

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 1

THE SIGNIFICANCE OF THE PUBLIC DEBATE FOR THE SCIENCE REVIEW

1.1 THE THREE STRANDS OF THE ‘GM DIALOGUE’

The decision to have a ‘GM Dialogue’ was announced by the Secretary of State for Environment, Food and Rural Affairs, Mrs Margaret Beckett MP, in July 2002. The three strands of the dialogue were: a Public Debate, as recommended by the Agriculture and Environment Biotechnology Commission (AEBC); a study of the possible costs and benefits of GM, undertaken by the Prime Minister’s Strategy Unit; and a review of GM Science undertaken by an independent science review panel, chaired by the Government’s Chief Scientific Adviser, Sir David King.

There was interaction between the three strands at an official level, so that each understood the intentions and progress of the other, but there was limited interaction at a public level. Neither the Strategy Unit nor the Science Review Panel reported in time to inform the Public Debate, ‘GM Nation?’. However, the Science Review drew explicitly on preparatory work for the Public Debate (as described below) and our First Report was published in July 2003 to allow comments from organisations and individuals to be considered in a second phase (see Annex 1), and to be able to take on board the results of ‘GM Nation?’.

1.2 THE KEY MESSAGES FROM THE PUBLIC DEBATE

The report from the Public Debate Steering Board ‘GM Nation?’ identified seven key messages:

- (1) People are generally uneasy about GM.
- (2) The more people engage in GM issues, the harder their attitudes and more intense their concerns.
- (3) There is little support for early commercialisation.
- (4) There is widespread mistrust of Government and multi-national companies.
- (5) There is a broad desire to know more and for further research to be done.
- (6) Developing countries have special interests.
- (7) The debate was welcomed and valued.

Of these, points 4 & 5 are most relevant to the Science Review process, and are discussed further below. Point 2 may also be relevant. Public concerns about scientific uncertainty were central to the task of the Science Review Panel.

The Science Review was framed to reflect public concerns by using the same foundation discussion workshop results (the Corr Willbourn Report) as used by ‘GM Nation?’ as well as issues raised on the Science Review website and discussions at open meetings. This appears to have been broadly successful. The main scientific issues raised in the ‘GM Nation?’ Report are treated in depth in the Science Review and are as follows:

- ‘Risk of contamination’, and ‘freedom of choice’, are both addressed in Chapter 7.2, looking at gene flow between crop varieties and co-existence¹.
- ‘Exercising precaution’, which is addressed in Chapter 3.2 as a regulatory issue.
- ‘Lack of reliable, independent scientific evidence’ – the Science Review was designed to address this question by reviewing available evidence and identifying gaps in knowledge and understanding, uncertainties and possible areas for further research.
- ‘Questioning the need for GM crops’ – the Science Review included information on potential benefits in Chapter 6.6 (horizon-scanning) and in other Chapters. The Strategy Unit Report covered this subject in more detail.
- ‘Environment at risk’ was addressed in Chapters 6 and 7 of the Science Review.
- ‘Health at risk’ was addressed in Chapter 5 of the Science Review.
- ‘Power of the multi-nationals’, ‘non-material values’, ‘doing right by developing countries’ and ‘labelling and liability’ are issues beyond the scope of the Science Review, except where labelling issues bear on co-existence, as discussed in Chapter 7.2.
- ‘Experience overseas’ – some of the emerging evidence of experience in other countries has been included in the Science Review, as has discussion about the extent to which use of GM in other countries constitutes evidence of safety.
- ‘Most resistance to trans-species applications and GM food’. The extent to which GM is ‘different’ and raises new issues, as well as the appropriateness of a ‘case-by-case’ approach to address any potential risks and benefits of GM products, are discussed in Chapter 4. The safety of GM food is discussed in Chapter 5.

1.2.1 Public Debate message 4: ‘There is widespread mistrust of Government and multi-national companies’

The ‘GM Nation?’ report claimed that this mistrust extended to scientists. People were concerned ‘that Government may not have adequate knowledge and advice to help them take the right decisions’.

¹ The AEBC has recently published a Report on co-existence: ‘GM Crops? Co-existence and Liability’. November 2003.

An important outcome of the Science Review is that many of the uncertainties and gaps in knowledge it addressed, for example in long-term impacts on health or the environment and the co-existence of GM crops with other crops, coincide with concerns expressed during the Public Debate. What the Science Review has done is make clear the extent of the uncertainties and their importance, and places them in the context of uncertainties associated with conventional crops and food. This confirms the importance of the Science Review's explicit treatment of uncertainty in its process and indicates that continued open public discussion of the nature and significance of uncertainties is crucial in re-establishing trust in science and its use in Government.

An important message from the Strategy Unit Report was that 'the overall balance of costs and benefits (of GM commercialisation in the UK) will depend on public attitudes, market demands, and on the ability of the regulatory system to manage uncertainties'.

1.2.2 Public Debate message 5: 'There is a broad desire to know more and for further research to be done'

Although often characterised as being 'anti-science', one theme throughout the Public Debate was for more research to be done. Our First Report highlighted areas where further research could contribute to greater understanding (on allergenicity, invasiveness, impact on farmland ecology, impact of gene flow to other crops or wild relatives, effects on soil ecology). The GM Crop Farm-Scale Evaluations (FSEs) are an example of current research to assess potential impacts of different weed management regimes on wildlife during cultivation of particular herbicide tolerant GM crops. The FSEs represent pioneering experiments which raise important broad issues about the way that the impact of agriculture on the environment is evaluated, and how a particular type of GM crop may influence this.

Research funding bodies in the UK and EU are continually identifying and sponsoring relevant scientific research on a broad front. This includes research relevant to assessing the environmental impact of crops and the safety of their products. We hope that the Science Review will influence their agendas.

'GM Nation?' also identified a desire for a body of factual scientific information about the production, cultivation, risks and benefits of GM crops. One of the primary aims of the Science Review was to discuss the status of our scientific knowledge, and agree, where possible, on the level of understanding. Where there was disagreement, this was outlined in our Report, together with ways this might be resolved. Our First and Second reports form a contemporary body of knowledge on GM crops and food. Further investigation and research will add to this, but one of the strengths of the Science Review process was that it also illustrated the potential to resolve divergent interpretations of scientific evidence, and divergent views on the adequacy of evidence, among its broad membership of scientists and non-scientists. Open acknowledgement of a divergence of views, as well as uncertainty, are important components of public trust in science as used by policy-makers.

1.2.3 Public Debate message 6: ‘Developing countries have special interests’

The role of GM crops in developing countries was raised repeatedly during the public consultation carried out in preparation for our First Report. This was one topic of discussion in the debate, but as it was beyond our remit, we could not treat it in detail. The outcome of the Public Debate suggests that the role of GM in developing countries is a subject of special interest, which may be judged on risks/benefit criteria and ethical issues that are different from those for GM crops in the UK. We hope that the scientific information gathered in the Science Review and subsequent assessment of the evidence will prove helpful to other countries in their own scientific deliberations and assessments (See also Section 4.3.1.).

1.3 IN CONCLUSION

In drawing conclusions concerning the significance of the Public Debate for the Science Review, we would like to return to some issues discussed in our First Report. We highlight there (page 8), that some of the questions over GM crops are not of a purely scientific nature, or even scientific at all but may be economic, social, ethical or even personal. Of those that are scientific, as in any field, the answers given by science will typically depend on the particular questions that are asked, the way they are framed and the assumptions that are made in analysis. Of course, scientific discipline and rigorously gathered data can narrow down the range of possible answers, and make an important contribution to various issues, but the science will often support more than one interpretation and may raise new uncertainties or reveal new gaps in knowledge. It is for these reasons that the provision of robust scientific advice to policy making, depends not only on the involvement of a wide range of specialist disciplines, but also on in-depth critical engagement with public values and concerns.

The Science Review was explicitly structured according to questions posed at an early stage in the Public Debate. The public also had an opportunity via the Science Review website to have an input into the Review, to engage with us and raise some concerns which we have addressed in our Second Report. Considerable efforts were made to ensure that the Science Review was as open as possible to inputs from stakeholders, interest groups and the wider public, as well as practising scientists. The conclusions contained in our First Report were carefully considered in relation to the findings of the Public Debate and other responses. Thorough attention was given to the diversity of scientific interpretations. In the end, conclusions drawn from the Science Review form one, albeit important, element to inform Government policy on the release, cultivation and consumption of GM crops. We believe there has been real value in the linkage between the Public Debate and the Science Review.

