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)

CONTENTS

Foreword by Sir David King, Government Chief Scientific Adviser

The GM Science Review was commissioned as part of the wider GM public dialogue by Mrs Margaret Beckett, the Secretary of State for the Environment, Food and Rural Affairs; with the agreement of the responsible Ministers in the devolved administrations. This report which supplements our earlier publication in July last year has, like the First Report, now been formally submitted to Mrs Margaret Beckett MP, Mr Allan Wilson MSP at the Scottish Executive, Mr Carwyn Jones AM at the National Assembly for Wales, and Mrs Angela Smith, Parliamentary Under Secretary of State at the Northern Ireland Office, to help inform government's decision making on GM crops and food.

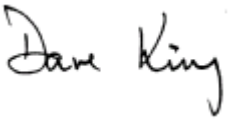
The Review has continued in its endeavour of taking an open look at the science relevant to GM crops and food, and to do so in a way that recognises the interests and concerns of the public as well as the science community.

The Science Review Panel's First Report was published in July 2003 and attracted wide public and media interest, both in the UK and abroad, with over 20,000 copies downloaded from the Review website. The Panel invited comments on the report and we entered the second phase of our Review. During this second phase, the Panel met on four occasions to discuss comments received on our First Report and the extent to which these altered our conclusions. We also examined the report of the GM Public Debate 'GM Nation?', to consider whether there were any further issues we should address and we also looked to see if there had been significant developments in GM science over the summer that we should report on. In particular, we considered the results of the UK Farm-Scale Evaluations of GM crops.

Once more, I extend my thanks to the Panel members for the time they have given and the cooperative way in which they have worked on this Second Report. As with the First Report, we have respected differences in views and recognised that that Panel members do not individually cover all the areas of expertise. On this basis, I am pleased to say that as with the First Report, the Panel has taken collective ownership of the Second Report.

On behalf of the Panel, I would also like to thank all those who submitted comments. We have sought to take account of your submissions. And I know that the Panel would wish to acknowledge the dedication of the Secretariat, in bringing this Second Report to print.

Finally, the publication of this Second Report marks the end of the GM Science Review and its Panel. Science moves on and the scientific debate over GM issues will continue. Scientific GM advisory committees such as the Advisory Committee on Releases to the Environment (ACRE), the Advisory Committee on Novel Food and Processes (ACNFP) and the Advisory Committee on Animal Feedingstuffs (ACAF) will continue in their task of advising the UK government on the safety of GM food and feed and impacts on the environment and we hope this Review facilitates their work. We also hope that we have succeeded at least in part, in our ambition of engaging in scientific issues of public interest to raise the level of discussion about GM matters in the UK. In conclusion, I note 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. I believe this initiative provides important lessons and a positive model for any such exercise in future.



22 January 2004

Members of the GM Science Review Panel

Professor Sir David King FRS (**Chairman**)	Chief Scientific Advisor, HM Government
Professor Howard Dalton FRS (**Deputy Chairman**)	Chief Scientific Advisor, Department Environment, Food and Rural Affairs
Dr Michael Antoniou*	Reader in Molecular Genetics, GKT School of Medicine, King's College London
Dr Mark Avery	Director of Conservation, Royal Society Protection of Birds, Bedfordshire
Professor Janet Bainbridge OBE	Director Science and Technology, University of Teesside; Chair of the Advisory Committee on Novel Foods and Processes
Dr Chitra Bharucha	Consultant Haematologist; Chair of Advisory Committee on Animal Feedingstuffs
Professor Dianna Bowles OBE	Director of CNAP, Department of Biology, University of York
Dr Simon Bright	Syngenta, Jealott's Hill International Research Centre, Berkshire
Dr Andrew Cockburn	Monsanto, Trumpington, Cambridge
Professor Mick Crawley FRS	Imperial College, Silwood Park, Berkshire
Professor Philip Dale	John Innes Centre, Norwich
Professor Mike Gale FRS	Deputy Director, John Innes Centre, Norwich
Professor Mike Gasson	Food Research Institute, Norwich; Chair of the Advisory Committee on Novel Foods and Processes
Professor Alan Gray OBE	NERC Centre for Ecology and Hydrology
Professor John Gray	Department of Plant Science, University of Cambridge
Professor Pat Heslop-Harrison	Department of Biology, University of Leicester
Ms Julie Hill MBE	Programme Adviser, Green Alliance; Deputy Chair of the Agriculture and Environment Biotechnology Commission
Dr Brian Johnson	Head of Agricultural Technologies, English Nature, Somerset
Professor Chris Leaver FRS	Head, Department of Plant Sciences, University of Oxford
Dr Bruce Pearce*	Deputy Research Director and Head of Operations, Elm Farm Research Centre
Professor Jules Pretty	Director of Centre for Environment and Society, University of Essex
Revd. Professor Michael Reiss	Institute of Education, University of London
Professor Bertus Rima MRIA	Medical and Biological Centre, Queens University, Belfast
Professor Bernard Silverman FRS	Master, St Peter's College, Oxford
Dr Andrew Stirling	Science Policy Research Unit, University of Sussex
Professor William Sutherland	School of Biology Science, University of East Anglia, Norwich
Professor Michael Wilson FRSE	Chief Executive, Horticulture Research International
Professor Peter Young	Professor of Molecular Ecology, Department of Biology, University of York

