, GeneWatch UK, Greenpeace, The Soil Association & Dr M Antoniou. See Annex I.

⁶² Green Party. See Annex I.

⁶³ ISIS. See Annex I.

⁶⁴ Swindon FoE. See Annex I.

⁶⁵ ISP. See Annex I.

crops⁶⁶. The implications of GM crops for the developing world have also been considered in reports by the Nuffield Council on Bioethics⁶⁷.

4.3.2 Food allergies from GM crops

Summary conclusions from First Report

‘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 a potential 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. 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.’

⁶⁶ ‘Field Work: weighing up the costs and benefits of GM crops.’ An analysis by The Prime Minister’s Strategy Unit (SU). July 2003. <http://www.number10.gov.uk/output/Page4131.asp>

⁶⁷ ‘Genetically modified crops: the ethical and social issues’, May 1999 & ‘The use of genetically modified crops in developing countries’, June 2003, Nuffield Council on Biotechnology. <http://www.nuffieldbioethics.org/gmcrops/index.asp>

The allergenicity testing methodology

Main points made by commentators

Several contributors raised concerns about the adequacy of current or proposed testing methodologies for allergenicity in GM crops.^{68 69 70} (Germolec *et al.* 2003; Kleter & Peijnenburg, 2002; Jank & Haslberger, 2003; Bernstein *et al.* 2003). One group of contributors stated that we had falsely implied in our Report that the regulatory process would always detect novel allergens in GM foods⁷¹, whilst others^{72 73} stated that, if anything, there was less of a risk of undetected allergens in GM compared to conventionally bred plants.

The Panel's response

In our First Report we concluded that no regulatory process will always detect novel allergens in GM foods. In Section 5.3 we considered food allergies from GM crops and on page 89 we state that 'Absolute certainty about lack of allergenicity cannot be achieved (EC, 2003) in this or any other risk assessment'. Individual people vary considerably in their sensitivity to different protein food allergens; the acceptable proportion of people that are adversely affected is a political judgment. The genetically determined hypersensitivity to certain allergens (atopy) can also appear and disappear in the same individual at different stages of their life. This section of the First Report makes a strong point in acknowledging our relative lack of knowledge about aspects of allergenicity and the limitations of current safety assessment procedures for any novel food.

In Section 5.3.4 of our First Report (page 89) we state: 'The main area of contention appears to be the value of specific tests and if, and how, they can be improved'. Similarly, at a conference on the Assessment of the Allergenic Potential of Genetically Modified Foods in the USA in 2001⁷⁴, it was stated that: 'Potential benefits that may be derived from biotechnologies involving genetically modified organisms could be enormous. Potential risks of allergenicity possibly associated with their use will likely be manageable, provided appropriate information is available to decision makers' (Selgrade *et al.* 2003). This last sentence embodies the issue under discussion. To recapitulate, four different types of test regimes have been proposed in decision making that aim to structure the assessment scientifically.

Computer evaluation of sequence similarity

The issue of requiring six (increases chance of false positives) or eight (increases chance of false negatives) or 35% (28) out of 80 amino acids⁷⁵ is a judgement which must be made by regulatory committees. And the suggestion by Kleter & Peijnenburg (2002) to include an analysis of surface availability of the epitopes is an additional consideration. Although, as we reported on page 82, conformational epitopes will be destroyed and new linear epitopes may be unmasked by food processing, or by denaturation of the protein by stomach acid, or by degradation of protein in the digestive process. The potential shortcomings of this technology have been outlined on page 87 of our First Report and need no repetition.

⁶⁸ R Scullion. See Annex I.

⁶⁹ Syngenta. See Annex I.

⁷⁰ Green Party. See Annex I.

⁷¹ Combined comment from: Econexus, The Five Year Freeze, Friends of the Earth, GeneWatch UK, Greenpeace, The Soil Association & Dr M Antoniou. See Annex I.

⁷² John Innes Centre. See Annex I.

⁷³ ABC. See Annex I.

⁷⁴ See issue of Environmental Health Perspectives, **111**(8), June 2003.

⁷⁵ Note misprint on page 82 of the First Report.

Resistance to proteolytic digestion

We indicated on page 83 that there is no universal correlation between stability in gastric fluid and allergenic potential.

Serum screens

This area of evaluation is hampered by a lack of availability of sera and more effort should be made to collect these. However in principle, the clinical testing involved in protecting the susceptible human population (about 1 in 50 people) potentially provides a good way forward. Genetic variation in the human population will make prediction of individual allergic responses impossible at present.

Collections of sera will need to be built up and as well as collections of standardised known allergens and non-allergens as positive and negative controls in tests. Testing of those who have occupational exposure to allergens has been proposed. This could be helpful and this area has been little studied, yet could be of public health significance (Bernstein *et al.* 2003).

Animal tests

There is strong support for the development of better animal tests that would allow a dose response measure to be derived for allergens (Selgrade *et al.* 2003). This might allow quantification of relative sensitising dose of the potential allergens as well as quantification of responses. There are no theoretical impediments to develop the appropriate tests for identified potential allergens similar to that relied upon in toxicity testing for human and animal medicines. Tests for the allergenicity of whole foods are more difficult as in most cases it would be impossible to feed animals the required amounts of GM containing food without seriously distorting their normal diet and hence inducing pathogenic effects.

Studies from the 2001 conference mentioned on the previous page and those of expert groups in the EU (EC, 2003) come to similar conclusions as our First Report. They support suggestions for future research elaborated in Section 5.3.8 (page 89) of our First Report. Apart from basic research in allergic responses in humans, a number of areas of research aiming at hazard identification were proposed in the paper by Selgrade *et al.* (2003). These were:

- development evaluation and validation of animal models;
- establishment of serum banks;
- improved human skin test technology;
- identification and banking of known allergens and non-allergens;
- a systematic approach to recording adverse events (case studies);
- development of relative potency, and thresholds for sensitisation and elicitation of the allergic reaction; and
- development and refinement, standardisation of tests protocols.

None of these issues are GM specific as all apply to other novel foods and conventional breeding methods. GM technology may have highlighted the need for improved assessment but is actually more able to deal with the issues since comparability with parent crops is more straightforward because exact gene and protein sequences are known. An influence on the variation in the composition of food stuffs and crops comes from agronomic practices, the weather, the soil type, the plant variety etc. In fact, comparisons between parent plants and GM derivatives grown under similar conditions allow this type of comparison to be put on a more solid scientific basis opening up the potential for application of proteome, metabolome and transcriptome analysis to deal with potential issues such as unintended effects.

The allergenicity of GM pollen

Main points made by commentators

Two commentators^{76 77} were specifically concerned about the lack of research into the potential allergenicity of GM pollen.

The Panel's response

In our First Report we mentioned the need to test for the allergenicity of ingested or inhaled pollen, for any new means of food production (page 61). Exposure routes, other than ingestion, were also mentioned on page 102, where we referred to the Royal Society 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. In Section 5.3.8 of our First Report we said that '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.' This applies as much to GM and non-GM pollen as it does to GM and non-GM foods.

4.3.3 The fate of transgenic DNA

Summary conclusions from First Report

'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

⁷⁶ R Patterson. See Annex I.

⁷⁷ Munlochy Vigil. See Annex I.

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 is 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 and over prescription and non-compliance in medical use, 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 distinctiveness of transgenic DNA

Main points made by commentators

Several commentators^{78 79 80 81} questioned our analysis that ‘transgenic DNA is no different from other DNA consumed as part of the normal diet and it will have a similar fate’ (page 90 of our First Report). Some stated that it clearly was different, because it contained a novel combination of genetic material from different species and genetic material often designed to enhance horizontal gene transfer (HGT) and recombination. One commentator⁸² agreed with our assessment, and one⁸³ stated that we had misleadingly implied that there was an increased opportunity for bacterial DNA present in a GM crop to transfer to gut bacteria *per se*.

The Panel’s response

We accept that the molecular configuration of transgenic DNA can be different from other DNA. The short statement on page 90 of our First Report would have been more accurate if it had read ‘Transgenic DNA is no different from other DNA in its behavior when consumed as

⁷⁸ ISIS. See Annex I.

⁷⁹ ISP. See Annex I.

⁸⁰ D Gledhill. See Annex I.

⁸¹ Green Party. See Annex I.

⁸² ABC. See Annex I.

⁸³ Syngenta. See Annex I.

part of the normal diet and it will have a similar fate'. We still support the conclusion in our First Report that there is no evidence that transgenic DNA *per se* behaves differently from any other DNA with respect to its fate following consumption in food, whilst also recognizing the need for more research in areas such as gene transfer in the gastro-intestinal tract (see below).

The evidence for horizontal gene transfer in the GI-tract

Main points made by commentators

It was stated by one group of commentators that there had been insufficient research in this area (i.e. little was known about HGT within the GI-tract of humans and animals) and that we had downplayed the risks and uncertainties in assessing the present state of knowledge⁸⁴. It was stated by several commentators that more research was needed on the variation in the behaviour of the human GI-tract between individuals^{84 85 86} (e.g. susceptibility to gut disorders and those with an unhealthy GI-tract).