Section 2

NEW PUBLISHED RESEARCH

2.1 INTRODUCTION

We have considered a number of scientific papers and reports, published after we produced our First Report in July 2003. Box 1 lists those that we have examined in this section. A number of other papers and reports, that were not referenced in our First Report and which were identified by commentators or Panel members in response to these comments, are considered in Section 4.

Box 1: New published research considered by the Science Review Panel

Agrawal AA & Kotanen PM (2003) Herbivores and the success of exotic plants: a phylogenetically controlled experiment. *Ecol. Lett.* **6**, 712-715.

Bais HP, Vepachedu R, Gilroy S, Callaway RM & Vivanco JM (2003) Alleopathy and exotic plant invasion: from molecules and genes to species interactions. *Science*, **301**, 1377-1380.

Chowdhury EH, Kuribara H, Hino A, Sultana P, Mikami O, Shimada N, Guruge KS, Saito M & Nakajima Y (2003) Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11. *J. Animal Sci.* **81**, 2546-2551.

Defra (2003a) Quantifying landscape-scale gene flow in oilseed rape. Research Report RG0216. <http://www.defra.gov.uk/environment/gm/research/epg-rg0216.htm>

Defra (2003b) Gene flow monitoring from the GM crop FSE sites: monitoring gene flow from the GM crop to non-GM equivalent crops in the vicinity. Part 1: forage maize. Research Report EPG 1/5/138. <http://www.defra.gov.uk/environment/gm/research/epg-1-5-138.htm>

Defra (2003c) The potential for oilseed rape feral (volunteer) weeds to cause impurities in later oilseed rape crops. Research Report RG0114. <http://www.defra.gov.uk/environment/gm/research/epg-rg0114.htm>

Defra (2003d) Modelling the effects on farmland food webs of herbicide and insecticide management in the agricultural ecosystem. Research Report EPG 1/5/188. <http://www.defra.gov.uk/environment/gm/research/epg-1-5-188.htm>

Jackson AL, Bartz SR, Schelter J, Kobayashi SV, Burchard J, Mao M, Li B, Cavet G & Linsley PS (2003) Expression profiling reveals off-target gene regulation by RNAi. *Nature Biotechnology*, **21**, 635-637, Brief Communications.

Kleter GA & Peijnenburg AACM (2003) Presence of potential allergy-related linear epitopes in novel proteins from conventional crops and the implication for the safety assessment of these crops with respect to the current testing of genetically modified crops. *Plant Biotechnology*, **1**, 371-380.

Lin H-X, Rubio L, Smythe A, Jiminez M & Falk BW (2003) Genetic diversity and biological variation among California isolates of Cucumber mosaic virus. *J. Gen. Virology*, **84**, 249-258.

Romeis J, Battini M & Bigler F (2003) Transgenic wheat with enhanced fungal resistance causes no effects on *Folsomia candida* (Collembola: Isotomidae). *Pedobiologia*, **47**, 141-147.

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

Tepper D, Garcia-Gonzales R, Mansouri H, Seruga M, Message B, Leach F, & Perica MC (2003) Homology-dependent DNA transfer from plants to a soil bacterium under laboratory conditions: implications in evolution and horizontal gene transfer. *Transgenic Research* **12**, 425-437.

Vlasák J, Šmahel M, Pavlík A, Pavingerová D & Bříza J (2003) Comparison of hCMV immediate early and CaMV 35S promoters in both plant and human cells. *J. Biotechnol.* **103**, 197-202.

Wilkinson MJ, Elliott LJ, Allainguillaume J, Shaw MW, Norris C, Welters R, Alexander M, Sweet J, & Mason DC (2003) Hybridization between *Brassica nap*a and *B. rapa* on a national scale in the United Kingdom. *Science*, **302**, 457-459.

2.2 RESEARCH PAPERS AND REPORTS

2.2.1 Agrawal AA & Kotanen PM (2003) Herbivores and the success of exotic plants: a phylogenetically controlled experiment. *Ecol. Lett.* **6**, 712-715

Abstract: ‘In a field experiment with 30 locally occurring old-field plant species grown in a common garden, we found that non-native plants suffer levels of attack (leaf herbivory) equal to or greater than levels suffered by congeneric native plants. This phylogenetically controlled analysis is in striking contrast to the recent findings from surveys of exotic organisms, and suggests that even if ‘enemy release’ does accompany the invasion process, this may not be an important mechanism of invasion, particularly for plants with close relatives in the recipient flora.’

In our First Report (Section 6.2.5, pp. 116-117) we considered the invasiveness of alien species in the UK. We felt that: ‘although a GM plant could theoretically become invasive, there was general agreement that equating current GM crops to exotic plants provides a very limited model for predicting the effects of gene flow and GM crops’.

The particular sample of alien plants considered by the authors did not have significantly lower rates of attack by native herbivores than matched native species. But equally, the list of alien plants did not contain any species that are regarded as serious invaders of natural habitats, many (if not most) of which do show lower rates of herbivory than native plant species. In Britain, garden plant species of alien origin have much lower rates of herbivory than do native plant species. The ‘enemy release’ hypothesis is still the most plausible explanation of why some alien plants become highly invasive in alien environments, where they are freed from their specialist herbivores and prove (serendipitously, of course) to be unpalatable to the native generalist and specialist herbivores. ‘Enemy release’ is not the only factor involved, because many species freed from their enemies still do not become invasive. This study does not change the conclusions in our First Report.

2.2.2 Bais HP, Vepachedu R, Gilroy S, Callaway RM & Vivanco JM (2003) Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science*, **301**, 1377-1380

Abstract: ‘Here we present evidence that *Centaurea maculosa* (spotted knapweed), an invasive species in the western United States, displaces native plant species by exuding the phytotoxin (–)-catechin from its roots. Our results show inhibition of native species' growth and germination in field soils at natural concentrations of (–)-catechin. In susceptible species

such as *Arabidopsis thaliana*, the allelochemical triggers a wave of reactive oxygen species (ROS) initiated at the root meristem, which leads to a Ca²⁺ signaling cascade triggering genome-wide changes in gene expression and, ultimately, death of the root system. Our results support a ‘novel weapons hypothesis’ for invasive success.’

We considered whether GM plants could be invasive or persistent in Section 6.2 of our First Report. On page 112, we said that there was no evidence for allelopathic effects (or production of noxious products in general) in GM herbicide-tolerant crops studied so far.

Centaurea maculosa is highly invasive and this study shows it also to be allelopathic. But we don't know that it is invasive because it is allelopathic, it may for example be because it is unpalatable to cattle. A convincing case would be made by a phylogenetically controlled comparison of many plant species, which indicated that the allelopathic member of the pair was significantly more likely to be invasive than the non-allelopathic member. Such a study has not been done. This study does not change the conclusions in our First Report.

2.2.3 Chowdhury EH, Kuribara H, Hino A, Sultana P, Mikami O, Shimada N, Guruge KS, Saito M & Nakajima Y (2003) Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11. *J. Animal Sci.* 81, 2546-2551

This paper describes the fate of corn DNA fed to pigs and investigates both genes from the normal genome of maize and transgenic DNA. In both cases PCR methods demonstrated that DNA degradation was incomplete in the pig GI tract. The survival of 242 bp gene fragments of corn zein, 226 bp fragments of invertase, 1,028 bp fragments of ribulose-1,5-biphosphate carboxylase/oxygenase were detected in the GI tracts of pigs fed both GM and non-GM corn. In the case of the transgene cry1Ab, 110 bp and 437 bp gene fragments were detected only in pigs fed GM maize.

We considered the fate of transgenic DNA in the GI tract in Sections 5.4 and 5.5 of our First Report. These additional observations do not change our original conclusion that DNA is incompletely degraded in the animal GI tract.