The Secretariat

Dr Adrian Butt (**Secretary**)	OST/DEFRA
Mr Matthew Billson	OST
Mr Richard Pitts	OST
Mr David Trew	OST

* New members who joined the Panel in the Autumn of 2003.

EXECUTIVE SUMMARY

The GM Science Review Panel's First Report was published on 21 July 2003 and attracted wide public and media interest, with over 20,000 copies downloaded from the Review website. The second phase of the GM Science Review had three main purposes:

- to consider the issues raised in the Public Debate held in the summer of 2003 in the context of our First Report;
- to address new scientific developments that had taken place since the publication of our First Report, including the publication of the GM Herbicide-Tolerant Crop Farm-Scale Evaluations (FSEs) on 16 October 2003; and
- to consider reactions to the First Report received by letter and through the Review website and to consider to what extent these altered our conclusions.

Section 1: The significance of the Public Debate for the Science Review

We reviewed the Public Debate Report: 'GM Nation?', and consider that our First Report covered those scientific issues raised by the public that were relevant to our remit. See Section 1.2 for an analysis of points raised and for relevant references to the First Report. The GM Science Review Panel and the Public Debate Steering Board were assisted by the foundation discussion workshops[1]. These helped to frame the issues both for the Public Debate and for our Review. We noted that some of the concerns expressed in the course of the Public Debate coincided with scientific uncertainties already identified and discussed in our First Report. Far from being 'anti-science', there was a strong theme in the Public Debate for further research to be done.

Section 2: New published research

Any review can only be based on the state of knowledge and understanding at the time it is carried out, and new research results have continued to emerge since the First Report was published. In addition to our consideration of the FSEs in Section 3, we reviewed a number of other important recent scientific papers, concentrating on their possible impact on our conclusions. None of this newly published research significantly altered our earlier conclusions.

Section 3: The GM Crop Farm-Scale Evaluations

The FSE results were published after our First Report, and we considered them in detail in the second phase of our work. Our conclusions were submitted to the Advisory Committee on

[1] Organised by Corr Willbourn Research and Development.

Releases to the Environment (ACRE) for consideration before ACRE put its advice to Government. We judged the FSEs to be work of high scientific calibre. In essence, the experiments compared the impacts on aspects of biodiversity of herbicide regimes used on three GM herbicide-tolerant (GMHT) crops (maize, sugar beet and spring oilseed rape) with those used on conventional equivalent crops.

Our conclusions from the FSEs are as follows.

- 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 (i.e. the herbicide applications) associated with these GMHT crops, and are not a direct consequence of the way the crops have been produced.

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

- That the findings of the experiments were different for different crops with GMHT traits reinforces the general conclusion in our First Report that impacts of GM crops must always be assessed on a case-by-case basis.

Section 4: Feedback on the First Report

We reviewed the substantial range of comments and questions received by letter and on the website concerning the First Report, and considered to what extent these altered our conclusions. We were gratified to receive a broad range of responses, and pleased that respondents had the opportunity to raise new interpretations of the evidence or literature. We have responded when the feedback we received provided new information and/or extended the range of views previously discussed in the First Report. As part of the aspiration of the GM Science Review to provide a reference point/information source for the ongoing scientific and public consideration of this issue, the Second Report also provided an opportunity to clarify some of the points in the First Report.

Conclusion

Our First Report stated that we had found no scientific case for ruling out all GM crops and their products, but nor did it give them blanket approval. We emphasised that genetic modification is not a single homogeneous technology and that its applications need to be considered on a case-by-case basis.

Whilst our Second Report does not alter this general conclusion, we have been prompted to clarify a number of points and explore some issues in more detail. For that we are grateful to

all those who provided us with feedback. This feedback and newly published research has helped us refine a number of arguments and has taken the process forward in terms of the science. In as much as we have systematically examined the issue of GM crops in the UK and have looked for areas of uncertainty and risk that may be addressed by research, we hope the Science Review will inform present policy debates and future research agendas.

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-existence[1].

- '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 geneflow 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.

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.

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 napa* 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) Alleopathy 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 Ca^{2+} 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 crylAb, 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 $1/100^{th}$ 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 Liberator 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 & Rieseburg, 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 2μg/μl) 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 that 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 napa* 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 rotations[1], 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 advantages[2].