It was stated by two commentators^{85 87} that, despite limited research (Breibart *et al.* 2003; Toivanen *et al.* 2001) there was positive evidence for HGT in food and in the GI-tract that we had ignored or dismissed (Netherwood, *et al.* 2002; Bauer *et al.* 1999). Some commentators^{85 87 88 89} placed great importance on evidence for the survival of transgenic DNA in the GI-tract or the body, in some cases even equating this to evidence of HGT (Chowdhury *et al.* 2003).

The Panel's response

Our First Report included a detailed review of gene transfer potential in the human or animal GI tract and in the environment. We were clear that a series of studies demonstrated that consumed DNA is degraded during passage through the GI tract but that the process is incomplete, especially in the more proximal regions. The retention of biological activity and potential to transform bacteria was also discussed. Experimental studies of gene transfer from GM plants to naturally transformable bacteria clearly identified marker rescue as the most likely molecular mechanism. This prompted a conclusion that in GM plants, the restriction of homology to bacterial DNA was to be recommended, making it desirable to minimise bacterial sequences within the transgenic DNA. The existence of bacterial transformation in the environment was highlighted, as were the limitations to gene transfer imposed by the molecular aspects of transformation processes as we understand them.

The study of human ileostomists by Netherwood, *et al.* (2002) was discussed in some detail in our First Report (pages 92 and 96). This investigation used a PCR amplification approach to follow the fate of transgenic DNA after the consumption of meals based on GM soya. Transgenic DNA was detected in samples taken from the gastro-intestinal tract indicating that degradation was incomplete. An *in vitro* bacterial culture of this material was established and a positive PCR result was observed through several sub-cultures suggesting that the DNA target was being replicated. This has been taken as evidence for gene transfer taking place in

⁸⁴ Combined comment from: Econexus, The Five Year Freeze, Friends of the Earth, GeneWatch UK, Greenpeace, The Soil Association & Dr M Antoniou. See Annex I.

⁸⁵ R Patterson. See Annex I.

⁸⁶ J Clarke. See Annex I.

⁸⁷ ISIS. See Annex I.

⁸⁸ R Scullion. See Annex I.

⁸⁹ Munlochy Vigil. See Annex I.

the human gastro-intestinal tract. Our First Report recognised that this result was unexpected and warranted further investigation. However, it was pointed out that this observation was not a rigorous demonstration of gene transfer and might be explained by other interpretations (First Report, page 96). The existence of gastro-intestinal tract microbes that cannot be cultured is well known but in this case the positive PCR data have been interpreted to suggest that *in vitro* culture of a transformed bacterial species was achieved. However, the fact that a pure culture was not obtained and subjected to molecular characterisation remains significant.

As we mentioned on pages 98 and 99 of our First Report there is a need for more research into gene transfer in the gastro-intestinal tract, both *in vivo* and via appropriate models. More human studies would be valuable, although there are practical difficulties. Follow-up of the Netherwood study is desirable, to replicate and explain the positive PCR data and hence clarify their significance.

The significance of ‘recombination hotspots’

Main points made by commentators

Two commentators^{90 91} stated that ‘many GM DNAs possess ‘recombination hotspots’ making them extra-unstable, and hence more prone to horizontal gene transfer and recombination, with all the attendant risks’ and that we had suppressed this evidence. The CaMV 35S promoter (Ca MV p35S) was regarded as the prime offender by these, and one other group of, commentators⁹².

The Panel’s response

Evidence for the alleged existence of a recombination hotspot in the cauliflower mosaic virus CaMV 35S promoter was considered quite fully in our First Report (Section 5.2.3, page 70 and Section 7.5.3, pp. 244-5). We concluded that there was no biologically plausible mechanism or evidence for the consumption of the CaMV p35S leading to any adverse health effect. Small regions of DNA homology are likely to be found in many different DNA sequences and they may provide a mechanism for rare integration events.

Other components of GM DNA were cited as recombination hot spots but in these cases other phenomena that may or may not be relevant to enhanced gene transfer are involved. With respect to the borders of integrating plasmids, the mechanism of *Agrobacterium* T-DNA integration is controlled by genetic components of the bacterial vector system and these do not remain in the GM plant. Restriction endonuclease sites present on DNA polylinkers are dependent on specific restriction endonucleases for functionality, which are unlikely to be present in a GM plant. Also, these sequences are very widely distributed throughout all DNA. The presence of a bacterial plasmid origin of replication would provide a means of DNA maintenance should DNA be taken up by bacterial species able to support it. Plasmid replicons have restricted host ranges and this specificity limits impact on gene transfer potential. Importantly, our understanding of molecular mechanisms in bacterial transformation and experimental data on plant to bacterium gene transfer suggest that marker rescue is the most feasible mechanism and this restricts the relevance of plasmid replicons. Origins of transfer are bacterium-specific and would only be of relevance subsequent to a

⁹⁰ ISIS. See Annex I.

⁹¹ ISP. See Annex I.

⁹² Combined comment from: Econexus, The Five Year Freeze, Friends of the Earth, GeneWatch UK, Greenpeace, The Soil Association & Dr M Antoniou. See Annex I.

primary plant to bacterium gene transfer event. Whilst making these points it is important to reiterate a key conclusion from our First Report that gene transfer potential would be reduced by the minimisation of bacterial DNA sequences that are integrated into GM plants. Many of the genes introduced into GM plants are based on bacterial gene sequences, but are synthesised *de novo* in the laboratory to include more appropriate codon usage for more efficient expression in plants (e.g. the *Bt* toxin). This reduces the likelihood that they would be expressed if reintroduced into bacteria.

The transcription activity of CaMV p35S is considered in relation to the recent paper by Vlasák *et al.* 2003 in Section 2.2.14.

Broader horizontal gene transfer issues

Main points made by commentators

One group of commentators⁹³ stated that our First Report had inadequately addressed: differences in the GI-tract between species; the use of transgenes from higher organisms that can be transferred to microorganisms; and HGT in relation to yeasts and other non-bacterial microorganisms in the GI-tract.

The Panel's response

In general, commentators seemed satisfied with the scope of our Review. As we indicated above, in our response on the evidence for horizontal gene transfer in the GI-tract, more research is needed into gene transfer in the gastro-intestinal tract. This is true of these other areas mentioned by commentators.

Antibiotic resistance markers

Main points made by commentators

Several commentators^{93 94 95 96} stated that we had inadequately addressed the issue of the use of antibiotic resistance markers in relation to horizontal gene transfer.

The Panel's response

Our First Report identified concerns that an antibiotic resistant gene present in a GM plant might be transferred to bacteria potentially leading to the development of antibiotic resistant pathogens. This issue was discussed both with respect to the likelihood of a trans-kingdom plant to bacterium gene transfer event taking place in the first place, and with respect to the consequences should such an event occur. Our First Report highlights scientific arguments and data that suggest a postulated gene transfer event would be of very low likelihood and valid arguments that any subsequent impact would be minimal. Marker rescue driven by DNA homology between bacteria and GM plants has been demonstrated experimentally and this led to the comment that minimisation of bacterial DNA sequences in GM plants was desirable. Clearly, antibiotic resistance genes fall into this category and a clear distinction was made between the use of the *nptII* gene to select plant transformation and the unnecessary inclusion

⁹³ Combined comment from: Econexus, The Five Year Freeze, Friends of the Earth, GeneWatch UK, Greenpeace, The Soil Association & Dr M Antoniou. See Annex I.

⁹⁴ ISIP. See Annex I.

⁹⁵ ISP. See Annex I.

⁹⁶ D Gledhill. See Annex I.

of other genes. The point was made that alternative selection processes and marker removal systems exist, but their safety also needs to be established.

4.3.4 The effect of GM derived feed in the food chain

Summary conclusions from First Report

‘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, conventional 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.’

Main points made by commentators

Commentators did not raise any significant issues that exclusively fell into this area (which we addressed in Section 5.5 of our First Report). Issues with some relevance to GM derived feed are considered in Sections 4.3.1 to 4.3.3.

4.4 CHAPTER 6: ENVIRONMENTAL IMPACTS OF GM CROPS

4.4.1 Invasiveness/persistence of GM plants

Summary conclusions from First Report

‘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.’

Main points made by commentators

Some commentators^{97 98} noted the prominence given to the PROSAMO experiment in this chapter, and drew attention to what they considered its limitations. These included the comment that invasiveness of undisturbed habitats was only studied when the crops concerned might be invasive of disturbed habitats, and that only a limited number of GM types were tested.

Some commentators felt that invasions of arable fields are an important nature conservation issue. Also more prominence should have been given to the invasive potential of crop/wild plant hybrids as discussed in the work of Snow & Pilson (2003).