**2.2.4 Defra (2003a) Quantifying landscape-scale gene flow in oilseed rape (OSR). Research Report RG0216
<http://www.defra.gov.uk/environment/gm/research/epg-rg0216.htm>**

This report covers work at the Scottish Crop Research Institute on hybridisation rates in a landscape in eastern Scotland where oilseed rape (OSR) is widely grown. The results confirm earlier work by this group and others that show considerable site to site and year to year variation in patterns of cross pollination; with rates dropping rapidly over the first few tens of metres but declining only slightly with distance beyond that. Long distance pollination occurred on male sterile plants at 5 and 26 km from the nearest known pollen source (thought to be due to normal events but difficult to verify). Insects (including bees and pollen beetles) are the main agents of long distance pollination. The study reports that male-sterile 'bait' plants overestimate crossing in male fertile plants by an order of magnitude.

The study adds detail but does not materially affect the conclusions in Section 7.2 of our First Report: that separation will not guarantee seed purity, but might be used to reduce cross pollination levels to below 0.1%.

2.2.5 Defra (2003b) Gene flow monitoring from the GM crop FSE sites: monitoring gene flow from the GM crop to non-GM equivalent crops in the vicinity. Part 1: forage maize. Research Report EPG 1/5/138
<http://www.defra.gov.uk/environment/gm/research/epg-1-5-138.htm>

This study takes advantage of the GM Crop Farm-Scale Evaluations (FSEs) to monitor actual rates of hybridisation in the non-GM crops adjacent to the GM herbicide-tolerant (GMHT) maize crops. The findings are in line with expectations and confirm the statements in our First Report (Section 7.2). Rapid decreases in cross-pollination occurred within 20 metres and low levels were found at 80 and 200 metre separation distances. Patterns varied from site to site, but not significantly across sites between years. Separation distances of 24.5 metres would be required to reduce GM presence below a 0.9% threshold, and 80 metres is sufficient to achieve a threshold below 0.3% in maize crops.

2.2.6 Defra (2003c) The potential for oilseed rape feral (volunteer) weeds to cause impurities in later oilseed rape crops. Research Report RG0114
<http://www.defra.gov.uk/environment/gm/research/epg-rg0114.htm>

The Scottish Crop Research Institute and Central Science Laboratory undertook this study. The project examined whether feral OSR could persist in the environment for long enough and in high enough numbers to cause impurities in later crops.

There was a range of scientific objectives for the project. However, they can be summarised as follows:

- (1) To consult widely with the industry and review the literature to define the perceived implications of introducing GM crops and the current situation with feral OSR.
- (2) To develop existing model systems and operate them to produce output to demonstrate likely implications to the seedbank, emerging plants and harvested yield over a series of years.
- (3) To compare the outputs of the model with measurements on feral populations in the field.

Feral OSR has become common in arable seedbanks since its introduction in the 1970s and appears to have persisted in some cases for 10 years. The typical seedbank population density of feral OSR is 100 m⁻², which is small when compared to total weed seedbanks, but similar to the established stand density of an OSR crop. With OSR grown only every two to four years, as a break crop in a cereal rotation, and if only 1/100th of the feral seedbank germinated it would have a large impact on the impurity in the OSR crop (with a current EU threshold of 0.9%).

There is currently little information available on the persistence time and population density of feral OSR arising from GM varieties. Therefore, the models were developed and employed to examine the effects of various management practices on impurity in the seedbank, emerging feral plants and harvested yield.

The study modelled the arable system of two years of winter wheat and one year of winter OSR, with no attempt to control the feral population after the first harvest. It took 16 years after the initial OSR crop for impurity in yield to fall below 1%. However, the sensitivity of the system is such that if maximum germination were reduced from 90% to 80% the impurity after 16 years would be 10%. However, these suggested outcomes need to be seen as what could occur but would be unlikely in practice. It is highly unlikely that broadleaf weeds (including feral OSR) would not be managed during the winter wheat years of the rotation, except in field margins, which are not included in the model.

Where rigorous controls of the feral OSR were introduced into the model (involving complete eradication of emerged plants and prevention of seed production during wheat crops), the seedbank contamination, interpreted as yield contamination, was 0.01% after the 16th year and below the 1% threshold by the 10th year. Reducing seed lost at harvest from 5% to 1% could achieve this threshold within five years. However, as impurities also arise through sown seed, by gene flows between fields, and via survival of volunteer plants in field margins, thresholds of this order and timeframe would be difficult to attain in practice.

The model is being tested and refined against data collected in fields in which GM crops have been grown, ie the FSEs.

This study adds to the evidence discussed in Section 7.2.3 of our First Report, but does not change our original conclusions on crop to crop gene flow.

2.2.7 Defra (2003d) Modelling the effects on farmland food webs of herbicide and insecticide management in the agricultural ecosystem. Research Report EPG 1/5/188
<http://www.defra.gov.uk/environment/gm/research/epg-1-5-188.htm>

The objective of this report is to provide the framework for predicting the consequences of changes in farmland management and especially the introduction of GMHT crops. It also points out the areas where there are gaps in our knowledge and where further information is required.

It reviews the available literature on factors determining the seed and invertebrate availability for farmland birds; summarises the available information on the dynamics of the common farmland weeds; reviews the diet of each farmland bird species and collates the available information on functional responses.

It reviews the possible approaches for predicting the responses to the changes in agricultural practice such as the introduction of GMHT crops, changes in rotation and changes in the abundance of stubbles.

The main model within the report incorporates six weed species (*Alepecurus myosuroides*, *Chenopodium album*, *Fallopia convolvulus*, *Papaver rhoeas*, *Poa annua*, and *Stellaria media*)

within three possible rotations and with varying degrees of overwinter stubble. It then explores the consequences of changes in weed seed density on the abundance of generalised granivorous birds.

The report was written prior to the release of the FSE data, so the approach taken was to consider the extreme case of 100% weed mortality associated with GMHT crops in order to provide an upper bound on the possible response. The next stage is clearly to incorporate the full FSE data within such models. Such an approach is clearly essential to determine the long-term consequences of GMHT crops and place these changes within the context of other changes in the landscape such as agri-environment schemes.

We considered the ‘new weed control strategies offered by GM herbicide-tolerant crops’ in Section 6.5 of our First Report and we noted the need for more studies on the impact of GMHT crops on farmland biodiversity in the UK. Since then the FSE results that have been published have helped to address this lack of knowledge (see Section 3). This study provides the basis for further work to help reduce uncertainty in this area.

2.2.8 Jackson AL, Bartz SR, Schelter J, Kobayashi SV, Burchard J, Mao M, Li B, Cavet G & Linsley PS (2003) Expression profiling reveals off-target gene regulation by RNAi. *Nature Biotechnology*, 21, 635-637, Brief Communications

Abstract: ‘RNA interference is thought to require near-identity between the small interfering RNA (siRNA) and its cognate messenger RNA (mRNA). Here, we used gene expression profiling to characterize the specificity of gene silencing by siRNAs in cultured human cells. Transcript profiles revealed siRNA-specific rather than target-specific signatures, including direct silencing of non-targeted genes containing as few as eleven contiguous nucleotides of identity to the siRNA. These results demonstrate that siRNAs may cross-react with targets of limited sequence similarity.’

Whilst this paper addresses siRNA in mammalian systems it draws attention to the possibility that the expression of genes other than the intended target may also be inhibited. In some cases this was explained on the basis of chance sequence similarity. 21-23 nucleotide siRNA has been widely used for inactivating gene expression in mammalian cells because introduction of large RNA molecules into mammalian cells often triggers cell death (apoptosis). In this regard, it is not unexpected that such small siRNA could silence off-target gene expression. However, this is not really relevant in plant systems where, broadly, the RNA-interference (RNAi) approach is used to silence a specific target gene. We mention gene silencing and RNAi in our First Report, mainly in Section 7.5, and this study does not change our conclusions.

Although specific RNA silencing can be induced by the shortest siRNA of 23 nucleotides in plant cells, suppression of plant gene expression is always achieved by long sequences (often more than a hundred nucleotides, and in many cases the whole target gene sequence), which have high specificity to the target gene. As a consequence, no off-target gene down-regulation by RNA silencing has been reported in plants, as the long RNAi molecules do not signal cell death in plants. For example, 700-nucleotide green fluorescent protein (GFP) works well in plants to silence a GFP gene but would kill animal cells. A good example of the use of RNAi in plant systems is the development of delayed ripening tomatoes in which the gene for

polygalacturonase is targeted so as to reduce pectin degradation during the fruit ripening process.