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

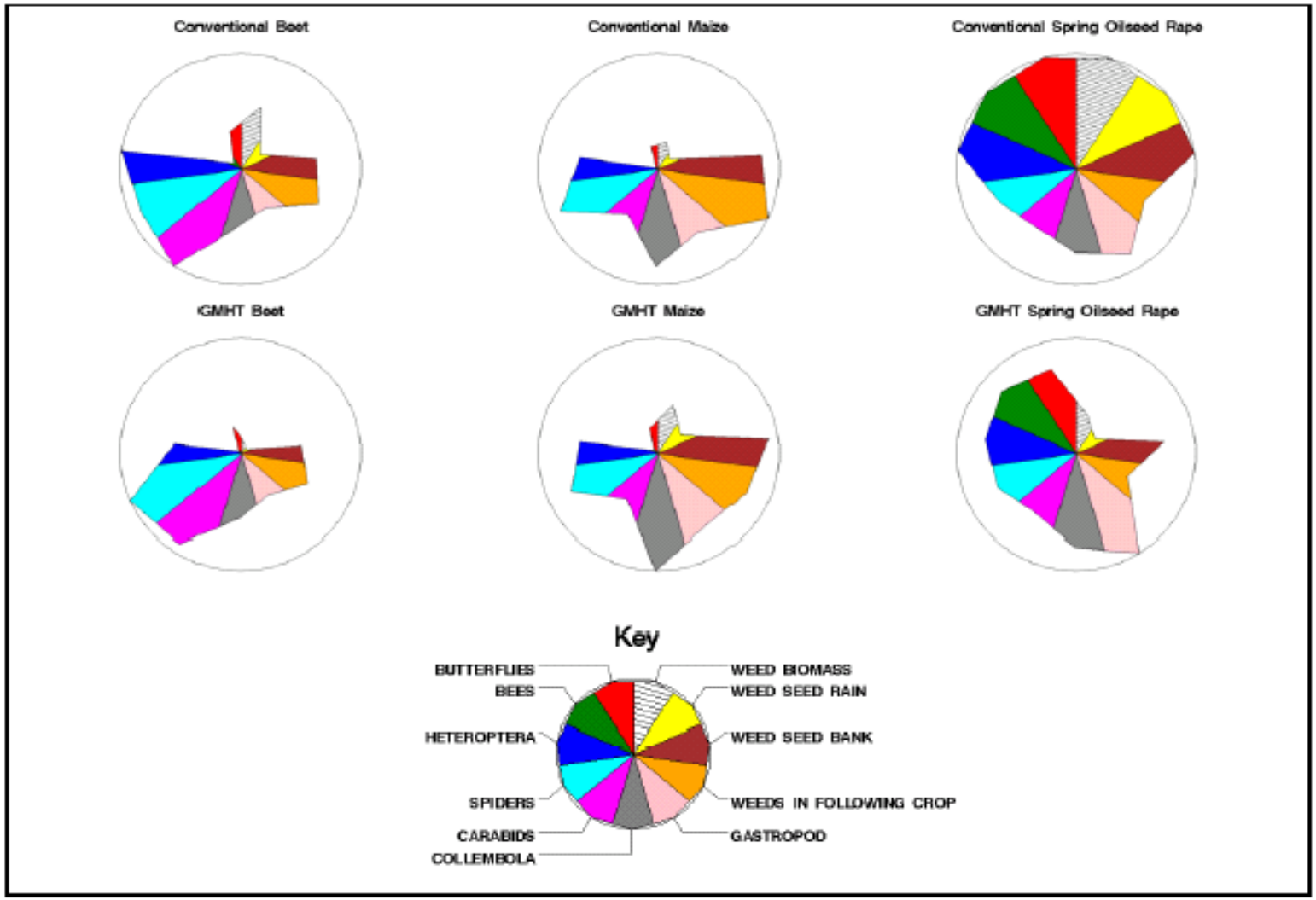
[2] E.g. environmental life cycle analyses might show energy savings, for example, in herbicide production, or fuel usage in tractor passes, or emissions. Organic farming may offer similar advantages over conventional systems.

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 crops[3]. 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.



[3]Reproduced 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.*

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 website[1] 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 commentators[7] 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 group[8] 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 suggested[13] 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 as 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 is 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 as 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 agriculture[15]. 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 publication[18].

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 publication[19]. 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 commentators[20] 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 commentator[21] 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 potatoes[30] and the Scottish Parliament's Health Committee enquiry[31] 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 73[50] that the Food Standards Agency (FSA) had commissioned further research in this area. The results of this research were published during the summer of 2003[51].