Other commentators⁹⁸ believed that generalisations about invasiveness based on specific examples were not valid and pointed to the work on viruses by Cooper *et al.* (2001) and Quemada (1990). The relevance of various models was questioned⁹⁷. Furthermore, they questioned the data that invasiveness would be determined by the additional trait(s) and not some unanticipated or pleiotropic effect as mentioned in this chapter. It was pointed out that small genetic changes could have large effects (Williamson 1993) and that experiments testing all eventualities are difficult to design (Parker & Kareiva 1996). Equally, long-term evolutionary processes were not considered. The comment that sterility traits might be used to prevent invasions discounted the fact that some invasive plants are vegetatively propagated.

The Panel’s response

The PROSAMO experiment (Crawley *et al.* 2001) remains the only study comparing the invasiveness of GM plants and controls, and was designed to test whether the transgenic

⁹⁷ Combined comments from: Econexus, The Five Year Freeze, Friends of the Earth, GeneWatch UK, Greenpeace, The Soil Association, and Dr Antoniou. See Annex I.

⁹⁸ British Statutory Nature Conservation Agencies: Countryside Council for Wales, English Nature, Joint Nature Conservation Committee and Scottish Natural Heritage. See Annex I.

process might produce plants that could be more invasive of natural habitats than their conventional counterparts. It included a perennial plant (potato) and all replicates included an experimental disturbance treatment. It showed that the transgenic plants were not more invasive or more persistent than their conventional counterparts under conditions where the transgene was selectively neutral; there was no herbicide treatment so the HT genes provided no known advantage to the GM plants. Thus the experiment provides important data about the starting point for assessment of other GM plants, because it demonstrates that transgenesis does not *per se* make plants more invasive. For complete risk assessment, constructs and genes used for specific transformations must be assessed for invasiveness on a case-by-case basis.

Invasions in arable fields can be of both agronomic and nature conservation importance in the UK and are referred to in our First Report regarding the potential problems of increased herbicide resistance in arable weeds (Chapter 6.4, page 137). The subject of crop/wild plant hybrids was discussed in Chapter 7 on Gene Flow and their potential for invading arable and natural habitats recognized as one of the possible consequences of gene flow.

Snow *et al.* (2003) demonstrate increased fecundity in hybrids from wild sunflowers crossed with *Bt* sunflower, pointing out the potential for increased fitness because of the transgene. The study is not designed as a complete population study of the kind appropriate for an assessment of the risk of invasion and is considerably different in aims to the PROSAMO experiments. Seed production was greater in plants protected by the transgene from insect herbivores, with a significant increase of 55% noted in one locality. The study shows an effect on fecundity but does not demonstrate that recruitment is seed-limited, which would relate the results to fitness and potential for invasiveness. 55% more seeds may not mean 55% more plants in the next generation and field studies, which would be desirable, need to last more than one whole generation to address questions of fitness and (trans-) gene ecology.

Our First Report supports the idea of case-by-case assessment and points out that generic risk assessment or approval of all GM crops is inappropriate; the evidence discussed above shows the starting point for case-by-case assessment based on our understanding of population biology and genetics (and underpinned by the paradigms of evolutionary biology).

There are minimal data or models for long-term evolutionary processes associated with transgenic crops, so these were not considered in detail and we acknowledge that such uncertainties should be tackled by a programme of monitoring, as is starting to be done with conventional agriculture. The limitations of monitoring were discussed.

In our First Report we discuss how genetic isolation methods (including sterility) can reduce gene flow (page 207), and potential for transfer of sterility genes from GM crops into wild populations (page 222). Sterile GM plants, like vegetatively propagated alien species, could become invasive and this potential should be considered as part of the case-by-case assessment.

4.4.2 Toxicity to wildlife

Summary conclusions from First Report

‘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.’

Main points made by commentators

Criticisms⁹⁹ included that the section did not explore some of limitations on assessing the specificity of toxins, given that testing is often on a restricted range of species; the lack of mention that the ecological role of *Bt* is unknown, which increases uncertainties; the section mentioned, but did not review, the evidence for changes in some non-target pests with Joubert *et al.* (2001) on cotton quoted. Another commentator¹⁰⁰ noted that 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. Also studies of the impacts on vertebrates (especially birds known to eat crops) of commonly-used GM-derived endotoxins are lacking in the scientific literature.

One commentator¹⁰¹ felt that it is necessary to compare the potential effects on wildlife of *Bt* crops, limited to a few insects and only those ingesting the crop, with current methods of reducing crop loss using broader spectrum insecticides applied over the top of the crop.

One commentator¹⁰² noted that this section refers to two pests not currently a problem in the UK, but that with climate change that situation could change.

Other commentators mentioned concerns about soil. One¹⁰³ quoted our conclusion on page 132 that ‘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’ but suggested that may be because the needed research in this area has not been done. On page 135 we stated that ‘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’. One¹⁰² of our commentators agreed that this was among the most striking gaps in knowledge that show little sign of being filled.

Other commentators¹⁰² highlighted two issues: reductions in insecticide use; and impacts on non-target fauna. In addition, the commentator was critical of the scientific rigour and quality of some of the studies cited in this section of our First Report. They agreed that one paper (Phipps & Park, 2002) clearly established reduced insecticide use in countries where GM crops had been planted. They pointed out that other studies cited in our First Report (such as: Carpenter *et al.* 2002, Carriere *et al.* 2003)¹⁰⁴ did not present rigorous scientific data on reductions of insecticides associated with GM insect resistant crops. They concluded that although Phipps & Parks present data supporting the contention that insecticide use is reduced in areas where GMIR crops are used, there are no studies that directly relate such reductions to the large-scale use of these crops.

⁹⁹ Combined comments from: Econexus, The Five Year Freeze, Friends of the Earth, GeneWatch UK, Greenpeace, The Soil Association, and Dr M Antoniou. See Annex I.

¹⁰⁰ Green Party. See Annex I.

¹⁰¹ Agriculture and Biotechnology Council. See Annex I.

¹⁰² British Trust for Ornithology. See Annex I.

¹⁰³ D Gledhill. See Annex I.

¹⁰⁴ Also: ‘Plant biotechnology: Current and potential impact for improving pest management in US agriculture: an analysis of 40 case studies.’ Gianessi *et al.* (2002) <http://www.ncfap.org/40CaseStudies.htm>.

The Panel's response

Risk assessment is a pragmatic process which may not provide all the answers, but tries to focus on the most important parameters. Although it may not be feasible to perform laboratory tests on all species that could potentially be harmed, appropriately designed field studies may enable monitoring of a wider range of species and may also be an important element of risk assessments. Commentators' criticisms about testing of inappropriate species mostly relate to risk assessments on *Bt* plants that were done over ten years ago for commercial release in the USA, whereas more recent risk assessments have increasingly focused on identifying ecologically relevant species in the field and testing them in the lab, e.g. Hilbeck (2002). We recognize that particular scrutiny should be paid to applications for commercial release to determine whether ecologically relevant species have been tested.

Lack of knowledge on the ecological role of *Bt* was mentioned in our First Report (page 121).

The commentator referred to the work of Joubert *et al.* (2001). However, this paper is unrefereed.

We agree that studies of the impacts on vertebrates (especially birds known to eat crops) of commonly-used GM-derived endotoxins are lacking in the scientific literature. However risk assessments are carried out using the laboratory evidence-based assumption that the *Bt* toxin is not harmful to vertebrates. Therefore it is not generally expected in risk assessments on individual releases that extensive data is needed to demonstrate lack of adverse effects on populations of vertebrates in the field.

The majority of field studies that have been carried out at research stations on ecological effects of *Bt* compare *Bt* crops with conventional pest control methods. One problem is that on farms, additional pesticides are often used on the GM crop to deal with non-target pests. Following the success of the FSE experimental protocol, similar studies could be carried out on *Bt* crops to compare their impacts with conventional pesticide treatment in realistic commercial situations.

So far as the impact of climate change on pest distribution is concerned, there would be little point in trying to carry out experiments that do not reflect current agronomic practice. If and when these pests become a problem in the UK, and *Bt* crops are proposed for commercial growing as a solution, comparative studies could be informative.

We agreed in our First Report that more field research on the impacts of pest- and disease-resistant GM crops on soil micro-organisms and processes should be carried out. However experiments would have to be crop specific and apart from *Bt* maize, no other GM pest or disease resistant crops are even close to commercialisation in the UK. However, we also have to be realistic about agricultural soils, which in general are already highly modified by conventional cultivation, e.g. ploughing, fertilizer and pesticide applications, compression, erosion, etc. Evidence so far from *Bt* crops suggests that impacts of individual crop varieties are likely to be far less than differences between agricultural practices.

Additionally, it might be useful in future to gather evidence for changes in pesticide inputs following introduction of GM insect resistant crops. Key questions could include: Are effects different for different crops? What are the longer-term trends? What is the relationship between inputs and impacts?

We agree with the contributor that the data presented in Phipps & Park (2002) support the view that commercial use of such crops is correlated with reductions in insecticide use.