2.2.9 Kleter GA & Peijnenburg AACM (2003) Presence of potential allergy-related linear epitopes in novel proteins from conventional crops and the implication for the safety assessment of these crops with respect to the current testing of genetically modified crops. *Plant Biotech. J*, 1, 371-380

This paper by Kleter and Peijnenburg indicates that there may be allergy-related linear epitopes in proteins in the mitochondria of non-GM male sterile plants due to rearrangements within the mitochondrial genome. Most of the protein sequences used in this paper had been predicted based on DNA sequences. In mitochondria, however, many messenger RNAs (mRNAs) are modified ('edited') so that the sequence of the DNA does not at all represent the actual sequence of the mRNA and the protein. The authors therefore also included protein sequences derived from the edited forms of the corresponding mRNAs that had previously been described in literature. If one analyses the sequences of the DNA of the mitochondria of the male sterile plants examples can be found of stretches of six or seven amino acids that are identical to linear epitopes in some recognised allergic proteins. Essentially, the paper makes the point that these potential proteins are not subject to the same scrutiny as GM transgenic proteins. Furthermore, in our First Report we did indicate that conventionally bred crops are not subject to the same degree of scrutiny as GM ones. So, this paper does not appear to shed new light on the allergenicity issue.¹

2.2.10 Lin H-X, Rubio L, Smythe A, Jiminez M & Falk BW (2003) Genetic diversity and biological variation among California isolates of Cucumber mosaic virus. *J. Gen. Virology*, 84, 249-258

The data presented in this paper provide further evidence in support of the wider molecular virological principles and conclusions contained in our First Report (Section 7.5, pp. 235-249).

GM plants resistant to virus diseases have been available since 1986 and, despite numerous actual research and commercial benefits, concerns have been raised over the possible development or emergence of new viruses that could overcome the genetically engineered resistance and affect virus and/or host plant ecology. It has been proposed that this could occur either through genetic recombination between an infecting mutant or related virus and the virus-derived transgene in the plant; or that a pre-existing natural sub-population of the target virus could exploit the new niche created by the GM resistant plants. Alternatively, since RNA viruses have a high rate of error-prone replication and hence genetic variation/evolution, the resistant GM plant could exert a strong selection pressure on the replicating population of viral RNAs (itself a 'quasi-species') to favour one sequence variant which then dominates the virus population. This detailed and systematic study by Lin *et al.* addresses these possible scenarios.

Cucumber mosaic virus (CMV) is one of the most economically important plant viruses, with a wide range of hosts in over 365 genera, in over 85 families. CMV is endemic in all cucurbit-growing areas of the world and effective 'conventional' genetic resistance is lacking in most varieties. Transgenic yellow crookneck squash plants expressing the coat protein (CP) genes

of Zucchini yellow mosaic virus and Watermelon mosaic virus ('Prelude II'), and CMV strain C ('Destiny III'/'Liberator III') were developed and marketed in 1995 and have shown good field resistance against their target (two or three) viruses.

No symptoms were seen, or CMV recovered from any transgenic Destiny III or Liberato III plant in two separate replicated field trials in 1999, despite widespread CMV-infections in adjacent non-GM susceptible or naturally resistant cucurbits, or in GM squash plants resistant to viruses other than CMV. Thus, 63 field isolates of CMV were obtained and compared with three 'standard' CMV strains (Fny, Q and Ls) and 18 CMV field isolates collected between 1985-1994 (before transgenic CMV-resistant squashes were available) from various plants and areas of California. All 81 field isolates were later used for extensive CMV CP gene and downstream RNA sequence comparisons by single-strand (RNA) conformation polymorphism (SSCP); however, only 68 of the isolates could be purified from other co-infecting plant viruses.

The packaged genome of CMV consists of three different RNA components, a sub-genomic RNA that codes for the coat protein (CP), and (occasionally) one of several small 'parasitic' satellite RNAs. CMV thus has a complex genetic structure ideally suited to all manner of genetic reassortment, recombination and variation/mutation during RNA replication and/or (mixed) infections in susceptible cells. It is therefore notable that the authors found no correlation between CP gene sequence, geographical origin, collection year, original host plant species, or the ability of an isolate to overcome 'conventional' or transgenic resistance in melon or squash.

The genetic diversity of all 81 Californian field isolates of CMV was analysed by SSCP, which classified them into 14 groups.

The 68 purified CMV isolates fell into five pathotypes based on their ability to infect and cause particular symptoms on three different cucurbit test plants (Dixie, a susceptible yellow crookneck squash; Destiny III (CMV-C CP transgenic Dixie) and Freeman (a conventional resistant cucumber variety)). The authors showed that 33 out of the 68 purified Californian field isolates of CMV could indeed infect young transgenic yellow crookneck squash plants ('Destiny III') when inoculated mechanically, at artificially high virus concentrations (c.f. normal aphid transmission). Significantly, 16 of these 33 isolates had been collected and stored between 1985-1994, before any genetic selection pressure to evade GM resistance existed. The remaining 17 isolates came from plants infected naturally in the field trials described above. No association was found between CMV CP gene sequence and biological characteristics such as the ability to infect natural (Freeman) or GM (Destiny III) resistant plants.

SSCP analysis was shown to be very sensitive for minor sequence variations, and that 32 out of the 33 CMV isolates were indistinguishable for a given isolate whether it came from Dixie (non-GM) or Destiny III (GM) isogenic hybrid pumpkins. One isolate (CK41) did show different but overlapping SSCP profiles between the two pumpkin types that may have been due to some selective effect of the transgenic resistance. However, further detailed analysis of CMV CP sequences from different plants revealed that the original CK41 had consisted of two sequence variants (differing by 7 nucleotides) in the CP gene region. One variant (A) dominated in both GM and non-GM pumpkin, while the other (B) appeared in conventionally resistant (Freeman) plants.

Thus the authors conclude that the anomalous SSCP profile seen for CK41-infected GM Destiny III plants was not due to recombination between the transgene (CMV-C CP) and the infecting virus. (CMV-C and CK41 in fact differ in their CP genes by 4.06 nucleotides per 100 nucleotides, as the authors show.) Nor was CK41 due to any selection pressure by the transgenic resistance because the same sequence changes were seen in non-GM pumpkin plants. CK41 most likely arose in the field through a mixed infection by two CMV isolates.

The conclusions presented in Section 7.5 of our First Report remain valid and are supported by these recent data.

2.2.11 Romeis J, Battini M & Bigler F (2003) Transgenic wheat with enhanced fungal resistance causes no effects on *Folsomia candida* (Collembola: Isotomidae). *Pedobiologia*, 47, 141-147

In our First Report we made the point that effects of transgenes on non-target organisms should be investigated before commercial release (Section 6.3, Executive Summary page 14, and pp. 119-135). And we identified the need for better protocols to test, on a case-by-case basis, GM crop impacts on non-target species. This paper presents an example of this type of study. A virus of the fungus maize smut (*Ustilago maydis*) carries a gene for an anti-fungal protein (KP4), which has been transferred to transgenic wheat and gives the plants resistance to smut. Romeis *et al.* carry out a series of bioassays and a glasshouse plant study to see if the transgenic wheats have any effect on *Folsomia candida*, a representative of an important group of soil microarthropods that decompose organic matter in soil and are exposed to crop residues. A number of 'very sensitive parameters' including insect development, egg cluster size and egg viability were measured. None of the parameters were affected when feeding on dried roots of KP4-transgenic wheat compared to untransformed controls, and there were no differences in the arthropod population development in pots of transgenic and control wheat grown in the glasshouse. Differences were detected between wheat varieties and between feeding on wheat and yeast.

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

This is a very comprehensive review of the area of our First Report covered in Chapter 7.3 and is very similar in emphasis. It explains the differences between hybridisation, gene flow and introgression and discusses the natural barriers to transgene introgression from crops to wild relatives (including linkage to genes of domestication). Crops are categorised into very low, low, moderate and high risk of introgression and the potential and merits of various techniques for reducing gene flow are discussed.

Although a review, covering the same literature as in our First Report, Stewart *et al.* confirm that Warwick *et al.* (2003) (in press when we produced our First Report) have produced the first evidence of transgene escape to a wild relative from a commercially released crop (herbicide tolerance from *B. napus* to weedy *B. rapa* in Canada). They also report a new study (Burke & Rieseberg, 2003) of the consequences of introgression of a fitness-associated transgene into a wild relative (sunflowers), which showed that a disease resistance transgene did not increase the fitness of a wild plant.