The contributor's fifth point deals with evidence of impacts of GMIR crops on non-target organisms. They state 'there is actually no evidence of ...positive benefits in any peer-reviewed publications that we have been able to find'. They cite: Carpenter *et al.* 2002; Pimental & Raven, 2000; Musser & Shelton, 2003; and Gregory *et al.* 2002, which were papers reviewed in Section 6.6.4 of our First Report, and conclude that none of this literature presents convincing data supporting the hypothesis that the use of GMIR crops has neutral or positive effects on non-target fauna.

We agree with the conclusion for some of the studies cited, but the Carpenter *et al.* paper does cite some lab-based studies where no adverse effects were found, and whilst Musser & Shelton did not find a significant reduction in natural enemies of lepidopteran pests in fields of *Bt* sweetcorn, they found that using conventional insecticides had an adverse impact on these organisms. However the extensive work done on impacts of GMIR corn (maize) on non-target lepidoptera in the United States cited in Section 6.3.3 of our First Report (Sears *et al.* 2001), lends support to the view that, when compared to conventional insecticide use, GMIR corn has little if any impact on populations of non-target insects and may be beneficial. Section 6.3 of our First Report contains an extensive and more rigorous review of this issue than the section cited by the contributor.

We agree with the contributor that there is a need for more formal experimental science to investigate the impact of specific GMIR crops on biodiversity. We say in Section 6.3.6 (page 134) of our First Report that there is a need for more agronomically realistic ecological studies of this kind. We would emphasise that such experiments should be at field scale and must always compare the GMIR system with other ways of controlling insects, especially the use of insecticide sprays.

4.4.3 Development of resistance

Summary conclusions from First Report

'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.'

Main points made by commentators

Some commentators¹⁰⁵ felt that the section did not give enough consideration to the fact that GM uniquely enables the same gene construct to be used in a range of crops across the world, which in their view raises new areas of uncertainty in terms of emergence of resistance and could make it more problematic. For instance, one commentator¹⁰⁶ was concerned about the potential of moths in the U.S. to hinder efforts to stem development of resistance because they fed on corn all summer and flew south to eat cotton, citing Gould *et al.* (2002) in support of this view. One commentator¹⁰⁷ drew attention to new research by Sayyed *et al.* (2003) suggesting that some resistant insects might be able to use the toxins in GM plants as a supplementary food protein, which was an unexpected effect. Another commentator¹⁰⁸ contended that Glyphosate-resistant marestail has already infested over 200,000 acres of cotton in the U.S.A. and cited by Ho & Cummins (2002) as the source of this and other examples of resistant weeds.

One commentator¹⁰⁹ felt that any change in management that may increase the rate of appearance of weeds resistant to glyphosate is a concern, and wanted the Panel to consider a number of further questions. These included: whether characteristics of the use of glyphosate with GMHT crops could exacerbate the emergence of resistant weeds; what responses to resistance could be expected from UK farmers, and what would be the environmental consequences of these responses; in relation to perennial weeds, could reduced application rates and sub-optimal timing increase the potential for resistance in perennial weeds; and what would be the implications of GMHT cereals?

¹⁰⁵ Combined comments from: Econexus, The Five Year Freeze, Friends of the Earth, GeneWatch UK, Greenpeace, The Soil Association, and Dr M Antoniou. See Annex I.

¹⁰⁶ R Patterson. See Annex I.

¹⁰⁷ Munlochy Vigil. See Annex I.

¹⁰⁸ G Humphreys. See Annex I.

¹⁰⁹ E Cross. See Annex I.

Some commentators¹⁰⁵ stated that one of the main challenges with resistance management is whether farmers will be able, or willing, to comply with the systems put in place, and they felt that we had not fully consider evidence from the US that up to 33% of farmers did not comply, quoting a New Scientist report (Coghlan 2001)¹¹⁰.

One commentator¹¹¹ was concerned that ‘gene-stacking’ discussed on page 143 of our First Report was said not to be a problem because of the multiplicity of available herbicides But it failed to mention that this may imply the use of older, more toxic, herbicides although it did refer to English Nature’s concern about the impact of paraquat and diquat on hares. Another commentator¹¹² said that incidents of such harm are rare and quoted Edwards *et al.* (2000) in support of this view.

The Panel’s response

We believe that on this issue, as for other areas of our Review, the primary focus should be on peer-reviewed research.

Herbicides, insecticides and fungicides, are used in a range of crops and so the development of resistance is not new. If a herbicide is successful, it will be applied to all crops. Therefore, we feel that this is not a new issue.

The reference to an example of marestail invading cotton fields is misplaced, since the cotton in question is conventional, and not GM, cotton.

We agree that one of the main challenges with resistance management is whether farmers will be willing to comply with the systems put in place. However this is true for all regulatory frameworks and systems.

4.4.4 New weed control strategies offered by GM herbicide tolerant crops

Summary conclusions from First Report

‘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

¹¹⁰ Almost a third of US farmers broke rules for planting GM maize last year. *New Scientist*, 5 February 2001.

¹¹¹ J Bond. See Annex I.

¹¹² Syngenta. See Annex I.

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 GMHT 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.’

Main points made by commentators

Some commentators¹¹³ felt that this is a comprehensive and detailed account of our state of knowledge.

One commentator¹¹⁴ contended that the report cited by Chamberlain *et al.* (2001, not 2002) did not show that the FSEs are too small to assess the impacts on birds, and given its conclusions, it was strange that the work on birds was not continued for the full three years of the FSEs.

One commentator¹¹⁵ questioned the use of the reference to Dewar *et al.* (2000), and did not think it provided evidence for suggesting that GMHT crops could reduce aphicide use – the opposite could also be true.

Another commentator¹¹⁶ observed that there is an impact of pesticides on soil and its micro-organisms, both of which are diminishing in quantity and quality across the world, and our First Report had not adequately evaluated the possible harmful effects of the accumulated use of glyphosate or glufosinate against this background.

One commentator¹¹⁷ drew attention to new research (Defra, 2003d) on modelling the effects on farmland food webs of herbicide and insecticide management in the agricultural ecosystem October 2003 and the need to consider this in the Review.

¹¹³ Combined comments from: Econexus, The Five Year Freeze, Friends of the Earth, GeneWatch UK, Greenpeace, The Soil Association, and Dr M Antoniou. See Annex I.

¹¹⁴ British Trust for Ornithology. See Annex I.

¹¹⁵ E Cross. See Annex I.

¹¹⁶ Bristol Area SERA Group. See Annex I.

¹¹⁷ Munlochy Vigil. See Annex I.

The Panel's response

The comparison we made in our first report was with conventional agriculture and thus we compared the impacts of the use of glyphosate and glufosinate against the alternatives.

The paper by Dewar *et al.* (2003) explored short-term consequences of introducing GMHT crops on farmland biodiversity by examining the possibility that spraying can be delayed in GMHT crops because the weed control is easier and possible even for large weeds. This showed that delayed spraying leads to increased weed biomass, which then leads to higher arthropod densities. This effect is increased if this is coupled with initial band spraying. Freckleton *et al.* (2003) review weed phenologies and create a population model which shows that many weeds are unlikely to benefit in the long run as spraying is generally delayed insufficiently to allow most species to set seed. The effects shown in Dewar's experiments are thus likely to be short term. Freckleton *et al.* (2003) do however show that if spraying is stopped earlier in the season then a viable population of large emerging weed species can persist.

The Defra report was published after our First Report, but is described in Section 2.

4.4.5 Horizon scanning

Summary conclusions from First Report

'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.'

Main points made by commentators

Some commentators¹¹⁸ felt that, perhaps inevitably, the section was superficial and of questionable value to the review. The account of prospects for biofuels was given as an example of an area that had not been sufficiently explored, and Murphy (2002) cited as providing a more critical evaluation.

Some commentators¹¹⁸ also noted that despite our First Report emphasising the need to make comparative evaluations with other non-GM options, this section failed to do so. Similarly, one commentator¹¹⁹ felt that the report focused on the perceived risks associated with GM crops and foods but did not address the risks associated with not fully exploring or evaluating the opportunities to use GM crops.

Another commentator¹²⁰ asked whether the Panel had any evidence as to whether the unexpected effects of GM traits mentioned in the 'Limitations on Science' section (Saxena & Stotzky 2001 on lignin in *Bt* corn, soya bean stem splitting) might limit the practical application of any of the traits mentioned in the horizon scanning section, particularly where multiple traits were involved. Do the risks of unforeseen effects on a plant increase as the genetic modifications make it more numerous or complicated, and will we have to make choices between traits because plants will only be able to cope with so much change? If so, these factors should be fed into horizon-scanning exercises. The same commentator queried whether unexpected effects such as raised lignin levels could have an effect on food chains.

The Panel's response

Comparative evaluations with other non-GM options were not made, as we are unaware of any specific, peer-reviewed research on this issue. Nor did the report address the risks associated with not fully exploring or evaluating the opportunities to use GM crops as this was outside the scope of the Science Review. Readers are encouraged to read the Strategy Unit publication 'Field Work: weighing up the costs and benefits of GM crops (July 2003)'. Our First Report covers the paper by Saxena and Stotzky (2001) in detail on page 187.

4.4.6 Changes in agricultural practice

Summary conclusions from First Report

'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

¹¹⁸ Combined comments from: Econexus, The Five Year Freeze, Friends of the Earth, GeneWatch UK, Greenpeace, The Soil Association, and Dr M Antoniou. See Annex I.

¹¹⁹ John Innes Centre. See Annex I.

¹²⁰ E Cross. See Annex I.

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.’

Main points made by commentators

Some commentators¹²¹ felt that scenarios in this section were entirely based on the technology and failed to consider other changes or alternatives. They also felt that the analysis in this section was weak and partial, including the assessment of whether GM would reduce biodiversity, whether GM would increase crop rotations, and whether GM would bring economic benefits. They contended that *Bt* corn in Spain has not been universally successful, quoting a report by FOE and Greenpeace¹²². They also argued that GM would bring new problems to farmers including dealing with resistances, meeting traceability and labelling requirements, and that choice for the farmer would be undermined by GM crops. These points were not, in their view, adequately addressed in the section.

Another commentator¹²³ felt that the report does not make clear enough that the major environmental impacts of any new or existing crop are determined by how it is managed. It was suggested that what is required is a blueprint for farming that sets out society’s requirements for food, industrial raw materials, landscape and water management, and environmental goods. Technologies and management systems can then be applied to meet those objectives. In general, the report gave insufficient prominence to the role of politics and economics in driving and controlling the direction of farming and food production.

Another commentator¹²⁴ felt that this was a reasonable attempt to address a difficult subject, but should have focused far more on those GM crops close to consent decisions rather than indulging in much ‘look-forward’ speculation, an area where science is of little help. The same commentator was surprised that the general conclusion of this section was that the introduction of GM crops would be likely to result in more diverse agricultural landscapes when there is no evidence to support this. The section did not fully explore what is already known of the changes in agricultural practice associated with GMHT and GMIR crops, including work by Read & Bush (1998) and Read & Ball (1999).

¹²¹ Combined comments from: Econexus, The Five Year Freeze, Friends of the Earth, GeneWatch UK, Greenpeace, The Soil Association, and Dr M Antoniou. See Annex I.

¹²² The Impact of GM corn in Spain. Friends of the Earth & Greenpeace, August 2003.

¹²³ John Innes Centre. See Annex I.

¹²⁴ British Statutory Nature Conservation Agencies: Countryside Council for Wales, English Nature, Joint Nature Conservation Committee and Scottish Natural Heritage. See Annex I.

The Panel's response

Our First Report set out a range of potential scenarios for changes in agricultural practices following the adoption of GM crops. This stated that some GM crops could result in increased biological diversity in the landscape, and some could lead to greater homogenisation. The range of possibilities for both short- and long-term changes are set out in more detail in Pretty (2001).

We acknowledge that politics and economics are critical drivers for the direction of food and farming systems. There is likely to be significant change following the mid-term review of the Common Agricultural Policy in 2003 that will shift payments from food production subsidies to optimising the supply of environmental goods and services. This will not lead to a new 'blueprint' for farming, but will raise new questions about what society at large wants from multifunctional agricultural systems using a wide range of production technologies.

The report by Brookes (2002) provides new data on *Bt* maize cultivation across Spain. This indicates that the FOE and Greenpeace study focused on maize in a small-scale trial only in regions where there was low pest pressure. Elsewhere in the country, *Bt* maize had been cultivated by farmers with agronomic and economic success to date compared with conventional varieties. The question of the development of resistance is dealt with in Section 4.4.3.

4.4.7 Limitations of science

Summary conclusions from First Report

'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.'

Main points made by commentators

Some commentators¹²⁵ felt that the section made excessive claims for the FSEs, particularly in the summary, which did not reflect other important areas of uncertainty discussed in the text. They did not agree that indirect effects of GMHT crops posed the only significant risk, which was the impression given by this section.

¹²⁵ Combined comments from: Econexus, The Five Year Freeze, Friends of the Earth, GeneWatch UK, Greenpeace, The Soil Association, and Dr M Antoniou. See Annex I.

The same commentators were also looking for a discussion of overarching issues, which in their view included: the practical constraints of experimental design (including financial); the problems of extrapolating from small to large and commercial scale; the difficulties of detecting small incremental changes which become significant only over long time periods; lack of knowledge of complex ecological interactions; and different types of error and constraints on modelling. They also felt that the limitations of the case by case approach were not sufficiently evaluated, which included the exclusion of comparative assessment of different options; that cumulative impacts are not adequately dealt with, and an excessive emphasis on the introduced trait which diverts attention from pleiotropic impacts.

Another commentator¹²⁶ agreed with our First Report that the regulatory processes must consider each GM crop as an individual case, but emphasized the need for this process to embrace the principle of risk management. The same commentator supported the view that large scale studies are needed to help determine the impacts of GM crops on wildlife, non-target organisms, soil and below-ground processes, and contended that a nationally co-ordinated, properly designed and adequately funded monitoring scheme is required to detect future changes in biodiversity.

The Panel's response

The FSEs, which were not published at the time of our First Report, are considered in Section 3. They aimed to look at 'indirect' effects of GMHT crops, but as indicated by the scale required to detect significant effects and as suggested by the commentator, such effects are difficult to find unless large and quick. As in the FSEs, such analysis is often required in a comparative context because of the continuing changes imposed by conventional and alternative agricultural strategies. Data and methods to measure direct effects are discussed in Chapter 6.3 and elsewhere in our First Report. Our review checklist (page 39, question 5) specifically considered options for monitoring of GM crops (as well as food/feed) after release. Current regulatory oversight requires applicants to consider direct, indirect, immediate and delayed effects. The difficulties in measuring fitness are discussed in Section 4.4.1.

4.4.8 Monitoring

Main points made by commentators

One commentator¹²⁷ felt that our First Report did not place enough weight on the value of monitoring and may exaggerate the difficulties. This commentator felt that there has been little direct attempt to monitor the impact of individual agronomic practices until now, and even expensive schemes designed to provide environmental benefits have been poorly evaluated, citing Kleijn & Sutherland (in press) in support of this view. The authors of this paper argue powerfully that evaluation of the effectiveness of such schemes should become an integral part of their introduction and the same applies to the monitoring of other major agricultural developments.

¹²⁶ Institute of Biology on behalf of the Biosciences Federation. See Annex I.

¹²⁷ British Trust for Ornithology. See Annex I.

The same commentator believed that the Panel should look more critically at the statistical underpinning of reports of ‘no effect’. This was echoed by a number of other commentators who questioned the use of anecdotal alongside data-derived evidence.

The Panel’s response

The value of monitoring was highlighted in the Report (page 135). ACRE has recently published guidance on monitoring which should help in the development of effective monitoring strategies¹²⁸.

We stand by our original conclusion that it will be very difficult to pick up the changes due to the introduction of GM crops alongside the other considerable changes that are likely to occur within UK agriculture in the near future as a result of economic, social, climatic and technological changes. General monitoring of biodiversity is clearly important. It was such monitoring established by the British Trust for Ornithology that resulted in the detection of the direct effects of organophosphate pesticides on birds and later detected the changes in farmland birds.

On the issue of statistical underpinning, we believe that an ‘estimation approach’ is preferable to hypothesis testing. In particular, we concur with the FSE statisticians and the contributor, that it is preferable to produce confidence intervals for parameters of interest, rather than simply carrying out a hypothesis test of ‘no effect’. Power analysis is helpful in designing an experiment, but a confidence interval, as the contributor points out, explicitly gives the range of effects consistent with the data observed. The published analysis of the FSE experiments is an excellent piece of statistical work.

In contexts such as the FSEs, it is entirely appropriate to use a design, which achieves power of 80% at a given level of an effect. Power of 80% for an effect of magnitude 1.5 means that if the true effect is 1.5 then the chance of rejecting the null hypothesis of ‘no effect’ is 0.8. It does not mean that an effect of magnitude 1.5 is ruled out if the null hypothesis is not rejected. The question ‘will an effect of size 1.5 normally be detected?’ is different from ‘if the null hypothesis of no effect is accepted, could the true effect be as large as 1.5?’

Detailed publication of data always makes it possible to analyse the data in a different way. While Bayesian methods are undoubtedly increasing in prevalence in many areas, their use in the biological literature is not yet standard, and for contexts such as the FSE's, it is completely appropriate to use the classical approach adopted.

¹²⁸ Available at: www.defra.gov.uk/environment/acre/advice/advice21.htm

4.5 CHAPTER 7: GENE FLOW, DETECTION AND IMPACT OF GM CROPS

4.5.1 Gene flow between crop varieties

Summary conclusions from First Report

‘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 GM 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.’

Main points made by commentators

One group of commentators¹²⁹ felt that the Review was over-optimistic about the prospects for limiting crop-to-crop gene flow. One commentator¹³⁰ provided a detailed critique of the

¹²⁹ ISP. See Annex I.

¹³⁰ Scientists for Global Responsibility. See Annex I.

separation distances suggested by the Ingram (2000) report. Other commentators^{131 132} felt that coexistence was achievable.