2.2.13 Tepfer D, Garcia-Gonzales R, Mansouri H, Seruga M, Message B, Leach F, & Perica MC (2003) Homology-dependent DNA transfer from plants to a soil bacterium under laboratory conditions: implications in evolution and horizontal gene transfer. *Transgenic Research*, 12, 425-437

This paper describes DNA transfer from six species of donor plants to the soil bacterium, *Acinetobacter* spp. using *nptII* as a marker for homologous recombination. Transfer was detected for both nuclear and plastid insertions of *nptII*, using intact tobacco leaves and intact tobacco and Arabidopsis plants *in vitro*. Transfer varied with plant genome size and the number of repeats of the transgenic DNA in the donor plant. Most importantly, transfer was not detected in the absence of a homologous *nptII* in the receptor bacteria. This observation extends the reports cited in our First Report, but it does not change the conclusion that marker rescue is a mechanism for the recovery of plant to bacterium gene transfer events. The dependence on DNA homology in the recipient bacteria is most important and fully addressed in our First Report.

2.2.14 Vlasák J, Šmahel M, Pavlík A, Pavingerová D & Bříza J (2003) Comparison of hCMV immediate early and CaMV 35S promoters in both plant and human cells. *J. Biotechnol.* 103, 197-202

It has been suggested that if the gene transcription promoter sequence (p35S) from a natural, common plant DNA virus (*Cauliflower mosaic virus* (CaMV)) was active in animal or human cells, then it may pose certain unique health risks. And, that these risks would be distinct from any risks created by our ingestion of all other food DNA-derived genes and/or promoter sequences. For example, if the CaMV p35S sequence could be taken up and functionally integrated into the chromosomal DNA of human or animal cells *via* ingestion of (unprocessed) transgenic (or CaMV-infected) plant food could it:

- (1) inadvertently activate or enhance expression of growth regulator genes that may be located at the site of integration leading to possible hyperplasia/malignancy;
- (2) reactivate dormant viruses; or
- (3) recombine resulting in viruses with novel phenotypes (Ho *et al.* 1999)?

These proposals were discussed in our First Report (Section 7.5.3, pp. 244-245) and this paper is relevant to our response to the comments that we received on the significance of 'recombination hotspots' in Section 4.3 of this report.

Vlasák and colleagues cite earlier studies which have shown that the CaMV p35S sequence is active to varying degrees in all plant cells, in green algae, in yeast and in *Escherichia coli*, as well as in amphibian (*Xenopus laevis*) oocytes. In toad oocytes, various plant gene promoters, including p35S, and poly(A) signals were tested. p35S was found to be almost as efficient in driving reporter gene transcription as the mammalian SV40 promoter (Ballas *et al.* 1989). CaMV p35S was also found to be more efficient than the adenovirus-2 late promoter in HeLa cell-free transcription extracts (Burke *et al.* 1990). However, as both oocytes and HeLa cell

extracts have anomalous gene transcription activities, the authors conclude that more ‘typical’ animal cells should be studied.

Vlasák and colleagues report on the relative levels of expression (transcription and translation) of a series of CaMV p35S and human cytomegalovirus (hCMV) DNA constructs which incorporated a popular reporter gene, bacterial β -glucuronidase (GUS). Transient transfection assays were used to compare the efficiency of expression of each DNA construct in potato leaf protoplasts and in human 293T kidney epithelial-type cells. Their data show that CaMV p35S-GUS activity was detectable in 293T cells, although at a level 4 orders of magnitude (i.e. 10,000 times) less than that obtained with the hCMV-GUS constructs. More unexpected and notable was the observation that, in potato leaf protoplasts, the hCMV promoter was only 2 orders of magnitude (i.e. 100 times) less active than the highly plant-active CaMV p35S promoter sequence.

The authors conclude by saying their work ‘indicates that the potential hazards associated with the use of (CaMV) p35S may not be so serious as it is sometimes maintained’. The authors continue to say ‘it is questionable whether (the) relatively low transcription activity of (the) CaMV 35S promoter can induce such hazardous events in mammalian cells.’

The data presented are clear and convincing and add interesting new information to the field. However, the study also inevitably raises a number of new questions, such as: how, and why, is the hCMV promoter so active in plant cells; and what is the biological significance, if any, of this result?

Pairwise comparisons of each promoter-GUS gene construct were performed in three replicate experiments in each cell type. Absolute levels of GUS expression varied 2- to 4-fold between experiments, thus only relative levels of GUS gene expression are presented (+/- standard deviations). There was no quantification of the absolute or relative amounts of plasmid DNA (used at 2 μ g/ μ l) transfected into each cell sample using quantitative PCR, or by using a common promoter-reference gene construct, or by expression levels of a marker transcript. Some other points to bear in mind are:

- (1) The CaMV p35S promoter ‘showed very low but measurable activity...in human 293T-cells (0.01% activity of that revealed when using hCMV)’. In human 293 cells, without the SV40 T-antigen, both p35S and hCMV promoters gave 10- to 20-times less GUS activity than in 293T cells. Nevertheless, these transient (non-integrated DNA) assays clearly confirm that CaMV p35S can have some detectable activity in animal cells.
- (2) The hCMV promoter is generally known to be the most efficient constitutive control element used in transient transfection assays in mammalian cells as evidenced by its broad use in commercially available gene expression vectors and its use to drive therapeutic gene expression within a human gene therapy context. Likewise, in most plant cells, the CaMV p35S promoter is known to be one of the most active, constitutive promoters available, hence its widespread use in transgenic plants. CaMV-driven transgene transcription (and optimal mRNA translation) typically produce a new protein at between 0.01-0.5% of total plant cell protein. Thus differences in the relative levels of activity of p35S and hCMV may be exaggerated, as each represents a highly efficient promoter in its own host cell types. Moreover, many endogenous housekeeping gene promoters compare very poorly in activity with

either CaMV p35S or hCMV, even in their homologous host cells. Thus, even very low levels of heterologous promoter-driven expression may be physiologically relevant, depending on the nature of the new gene expressed or sequence affected.

- (3) As already noted for HeLa cell extracts and *Xenopus* oocytes, transcription factor activity varies greatly between cell types. It is therefore possible that CaMV p35S may function more efficiently in some animal cell types than in others, as it does for example in different plant cell types (meristem vs vascular vs mesophyll cells) *in vivo*. Thus, it would have been useful if more than one human or animal cell line had been incorporated in this type of analysis (i.e. more than 293 and 293T cells). Since the primary point of contact of intact or fragmented CaMV p35S-containing GM plant material would be various cell-types lining the GI tract, studies using a model for gut epithelial cells (e.g. Caco-2 cells) would have been appropriate. The unexpectedly high relative level of hCMV promoter activity in potato leaf protoplasts (and other plant cell types?) illustrates the unpredictability of such events and the need for sound experimental data.
- (4) Although encouraging and interesting, these studies used convenient and relatively rapid transient transfection assays, in which various plasmid DNAs harbouring the gene constructs under test were not stably integrated into the heritable (nuclear) chromosomal DNA of the host cell (i.e. they all remained episomal). Assays measuring the relative (in)activity of p35S-GUS, or hCMV-GUS (or any other gene control sequence) integrated into the DNA of a heterologous cell may be generally informative, but can never eliminate completely the remote chance that a site-specific integration event (in the DNA of an homologous or heterologous host cell) could generate an unexpected result. Indeed, it is well documented that in both 'traditional' mutation breeding and modern GM breeding, greater or lesser numbers (respectively) of progeny plants are discarded, having undesirable phenotypes (traits) attributed to unpredicted genetic events during production. Experiments such as those by Vlasak *et al.* can only ever confirm that the CaMV 35S promoter is intrinsically less active in animal/human cells than in plants, and hence likely to be less risky.

Although fundamentally dependent on the intrinsic level of activity of the CaMV p35S promoter, the hypothetical risks proposed above (Ho *et al.* 1999) also depend upon some detrimental site-specific integration event. The uptake, stable integration, functional activity and inheritance of any food-derived DNA sequence (functional or non-functional) remains speculative.

2.2.15 Wilkinson MJ, Elliott LJ, Allainguillaume J, Shaw MW, Norris C, Welters R, Alexander M, Sweet J, & Mason DC (2003) Hybridization between *Brassica napra* and *B. rapa* on a national scale in the United Kingdom. *Science*, 302, 457-459

This paper reports work referred to in our First Report (page 220) which attempts to provide an estimate of hybridisation rates in the UK between oilseed rape and the two forms of its wild relative *B. rapa*. Although rarer, hybridisation with the arable weedy ecotype are relatively higher than with the naturalised waterside form. The combination of methods used generate very high confidence ranges but, this aside, produce an order-of-magnitude estimate. Although absolute numbers of hybrids appear high (32,000 for waterside, 17,000 for weeds)

they represent around 0.04% of the wild *B. rapa* population. Most hybrids are produced in areas of sympatry (26,000 of the 32,000 waterside form) suggesting that regional variation could be exploited to reduce gene flow.

This study does not address the fate of the transgene (i.e. of plants bearing the transgene) in the new habitat into which it has been dispersed. Hybridisation is but the first step and estimates of hybrid fitness (dependent on the gene) and of introgression are important next steps. The number of hybrids found in this study represents a very small proportion of the wild *B. Rapa* population, but fitness and introgression are more important than initial numbers. Backcrossing, for example, would put the transgene into a genetic background that is increasingly dominated by wild-type genes and thus more likely to have increased fitness. Whether the transgene increases in frequency or the plants bearing the gene increase in abundance would need to be addressed by comparative field experiments over several years in the natural habitats in question.

¹ **Erratum**

The text of Section 2.2.9 is a revised version of that originally published. The authors of the paper considered in this Section had pointed out that the text in the original report did not properly reflect the content of their paper and it was agreed that it should be changed. This revision does not, however, change any of the overall conclusions or the conclusions on allergenicity in the Report.

Section 3

THE GM CROP FARM SCALE EVALUATIONS

3.1 INTRODUCTION

The first part of this Section briefly describes the research and some of the findings reported by the GM Crop Farm-Scale Evaluation (FSE) research team. This is followed by the Panel's interpretation of the evidence, followed by commentary on the broad implications of this work.

3.2 THE ISSUE

In Section 6.5 of our First Report, we drew attention to a lack of detailed knowledge of the potential impacts on biodiversity following the introduction of GM herbicide tolerant (GMHT) crops. Studies that systematically compared the effects of GMHT and conventional management regimes were available, but usually confined to small-scale studies at research stations or observations in commercial fields. At least five studies had shown that using GMHT crops generally increased the efficiency and reliability of weed control in maize, beet, and oilseed rape (Read & Bush 1998, Strandberg & Pederson 2002, Wevers 1998, Read & Ball 1999a, Read & Ball 1999b). Only one commentary (Firbank & Forcella 2000) suggested that weed control was sometimes less efficient than conventional methods when GMHT varieties were used. The Panel also raised the possibility that the use of GMHT cropping systems could have an impact on spray damage to field boundary and hedgerow biodiversity.

The First Report also drew attention to potential impacts of GMHT cropping on taxa other than plants. In this case, there was little scientific information, although some research was available on possible impacts of delayed herbicide spraying of GMHT crops on insect populations. A modelling study had investigated potential impacts of changes in herbicide use associated with GMHT crops upon skylarks.

Section 6.5 of our First Report identified as an important issue whether GMHT management would be more or less harmful to wildlife than conventional cropping, and posed the key questions:

'Will delayed herbicide applications in GMHT crops allow more weeds, more invertebrates and, for example, improved breeding productivity of birds? Or will the efficiency and reliability of weed control mean fewer resources for seed predators such as granivorous birds, and declining weed populations in the long term?'

In Section 6.5.4 of our First Report, we reported that there is general agreement that there have been substantial declines in biodiversity in the UK in recent decades and that the evidence is stronger for birds and plants than for invertebrates. However, there is growing scientific acceptance that these declines have been caused by agricultural intensification. We noted that there is less evidence (particularly environmental evidence), and therefore less general agreement, to indicate the relative contributions of herbicides *per se* in these declines;

but that there is, however, general agreement that declines in weed seed resources have played a major role in the dramatic declines of seed-eating farmland birds.

In Section 6.5.7, we looked forward to the outcome of the UK Government-funded FSEs of GMHT crops to help resolve this issue. This study has been running for four years, investigating the impacts on biodiversity of herbicide regimes associated with GMHT maize, beet and oilseed rape compared to herbicide application on non-GM crops. The results of the studies on spring-sown varieties of these crops are now available; the results of research on winter-sown oilseed rape to follow next year.

3.3 THE RESEARCH

The FSEs are the largest manipulative experiment ever carried out on farmland ecology anywhere in the world, exceeding by more than threefold any comparable experiment undertaken previously (Perry *et al.* 2003). The researchers, a consortium of experienced ecologists and statisticians overseen by an independent steering committee, organised the planting of 273 trial fields around England and Scotland. Data from 201 fields were eventually used in the final analyses. Each field was divided into two: half was sown with the GMHT crop and half with its conventional equivalent. To avoid experimental bias, treatments were allocated randomly to field halves. Farmers managed the fields using their normal herbicide regime on the conventional half, and a spray regime on the GMHT part that was consistent with cost-effective weed control, based on recommendations from the agrochemical manufacturers and an industry body (SCIMAC). The researchers checked that these management practices were being carried out properly, and that herbicide applications on the conventional parts of fields represented normal farming practice.

Crops were grown as part of farmers' normal crop rotations. These three crops are 'break' crops, usually grown as part of a rotation that includes other crops such as wheat or barley. This means that the GM crop was often grown for only one year in a specific field, with the exception of a few maize sites where continuous cropping was practised.

Within the fields, researchers measured weed diversity and abundance, seed rain and weed seed banks. Assessments were also made of weed seed banks and weed seedlings in plots following the GMHT management. Within the crops, various standard methods were used to assess the relative abundance and diversity of invertebrates, including slugs and snails, insects and spiders. Bee and butterfly transects were also used to assess the foraging preferences of these insects. Plants and invertebrates were also assessed in field margins, field verges and in field boundaries such as hedges and ditches where these were present. Estimates of crop cover and development were also made to assess whether any differences between the characteristics of the GM and conventional crops *per se* could be affecting the results.

The strengths of this experimental design are:

- High statistical power derived from the large number of sites and split field design. This meant that the experiment was able to detect relative small changes in numbers of organisms affected by the different herbicide applications. The overall size of the trials was determined by a 'power analysis'. This used existing data on weed and invertebrate numbers and their variability to arrive at a target of 65 fields for each crop type. The targets were met over the duration of the experiment. The numbers of fields

were set to detect over 80% of 1.5 fold differences in abundance between treatments at a statistically significant level; in the event 82% of such differences were significant, fully in line with the targets.

- The wide variety of organisms sampled and measured (1.5 million invertebrates and 0.5 million seeds). Statistical analyses were not only able to detect variation in different taxa between treatments, but could also be analysed by looking at the effects of treatments on species grouped by trophic (feeding) level, revealing impacts on food webs.
- Field management was carried out by a group of farmers representative of the range of cropping practices used in different regions of the UK. This was more likely to reflect the realities of any commercial introduction of GMHT crops than previous small-scale experiments confined to research stations.
- A wide variety of sites reflecting different farming intensities were selected over a wide geographic range, spread over several years with varying weather conditions.

3.4 THE RESULTS

The results were published in October 2003 as a series of papers in a theme issue of the *Philosophical Transactions of the Royal Society* (Squire *et al.* 2003; Champion *et al.* 2003; Heard *et al.* 2003a & 2003b; Brookes *et al.* 2003; Haughton *et al.* 2003; Roy *et al.* 2003; Hawes *et al.* 2003). This group has also published a commentary on the implications of their work (ISBN 0-85521-036-2).

The results were remarkably consistent and clear. The results showed overall, that animal and plant life was most abundant in conventional oilseed rape fields, with more butterflies, weeds and seeds in these fields than in those of GMHT rape, where a different herbicide was used. A similar picture emerged for biodiversity in beet fields, but here bees were significantly less abundant on the GMHT side. There were, however, more springtails, small insects feeding on plant debris, in GMHT beet and rape fields in summer than in the conventional fields. The research showed that these were feeding on dead weeds after the GM crops had been sprayed. A ground beetle that feeds on springtails was also more abundant in the GMHT beet and rape fields.