Some commentators¹³³ felt that although the review recognized the inevitability of gene flow, and acknowledged important uncertainties in predicting gene flow between crops, it had not placed these issues in the context of public concerns, i.e. the extent to which, if at all, consumers will have a choice of GM free, UK produced food and whether UK organic farmers will be able to produce uncontaminated crops. They felt that the analysis and comment in this section should therefore have been directed at considering gene flow to non-GM crops at zero or limit of detection levels (0.1%) instead of 1%, and should have given fuller consideration to market and farming realities, including problems already experienced in Canada with HT OSR, and in Spain with *Bt* Maize. They also felt that the effects of multiplication over generations, increases in the area of GM crops, and feral populations were not properly addressed.

Some commentators¹³³ also felt that gene flow had significance beyond co-existence. They argued that the review should be amended to acknowledge that gene flow *per se* is important and to give proper consideration to the Mexican maize contamination incident (because of the need to protect agricultural resources) and the StarLink™ maize contamination incident (because of the possible implications for food safety). Another commentator¹²⁹ underlined the importance of the Quist & Chapela (2001) paper on the Mexican maize situation.

One commentator suggested several new references¹³⁴.

The Panel's response

In our First Report we reviewed the available evidence relating to gene flow. We came to the conclusion that there are uncertainties about how the factors determining co-existence in the UK will come together at commercial scale; including the combination of adventitious presence in seed, gene flow between crops in the field, and gene flow from volunteers of previous crops.

We acknowledge that the practicality for co-existence is dependent not only on the biology of adventitious presence, but also on the UK farming context. There is science available to at least guide, if not completely guarantee, the maintenance of thresholds. However, what is not known is how many farmers will want to adopt GM, how many will want to work to the organic movement's self-imposed *de facto* threshold of 0.1% (and it is possible that some retailers and conventional farmers will want to work to 0.1%), and how many will want to work to the statutory threshold of 0.9%. These scenarios were not examined in detail in our First Report, but it did state that ultimately co-existence would be determined by political and economic issues including legal thresholds. The Science Review did not look in detail at the issues of choice arising from co-existence. Many of these issues are covered by the AEBC's report.¹³⁵

¹³¹ Syngenta. See Annex I.

¹³² Agriculture and Biotechnology Council. See Annex I.

¹³³ Combined comment from: Econexus, The Five Year Freeze, Friends of the Earth, GeneWatch UK, Greenpeace, The Soil Association and Dr M Antoniou. See Annex 1.

¹³⁴ Munlochy Vigil. See Annex I.

¹³⁵ AEBC report: 'GM Crops? Co-existence and Liability'. November 2003.

As one commentator correctly pointed out, the growing of maize as sweetcorn for ‘pick-your-own’ or corn on the cob, presents a special case. If grown near to GM forage maize (or GM sweetcorn), the cobs on the perimeter of the field (and possibly in pockets within the field) could have higher levels of kernels pollinated by a GM male plant than is the average across the field, and there is no post-harvest means of averaging out the effect. This problem has been the subject of advice from ACRE¹³⁶.

The paper by Quist & Chapela (2001) remains controversial and unique in its treatment by Nature, but did not show introgression as such. Some kernels on different cobs of a landrace gave weak but positive PCR signals indicating the presence of a transgene possibly by F1 hybridisation with nearby GM maize crops, but did not show that the gene had been stably integrated. Stewart *et al.* (2003) (see Section 2) asserted ‘No published data have ever shown that transgenic DNA has been unintentionally introgressed into maize landraces or into any unintended maize genome’. However, such introgression is possible. Introgression is the stable integration in a heritable state of gene(s) from one species to another following hybridisation and further backcrossing. A PCR signal is the identification of a particular DNA fragment on a gel following a Polymerase Chain Reaction to amplify the DNA.

The StarLink™ case was discussed on page 81 of our First Report.

Our First Report also recognises the potential hazards of introducing GM pharmaceuticals into food crops. Strong recommendations against this have been made by all EU Regulatory bodies, and pharmaceutical crops are not likely to be grown in uncontained conditions.

Most of the other contributions are statements which are difficult to comment on in detail but which have been covered in Chapter 7 of our First Report. Some statements (‘there is little evidence on how different factors (seed purity, cross pollination, volunteers, gene stacking etc) will combine, which makes prediction of gene flow difficult’, ‘Non food GM crops must be separated from food varieties’, ‘GM volunteers can act as a reservoir of transgenes and lead to changes in farming practice that can affect biodiversity’ etc.) are incontestable. Some are based on misunderstanding (e.g. the Report does say that for some people gene flow of transgenes is undesirable *per se.*). Others, such as whether coexistence is possible, are speculative and include statements for and against, often quoting evidence from existing practice and experience with GM crops. Unless new published scientific evidence is given, as in the case of those papers dealt with in Section 2, it is probably not helpful to make further comment here.

A report by one contributor¹³⁷ makes an interesting contribution to the issue of separation distances between maize fields. It revisits early (1950) experiments of Jones and Brooks in North America (experiments that Ingram (2000) cited in our First Report), used to calculate separation distances. Using a different procedure (which the author should be encouraged to submit for publication) the values for percentage outcrossing with distance are very similar to those presented by Ingram. The report goes on to say that factors such as high winds and weather conditions would considerably influence the values, and have not been accounted for in the calculations on which Ingram based the separation distances.

¹³⁶ ACRE. Advice on separation distances for the cultivation of T25 maize. *A report by the Advisory Committee on Releases to the Environment* (2003). http://www.defra.gov.uk/environment/acre/advice/pdf/acre_advice28.pdf

¹³⁷ Scientists for Global Responsibility. See Annex I.

Most of the refereed papers suggested by one commentator have been covered in different parts of this Section, or in Section 2.

4.5.2 Gene flow from GM crops to agricultural weeds and wild relatives

Summary conclusions from First Report

‘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.’

Main points made by commentators

Some commentators¹³⁸ felt that this was an extensive review of the literature and did acknowledge areas of uncertainty. However it should also have included new data on frequency of gene flow to wild relatives in the UK from Wilkinson *et al.* (2003).

¹³⁸ Combined comment from: Econexus, The Five Year Freeze, Friends of the Earth, GeneWatch UK, Greenpeace, The Soil Association and Dr M Antoniou. See Annex 1.

One commentator¹³⁹ felt that there was too much faith in management practices, future genetic use restriction technologies, and pollen flow limiting technologies in the report. The same commentator felt that there was no sound scientific basis for the assertion that transgenes behave in the same way as resident genes, and pointed to research that indicated that Mendelian inheritance in transgenes is more uncommon than common (Breiztger & Tonks, 2003).

Some commentators¹³⁸ felt that there is evidence that hybridisation can occur between wheat and a wild relative in the UK.

The Panel's response

The new data on likely frequency of gene flow to wild relatives in the UK has been considered in our Second Report. It is actually data from a research consortium estimating the likely frequency of hybridisation (the first stage of gene flow) between oilseed rape and its wild relative *Brassica rapa* (Wilkinson *et al.* 2003). A critique is provided in Section 2.

Most of the arguments that transgenes behave differently in terms of their rates of transfer, and ability to persist, in the wild are based on the 'absence of evidence does not constitute evidence of absence' logic. It depends therefore on how long (over what period of time and on what scale globally) individuals believe such comparisons must be made. Statements in our First Report are based on the experience to date. One small piece of evidence quoted as supporting the idea that transgenic plants may be more outbreeding must be treated with caution. This note to *Nature* (Bergelson *et al.* 1998) on outbreeding in *Arabidopsis* compares two transgenic lines (with an unexplained difference between them in outbreeding rates) with a wild-type mutant against a background of unknown variation in rates of outcrossing. Follow-up research using isogenic lines in a fully comparative experimental design is needed.

Apomixis as a gene containment technology is far off from commercial development and its use and the potential risks do require a full evaluation.

A number of wild relatives of wheat (*Triticum aestivum*) have hybridised and introgressed with wheat in the wild (mainly in Turkey and Greece). A monograph by van Slageren (1994) lists 14 *Aegilops* species and two species of *Triticum* in this category. Several species of *Secale*, *Agropyron*, *Hordeum*, *Elymus* and *Isathyrostachys* have been crossed under artificial conditions with wheat – but no spontaneous hybrids with wild relatives of these species have been found in the UK. The presence of wheat specific genes in a wild plant of *Elymus caninus* is indirect evidence of past introgression (but could be genes shared by descent) and should alert us to seek further experimental evidence. It remains true that gene flow is less of an issue in wheat than oilseed rape for a large variety of reasons (no feral populations, many genes of domestication especially lack of dormancy, etc.).

¹³⁹ ISP. See Annex I.

4.5.3 Can DNA from GM crops transfer to soil microbes?

Summary conclusions from First Report

'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 of transfer 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.'