In maize fields a contrasting picture has emerged from the research. Conventional maize fields (where a powerful residual herbicide, atrazine, is commonly used) were found to be the least abundant in animal and plant life, with relatively more weeds, seeds, and insects occurring in the GMHT maize.

These results were consistent not only from year-to-year but also from area-to-area, indicating that how farmers manage their fields has a far greater effect on biodiversity than variations in weather or soil-type. The results showed that conventional beet and spring rape crops were in general, more abundant in plants, seeds and animals than the GMHT crops because the broad-spectrum herbicides used on the GM crops were more effective at controlling weeds than the selective herbicides used on conventional crops. Conversely, the residual herbicide regimes used in conventional maize are more effective, in general, at controlling weeds than the broad-spectrum herbicide applied over the GMHT maize.

Analysis of the impacts of the different herbicides on trophic levels within fields demonstrated that effects on weeds were reflected through food webs, with reductions in weeds causing reductions in invertebrates feeding on plants and consequent reductions in predators, although not all the organisms sampled in these studies declined. In maize and oilseed rape fields, the effects of GMHT management regimes on invertebrates were less marked than in beet.

Field margins can support a high diversity of plant species and are important for conservation within farmed landscapes in Europe. Margin vegetation was recorded in three components of the field margin. No marked effects of GMHT crops were found on plants and invertebrates living in the field verge or boundary. Effects in the tilled margins of fields were similar to those recorded within the crop, as they were subject to the same herbicide regimes. There was a significant reduction in seed-producing weeds, flowering plants and butterflies (in July) in the tilled margins of GMHT beet and oilseed rape. The effect was reversed in maize fields, where significant increases in weeds and flowering plants were recorded in the margins of GMHT fields. Effects on butterflies mirrored the effects on vegetation. The likely cause is the lower nectar supply in GMHT field margins and cropped edges. Few large differences were found for bees, gastropods or other invertebrates. Scorching of vegetation by herbicide spray drift was significantly higher on field verges adjacent to all three GMHT crops, although the areas affected were very small in relation to the total length of verge.

Figure 1 below provides a star plot containing mean values of major biodiversity indicators across conventional and GMHT of beet, maize and spring oilseed rape.

3.5 THE PANEL'S VIEW ON THE QUALITY OF THE EVIDENCE FROM THE FSES

The FSEs are one of the largest ecological experiments that have taken place on farmland. The Panel agrees that the data sets produced by this research are unusually large and that the statistical methods used both for design and analyses were valid and robust. The FSEs give a clear picture of the changes in biodiversity caused by the different herbicide regimes used on GMHT and conventional crops of maize, beet and spring oilseed rape.

The design of the FSE experiments attempted to capture the current range and intensities of farming practices across the UK. Given that the sites used in these experiments varied greatly in species composition, geographic location and crop management, we agree that the effects of GMHT management on biodiversity are a fair representation of what would actually happen if widespread adoption of GMHT crops and weed management regimes were to take place as set out in the FSEs.

The researchers conclude that because there were significant differences between treatments for each crop, but the effects were not the same for each crop, there was no evidence that treatment effects had arisen because the crops had been produced using transgenic technology. In addition, there were no significant effects on crop pests (rather than those that lived on weeds) suggesting that the crop itself had no effect on invertebrates. They showed that the differences could be explained entirely by the effects of contrasting herbicide regimes used on GMHT and conventional crops. We agree with this and therefore note that the conclusions of the experiment would apply equally if similar herbicide tolerance were to be

introduced into these crops using other forms of plant breeding, such as mutation breeding or marker-assisted breeding that are not regulated in the same way as transgenic technologies.

3.6 IMPLICATIONS FOR FARMLAND BIODIVERSITY

3.6.1 What we know

The results show that the adoption of GMHT beet and oilseed rape, if managed as they were in the FSEs, would result in fewer weeds, seeds, butterflies and bees (bee activity was only significantly lower in beet) in and around these fields. Not only was there a significant reduction in weeds in these crops, but also a large reduction in weed seed production and return of seeds to the soil, especially seeds from broad-leaved plants. These differences, if compounded over time, could result in a large decrease in population densities of arable weeds. On the basis of the available seed bank data, Heard *et al.* (2003b) conclude that there is the potential for an accelerated decline in the abundance of weed seed species under GMHT beet and spring oilseed rape management, in the order of an additional 7% per year for arable rotations¹, over and above the current generally accepted annual 3% decline in weed seed banks in the UK since the 1940s.

The results are clear and show that overall, GMHT beet and spring oilseed rape crops, if managed as they were in the FSEs, would provide fewer nectar resources for pollinating insects (bees and butterflies) and fewer weed seed resources for granivorous birds.

By contrast, GMHT maize resulted in more weeds in this crop in summer. This could result in more food resources for birds in and around GMHT maize fields, and raises the prospect of leaving weedy stubbles following maize cultivation, with potential benefits to wintering wildlife. Weedy stubbles do not usually result from our current atrazine-based weed control in conventional maize.

3.6.2 Uncertainty

The main uncertainties that remain concerning the impact of these GMHT crops on farmland biodiversity (were they to be given commercial approval in the EU) are the degree of uptake of the crops by farmers (acreage and distribution); the nature of the farms involved (e.g. would participating farmers tend to be from farms with current high or low weed burdens?); and how closely any future management of these crops mimic those studied in the FSEs (e.g. in particular, the similarity or otherwise of herbicide regimes). The significance of the impact on wildlife will also be dependent upon the wider landscape setting.

It is therefore not possible to predict the scale of potential effects at the current time. However, the evidence from the FSEs suggest that the herbicide regime associated with the large scale cropping of GMHT maize, compared with conventional maize, could be of benefit to farmland wildlife, with increased levels of weeds that may be of value to granivorous birds, whereas those associated with GMHT cropping of beet and spring oilseed rape will be of

¹ In a five course cereal rotation with a break crop grown every 5 years (e.g. *Watkinson et al.* 2000).

disbenefit compared with the conventional crops, providing fewer nectar resources for pollinators and fewer weed seed resources for granivorous birds.

It would be useful to develop ecological models using the raw data from the FSEs to investigate these issues further.

3.7 LOOKING TO THE FUTURE (What are the potential developments in this area and do they affect the Panel's conclusions?)

The interpretation of the maize results has been complicated somewhat by the recent announcement that atrazine will be banned within the EU because of its unfavourable environmental profile. Atrazine, a residual herbicide, was the most commonly used weed killer on conventional maize in the FSEs. It is possible that the herbicides that replace atrazine in maize will be as effective in weed control, but it is also possible that they will not. Further analysis to compare the biodiversity impacts of the GMHT maize management with the few conventionally cropped fields where atrazine was not used might be informative but, because of the low numbers of these fields, this may not be statistically sufficient.

The FSEs not only gave a clear and consistent answer to the important issue of impacts on biodiversity of GMHT cropping identified in our First Report, but also gave a deep insight into the wildlife that lives in and around the crops tested (Figure 1). This experiment was the first time that the impact on biodiversity of a novel cropping system has been assessed *before* large-scale commercial use of the system. The experimental protocol could be used not only to assess the wildlife impacts of future cropping systems but could also be used to look at the long-term impacts of existing systems such as winter cropping and general agrochemical applications on farmed land. The methods used in the FSEs could be used, for example, to assess the indirect impacts of herbicides and other agrochemicals used in conventional cropping, or to compare the biodiversity associated with conventional cropping to that in organic systems or other farming approaches such as integrated pest management (IPM). Analysis of the FSE data may suggest some alternative smaller-scale approaches for the future.

There have been vast changes to agricultural practice over the last 50 years – changes in crops, in farm management, rotations, change to autumn sown and spring sown crops. It is important to place GMHT crops in the context of past and future changes. The FSE data, and more that will follow, offer modelling opportunities to assess the longer term and large-scale implications of this work, and will contribute to informing debate on broader agricultural issues related to societal choices and the balance of natural resources.