Main points made by commentators

One commentator¹⁴⁰ thought that if soil bacteria modified by GM crops persisted, they would quite possibly persist indefinitely. It is possible that soil having once grown GM crops could never again be considered 'GM free'. They also felt that the greatest omission of Chapter 7 was lack of consideration of the likelihood that horizontal gene flow is more likely to occur from GM crops, or any GM organisms, than from non-GM crops or organisms. This is because the transgene will have probably exploited a weak point in the host DNA to recombine, so is more likely to become detached again; it will have been engineered to recombine easily, so has been specifically designed for horizontal gene flow; it may contain parts of bacteria or virus DNA, so would be more likely to recombine with bacteria or viruses than pure plant DNA.

The Panel's response

It is never possible to be certain that any microbe is completely absent from a complex environment such as soil. All that can be said is that it is below the lower limit of detection, which depends on the sensitivity of the method and the sampling itself, but is potentially much lower than the threshold of 0.1% currently accepted by the organic movement for GM in crops. This is as true for any GM bacteria that might arise in a GM crop as for disease-causing bacteria, for example, in a conventional or organic crop. An organism that remains below the limit of detection is unlikely to have any environmental or health effect. There is no known mechanism for the active transfer of genes from plant cells to bacteria, but free DNA derived from plants can be taken up by some bacteria. For this reason, the stability of transgenic DNA within the plant has been considered less relevant than its fate as the bacterium. This latter issue is addressed in our First Report, where it is concluded that some, but not all, transgenic DNA might be more likely to be taken up successfully by bacteria than average plant DNA.

4.5.4 Can genetic material in GM plants transfer to viruses?

Summary conclusions from First Report

'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.

¹⁴⁰ Green Party. See Annex I.

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.’

Main point made by commentators

One group of commentators¹⁴¹ were concerned about the possible risks arising from possible recombination and capsid exchange events between GM transgenes and viruses. We have dealt with comments on specific viral issues separately below.

The Panel’s overall response

Our First Report fully acknowledged laboratory-based studies in which a (more-or-less) genetically defective virus clone could be restored to a (near) wild-type phenotype by homologous RNA or DNA recombination in specially engineered GM plants that expressed an essential ‘restorer’ gene sequence. The desirability (or otherwise) of including viral replication signals in a transgene, their consequent impact on recombination frequencies, and the lack of evidence that such events happen between wild-type plant viruses and GM plants

¹⁴¹ Combined comment from: Econexus, The Five Year Freeze, Friends of the Earth, GeneWatch UK, Greenpeace, The Soil Association and Dr M Antoniou. See Annex 1. (Subsequent references to ‘some commentators’ in Section 4.5.4 means this group of commentators.)

in field trials, or in commercial production, are discussed extensively in our First Report, in many reviews since the late 1980's, and in the cited DETR Report (1999)¹⁴².

Our First Report also acknowledged a general problem that it is scientifically impossible to prove a negative (e.g. that gene flow (i.e. heritable genetic recombination) between a viral transgene sequence and a wild-type plant virus could never happen), and that negative results are intrinsically difficult to publish in peer-reviewed journals.

The occurrence and possible risks of non-homologous recombination were acknowledged in our First Report, as was the possibility of natural homologous or even non-homologous recombination (page 241, paragraph 6 and page 243, paragraph 2). But, there is no experimental evidence that such events occur in nature, or that any hypothetical risk posed is any greater in a GM plant than in any natural mixed infection of non-GM plants. Guided by the title of Section 7.5 in our First Report, the text considered all available scientific evidence for or against genetic recombination during RNA or DNA replication between mutated or native viral and transgene sequences. One consequence of such an event would be the generation (evolution) of a heritable new (recombinant viral) sequence (as screened for by Lin *et al.* 2003), possibly with new biological properties (as tested in Thomas *et al.* 1998). From both these papers, and from other research, there is as yet no *a priori* evidence for field-level RNA or DNA recombination between a GM plant and a wild-type virus isolate.

The commentators do not provide any new data or scientific evidence to support their assertions that recombination between any viral transgene in any GM plant and any field-isolate of a related or unrelated virus can occur, or has occurred. Equally they provide no scientific reason or evidence to support the assumption that any speculative ecological or environmental risk posed by such a theoretical recombinant virus would differ from that posed by new viruses which evolve constantly in nature, in non-GM host plants, through RNA/DNA mutation, genetic recombination, and/or genomic reassortment events during mixed infections.

Contrary to claims made by the commentators, those surveyed for the DETR Report (1999)¹⁴³ did not say that recombination was 'inevitable', or that 'the probability of horizontal gene transfer of transgenes is greater than for mixed infections'. Most respondents did, however, identify viral transcapsidation and RNA recombination as the most likely possible hazards of deploying GM crops with viral transgenes. This is stated clearly and openly in our First Report (Section 7.5.3, page 240, paragraph 1).

The commentators did highlight the fact that five independent naturally co-infecting viruses were recovered from a single plant (reported in Falk & Bruening 1994). Indeed, mixed virus infections of wild reservoir species and field crop plants are surprisingly common in nature (see below).

¹⁴² Safety of plant viral inserts. Research report No. 11, DETR 1999.

¹⁴³ Safety of plant viral inserts. Research report No. 11, DETR 1999.

The Panel's response on some specific viral issues

The susceptibility of GM crops to viruses

Some commentators implied that our First Report report ignored one of the main risks involved in the use of plant virus-derived transgenes, namely the potential for them to make crop plants more susceptible to viruses which are related or unrelated to the virus from which the transgene came. They contended that such GM crops may be especially susceptible to new infectious viral diseases.

The Panel's response

The First Report fully acknowledged the extensive published evidence for, and possible hazards posed by, natural virological phenomena such as transcapsidation, synergism, complementation and phenotypic mixing (pseudo-recombination) that do occur during mixed virus infections, and that may occur in GM plants expressing a particular functional virus-derived transgene. However, none of these events occur by, or result in heritable recombinant RNA or DNA. There are no reports of any GM plant becoming more susceptible to an existing or new plant virus in the field. Published data discussed in detail elsewhere in our Second Report (Lin *et al.* 2003; Thomas *et al.* 1998) and many other field studies (since 1988) on GM plants expressing viral transgenes have found no evidence of recombination under field conditions, although it is acknowledged that recombination events will be rare and therefore difficult to detect.

A 6-year long (1991-1996) field study by Thomas *et al.* (1998), using 65,000 potato plants from 954 GM-lines designed to express different *Potato leafroll virus* (PLRV) coat protein or RNA replicase transgene constructs, showed them not to be infected in the open field or the greenhouse by viruses that did not normally infect potato. Simply being GM had clearly not made the plants more vulnerable to infection by new or unrelated viruses. Nor were new viruses or viruses with altered biophysical, biochemical or biological properties detected in any case. Similar conclusions were reached from extensive field data on a range of common cucurbit-infecting viruses and GM plants collected by Lin *et al.* 2003 (see Section 2).

Virus transcapsidation and gene silencing

Some commentators stated that the potential for expression of viral capsid proteins to transfer viruses to new hosts by allowing them to be transported by unfamiliar vectors was only addressed briefly and inadequately (paragraph 135), and potential food safety risks, particularly of gene silencing, were also addressed briefly and inadequately (paragraph 136).

The Panel's response

Our First Report contained extensive discussion of the natural biological phenomenon of virus transcapsidation (Section 7.5.3, paragraph 2, page 242) and actual or possible transfer by a new vector, as well as how to mitigate this phenomenon. These issues were also covered extensively in the DETR Report (1999)¹⁴⁴ cited in our First Report.

Comments on gene silencing as 'an extremely poorly understood mechanism' to confer resistance to viruses conflict with the wealth of recent data on this topic (see Hannon 2002, for example). The reader is also referred to Section 2.2.8 of this report. Here we deal more extensively with the issue of silencing and the probable differences between plant and animal systems. Moreover, the commentators' text is contradictory on whether or not gene silencing

¹⁴⁴ Safety of plant viral inserts. Research report No. 11, DETR 1999.

poses some hypothetical risk as a ‘powerful biochemical pathway’ because it is, or is not, sequence specific. The commentators provide no reason or evidence for their proposal that gene silencing, a natural process, ‘presents a risk to food safety’. Reference to SARS and HIV as evidence for natural, newly evolved viruses (with no cited references) is made, despite there being no scientific connection to the issue of gene flow between GM plants and natural plant viruses.

Evidence for recombination

Some commentators felt that the main conclusion in our First Report, that recombination has not happened in the field, put too much reliance on one study (Thomas *et al.* 1998). In addition, the conclusion that any new viruses would be out-competed by other viruses was not supported by the evidence. Viruses are constantly evolving and can prosper despite locally adapted competition.

The Panel’s response

Although not common, DNA or RNA recombination events do occur during natural mixed virus infections in non-GM plants, and laboratory-generated homologous recombination has been reported between a genetically engineered defective virus and a transgenic host plant expressing a suitable restorer RNA or DNA sequence. Recombination in such cases can be detected as a result of the fact that a selective pressure (selection of viable, heritable non-defective virus genomes) is applied in which only recombined molecules can compete successfully.

Since 1982, all attempts to develop DNA, and later RNA viruses as vectors to express foreign gene sequences (including sequences from related or unrelated plant viruses that could comprise a resistance transgene in a GM plant) have shown that all foreign (unselected) viral sequences pose a ‘genetic burden’ to the parent virus. Why might this be the case? All wild-type viruses (of plants, animals etc) are constantly mutating; possibly reassorting and recombining (when two viruses co-infect the same plant cell); and being tested and selected for fitness. RNA viruses are especially variable because their replication machinery is error prone and they exist as a swarm of related sequences, which teeter on the brink of error catastrophe. What we define as ‘the virus’ is the ‘optimal’ sequence which occupies a very sharp ‘fitness peak’ in the sequence landscape. This sequence is the optimal sequence of the virus that maximises replication under the selection conditions applied (species and physiology of host plants; vector species, temperature, humidity, etc.). The inherent variation in the sequence swarm allows viruses to respond rapidly to different selection conditions.

Two decades of experimental evidence support the view that any newly-engineered recombinant virus is either non-viable, or competes poorly against its wild-type progenitor strain. Often, genetic reversion of the virus population to the original wild-type sequence occurs within one (or a few) infection cycles (passages) from host-to-host, even in the laboratory or greenhouse. On the basis of this extensive experience, as well as the theoretical framework described above, which is supported by the Thomas *et al.* (1998) and Lin *et al.* (2003) papers (see Section 2), any scenario in which a new virus could result from recombination between a transgene and a virus infecting a transgenic crop, is considered to have an extremely low probability of occurrence. Mutation and recombination will very likely have already selected the most successful genetic combination, each of which we recognise today as a defined natural ‘plant virus’. However, particular attention in risk assessments should be with events that could not happen in doubly infected plants where novel

recombination events may arise and which could introduce new dimensions into the dynamics of plant/virus co-evolution.¹⁴⁵

A 6-year-long, release experiment using GM Russet Burbank potato varieties (65,000 plants from 954 independent GM lines designed to express different *Potato leafroll virus* (PLRV) coat protein or RNA replicase transgenes) was prompted by ‘a search for evidence of transcapsidation, complementation, synergism, or genetic recombination in virus-infected transgenic plants’ (Thomas *et al.* 1998). Among others, three lines of two constructs (line 82 of 18844, and lines 129 & 350 of 18834; described by Thomas *et al.* (1998)) that contained a full-length PLRV replicase gene were then studied further, ‘selected and released for commercial production’ (Thomas *et al.* 2000). However, their extreme resistance to PLRV, and hence their reduced need for aphicidal sprays, have not yet been exploited commercially due to current policy decisions on GM foods by processors and retailers. The authors concluded that new viruses or viruses with altered sedimentation characteristics, symptoms, or host range were not detected in field-exposed or greenhouse-inoculated potato plants that expressed PLRV CP or replicase transgenes.

The scale, range and detail of the approaches used by Thomas *et al.* (1998) were adequate to detect any significant change in the particular viruses and their particular biological properties under investigation (alterations in host range and symptoms) and hence support the (albeit) negative conclusions of the paper.

Some commentators were critical about the prominence given to this study in our First Report and the sensitivity of the tests used and their relevance to measuring recombination in the field. On the first point, this study was given prominence because it gives us real world data under real large-scale conditions. The conclusions drawn are appropriate, as the authors point out in relation to sensitivity: ‘only the transgene/virus interactions that produced the specific alterations searched for in this study would have been detected in the studies reported here’; and in terms of scope, the study makes it clear that general conclusions cannot be drawn about recombination. We endorse this view, which is the case-by-case approach.

More recent published work (Lin *et al.* 2003) with GM squash plants that co-expressed several different plant viral coat protein genes, and using molecular tools to detect changes at the RNA level, showed that no new viruses were generated through RNA recombination. This study is described in detail in Section 2.2.10.

Further scientific studies will increase our knowledge in this important area. We identified a number of areas in our First Report (page 248), including research to compare the frequency of recombination events in natural mixed virus infections with events in a single virus-infected GM plant.

The containment of new plant virus outbreaks

Some commentators felt that Section 7.5 in our First Report implied that methods exist to control new virus outbreaks when this is true for only a handful of known viruses (paragraph 144). Even if new plant virus diseases are containable, this may take time and be at a large cost.

¹⁴⁵ Safety of plant viral inserts. Research report No. 11, DETR 1999.

The Panel's response

Phytosanitary controls exist to control existing and newly emerging plant viruses, with varying degrees of success. There is no reason to assert that these measures would be any less effective against any hypothetical, viable, new recombinant virus, however it may have been formed. There is no evidence to assume that 'new plant viruses diseases' would somehow be uniquely 'uncontainable' (take longer or cost more to control) through gene flow (recombination) any more than those which arise through natural evolutionary events (see 'swarm theory' and 'viral fitness' above).

4.6 THE GM SCIENCE REVIEW PROCESS

Our First Report was welcomed by a large number and wide range of respondents. Many of even the most critical commentators remarked that this initiative constituted a positive and potentially significant step in the context of other UK Government activities in this field. Although there was one individual who found the review a 'pseudo-drama and massive misjudgement', many readers appreciated the broadening and deepening of the general appraisal of GM science, linking it more closely with public concerns and questions and giving due attention to uncertainties, gaps in knowledge and divergent scientific perspectives. The Panel as a whole would strongly concur with the more widely held view.

The Panel was certainly unusually broad in its composition, both in terms of the disciplines and the perspectives on the GM issue that were represented. Given this diversity, it achieved a welcome degree of cohesiveness. Strong efforts were made to identify and build on common ground or, failing this, to document the reasons for remaining differences of view. There were comments welcoming the First Report as thorough and well-balanced.

However, concerns were raised by some commentators that, despite its relatively large size, the membership of the GM Science Review Panel was in some respects rather narrow. For instance, the relative absence of nutritionists and epidemiologists was highlighted. In looking at these comments, the Panel also discussed the point that membership did not reflect the full range of views on every subject area. Taking these comments together, the Panel noted that toxicology, rather than nutrition was more relevant to its remit, and there was a toxicologist as a member. The Panel recognised that he worked for the industry, and that they had also invited and received comments from the FSA and its expert advisory committees, which themselves have a wide range of members. The Panel observed that there are very practical limitations on the size of group that can engage in effective deliberation. It is simply not possible to include all shades of opinion from every relevant discipline. It was therefore important to put the First Report out for public comment. It was also noted that members networked outside the Panel and read the literature where points might not have been central to their expertise. Despite its large size, it is widely believed by Panel members that it operated effectively through much vigorous debate and that, overall, the size and disciplinary composition of the Panel was about right.

Concerns were also raised by a number of respondents to the effect that the Panel membership embodied a generally more favourable opinion of GM than is extant among the wider public. The Chair of the Panel, has observed that on many occasions in panel discussion it would not be possible to pinpoint the speakers' particular viewpoint; the discussion was on the merits of the scientific evidence and that it was an objective process.

A particular concern was raised by the NGOs in relation to a perceived asymmetry in the invitations to different constituencies to nominate Panel members. This seems to arise because biotechnology industry organisations were approached as a single body, whilst Greenpeace, Friends of the Earth, GeneWatch and The Soil Association were approached individually. It was felt that this led to the Panel including two scientists working for industry organisations, but no scientists working for the environmental organisations named above, although their two nominees were accepted. This does suggest that there is scope for improved communications for future exercises, including enhanced provision for direct scientific dialogue with the NGO community. The Panel again, however, emphasises the range of its membership, including those working for the Royal Society for the Protection of Birds (RSPB) and English Nature.

Another criticism was over the inclusion in the Panel of members of established GM advisory committees. There was concern that critical scrutiny of existing regulatory practice might somehow be restrained. In this regard, it should be emphasised that all members of the Panel served as individuals in an entirely independent capacity. A major conclusion of the Panel was that it was imperative that regulatory expertise keeps abreast of developments in GM science.

A further concern was voiced over the process by which the drafting task was approached in the early stages, in particular that the initial draft and early editing of one section was produced by an industry nominee. The Panel believes it is important to set this observation in the overall context of the production of the final report. After initial scoping meetings of the full Panel, sub-groups met to discuss the subject matter of the four main science chapters of the report and agreed the allocation of tasks. On this basis, individuals with particular knowledge produced first drafts of sections and took account of initial comments from the whole Panel. Partly to meet concerns that there might be a perception of partial editing, but also to take account of all the discussion in sub-group and in the seven main Panel meetings, and in e-mail exchanges on detail, the Secretariat then took on responsibility for the final editing process. The entire Panel took an active role from the initial stages through to agreeing the final documentation. The Panel believes that all Panel members had the opportunity to challenge and have their views heard, that the process was robust and wishes to underline that the Panel as a whole took ownership of the final document.

In conclusion, it seems that the GM Science review process, taken in the context of the UK Government's wider GM Dialogue, has been generally regarded as a very positive and useful exercise. This initiative provides important lessons and a positive model for any such exercise in future.