Looking at the broad context, the results underscore how crop production and wildlife are irrevocably linked in farming. These trials give numerical possibilities in allowing us to measure, interpret and manipulate the balance between resources for human beings and for wildlife. Striking the balance between the landscapes we want and the food we need is a much broader issue that is beyond the immediate context of this review and would need to consider changes in crop rotation practice, hedge and headland management and a broad range of wildlife stewardship objectives.

3.8 CONCLUSIONS

If all else remains constant and the three crops are introduced and managed in the way they were in the trials, then for GMHT beet and spring oilseed rape a significant reduction would be expected in weed biomass and weed seed return resulting in fewer nectar resources for pollinators and fewer weed seed resources for granivorous birds. For GMHT maize the opposite is expected. These effects arise from the crop management regimes associated with these GMHT crops (i.e. the herbicide application) and are probably not a direct consequence of the way the crops have been bred.

These data, and more that will follow, offer modelling opportunities to assess the longer-term and large-scale implications of this work, and will contribute to inform debate on broader agricultural issues related to societal choices and the balance of natural resources.

3.9 RELEVANCE TO OTHER PARTS OF OUR FIRST REPORT

3.9.1 Herbicide applications, loads and effects

As mentioned in our First Report, there is no clear relationship between amounts of herbicide and biodiversity impact (Section 6.5.3). The FSE results provide an example where less herbicide was added to the GMHT beet and oilseed rape compared with the non-GM counterparts yet the impacts on certain classes of wildlife were greater.

In Section 6.5.6, of our First Report, we identified a gap in knowledge in terms of information on number of applications; the number of active ingredients used and the number of tractor passes needed, in comparison to conventional weed control systems. Evidence from North America was equivocal. For example, a review of various studies on glyphosate-resistant soybean cropping showed results varying between a 7% increase and a 40% decrease in total herbicide use with the HT crop (Hin *et. al.* 2001). The fewer passes over fields brings with it other potential environmental benefits such as reduced energy costs and emissions. We noted that the herbicide cropping regimes for the GMHT varieties in the FSEs required lower inputs to achieve similar or greater levels of weed reduction in terms of numbers of active ingredients and tractor passes. The reductions in the application of these chemicals may have advantages².

3.9.2 Relationship to other studies

In our First Report the potential benefits of the GMHT herbicide regimes in terms of simplicity of weed control, the flexibility of weed control and potential benefits and biodiversity gains were discussed (Section 6.5.3). Two separate studies (Strandberg & Pedersen 2002; Dewar *et al.* 2003) considered in our First Report (Section 6.5.3) suggested that by using GMHT beet, applications of broad spectrum herbicides could be delayed, leaving weeds in the fields for longer. It was suggested that this might benefit farmland birds because more weeds would yield more invertebrates for them to eat. The FSEs did not test

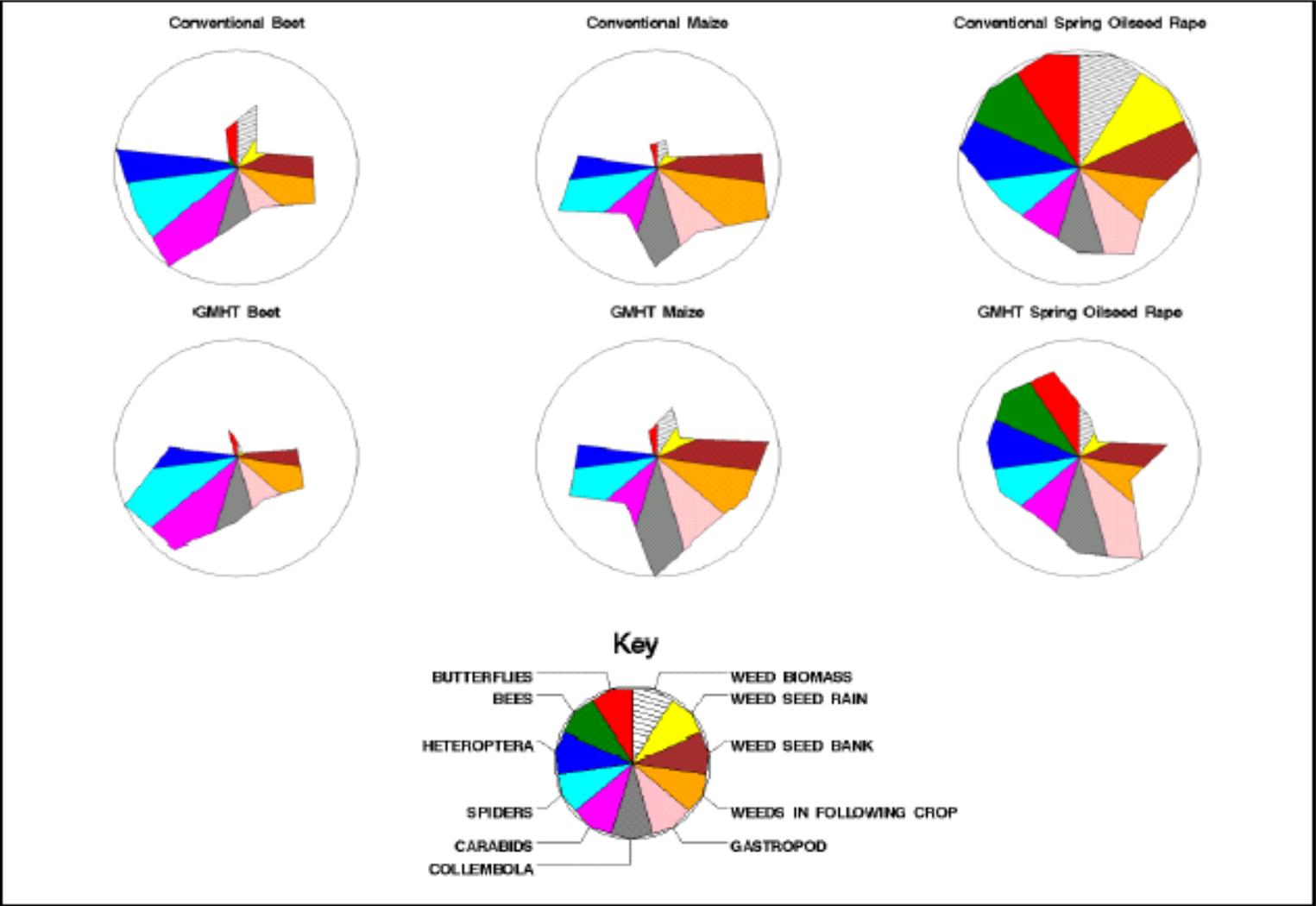
² E.g. environmental life cycle analyses might show energy savings, for example, in herbicide production, or fuel usage in tractor passes, or emissions. Organic farming may offer similar advantages over conventional systems.

this, because farmers were managing the GM parts of the fields to optimise crop yield, not biodiversity, and applied the herbicides earlier in the season. In these parts of the FSE fields, compared to the conventional parts, there were more springtails and their predators feeding on decaying weeds, but these occurred in late summer when breeding birds' chicks would be unlikely to be feeding in the fields. There was also very little weed seed available for wintering birds in the GMHT beet because although weeds were killed later than in conventional beet, mortality occurred before they had set seed.

3.9.3 Case-by-case assessment

Growing GMHT beet and oilseed rape had similar impacts on biodiversity despite the fact that they carried transgenes giving tolerance to two different herbicides, with beet tolerant to glyphosate, a translocated herbicide, and oilseed rape tolerant to glufosinate ammonium, a contact herbicide. That the conclusions of these experiments were different for different crops with GMHT traits reinforces our general conclusion in our First Report that impacts of GM crops must always be assessed case-by-case.

Figure 1: Star plots comparing mean values of major biodiversity indicators across conventional and GMHT treatments of beet, maize and spring oilseed rape crops³. For each indicator, the length of the star corresponds to the value relative to the maximum value found in any of the six combinations of crop and treatment; for example, the most gastropods were found in GMHT spring oilseed rape. The key diagram shows which section of the star plots star relates to which indicator.



³Reproduced with kind permission from Les Firbank from: 'The implications of spring-sown genetically modified herbicide-tolerant crops for farmland biodiversity: A commentary on the Farm Scale Evaluations of Spring Sown Crops' by L.G. Firbank *et. al.*