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 models[54]. 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 diets[57]. Yet another gave an incomplete reference to a study that appears to support Dr Pusztai's findings[58].

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] Munlochy 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.

crops[66]. The implications of GM crops for the developing world have also been considered in reports by the Nuffield Council on Bioethics[67].

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 allergencicity 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 foods[71], 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 2001[74], 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.

<u>Computer evaluation of sequence similarity</u>

The issue of requiring six (increases chance of false positives) or eight (increases chance of false negatives) or 35% (28) out of 80 amino acids[75] 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 distictiveness 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 commentator[82] agreed with our assessment, and one[83] 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 knowledge[84]. 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, commentators[92].

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 commentators[93] 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 commentators[98] 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 questioned[97]. 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 & Karieva 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

Criticisms[99] 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 commentator[100] 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 commentator[101] 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 commentator[102] 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. One[103] 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'. One[102] of our commentators agreed that this was among the most striking gaps in knowledge that show little sign of being filled.

Other commentators[102] 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 commentators[105] 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 commentator[106] 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 commentator[107] 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 commentator[108] 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 commentator[109] 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 commentators[105] 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 commentator[111] 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 commentator[112] 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 Scientis*t, 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 commentators[113] felt that this is a comprehensive and detailed account of our state of knowledge.

One commentator[114] 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 commentator[115] 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 commentator[116] 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 commentator[117] drew attention to new research (Defra, 2003d) on modelling the effects on farmland food webs of herbicide and insecticide management in the agricultural eco-system 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 commentators[118] 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 commentators[118] 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 commentator[119] 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 commentator[120] 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 commentators[121] 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 Greenpeace[122]. 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 commentator[123] 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 commentator[124] 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 commentators[125] 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 commentator[126] 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 commentator[127] 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 strategies[128].

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 commentators[129] felt that the Review was over-optimistic about the prospects for limiting crop-to-crop gene flow. One commentator[130] 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 commentators[133] 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 commentators[133] 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 commentator[129] underlined the importance of the Quist & Chapela (2001) paper on the Mexican maize situation.

One commentator suggested several new references[134].

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 ACRE[136].

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 contributor[137] 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 commentators[138] 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 commentator[139] 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 commentators[138] 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 commentator[140] 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 commentators[141] 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.

BIBLIOGRAPHY

The details of published papers and reports referred to in this Second Report, and which have already been included in the bibliography of our First Report, are not repeated here.

ACNFP (2000) *Advisory Committee on Novel Foods and Processes: Annual Report 1999.* Ministry of Agriculture, Fisheries and Food/Department of Health, Crown Copyright, PB 4869, Appendix VIII, 73-76. http://www.foodstandards.gov.uk/multimedia/pdfs/acnfp1999

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.

Ballas N, Broido S, Soreq H & Loyter A (1989) Efficient functioning of plant promoters and poly(A) sites in Xenopus oocytes. *Nucl. Acids Res*. **17**, 7891-903.

Ballester V, Granero G, Tabashnik BE, Malvar T & Ferre J (1999) Integrative mode for binding of *Bacillus thuringiensis* toxins in susceptible and resistant larvae for the diamondback moth (*Plutella xylostella*). *Appl. Env. Microb*. **65**, 1413-1419.

Bauer F, Hertel C & Hammes WP (1999) Transformation of Escherichia coli in foodstuffs. *Syst. Appl. Microbiol.* **22**, 161-8.

Bennetzen JL & Ma JX (2003) The genetic colinearity of rice and other cereals based on gnomic sequence analysis. *Current Opinion in Plant Biology*, **6**, 128-133.

Bergelson J, Purrington CB & Wichmann G (1998) Male promiscuity is increased in transgenic plants. *Nature,* **395**, 25.

Bernstein JA, Bernstein IL, Bucchini L, Goldman LR, Hamilton RG, Lehrer S, Rubin C & Sampson HA (2003). Clinical and laboratory investigation of allergy to genetically modified foods. *Environmental Health Perspectives* **111**, 1114-1121.

Betz FS, Hammond BG & Fuchs RL (2000) Safety and advantages of *Bacillus thuringiensis* protected plants to control insect pests. *Regul. Toxicol. Pharmacol*. **32**, 156-173.

Breitbart M, Hewson I, Felts B, Mahaffy JM, Nulton J, Salamon P & Rohwer F (2003) Metagenomic analyses of an uncultured viral community from human feces. *J. Bacteriol.* **185**, 6220-6223.

Breiztger P & Tonks D (2003) Inheritance and expression of transgenes in Barley. *Crop Science,* **43**, 4-12.

Brookes G (2002) The farm level impact of using *Bt* maize in Spain. Brookes West, Canterbury, 23pp.

Brooks DR, Bohan DA, Champion GT, Haughton AJ, Hawes C, Heard MS, Clark SJ, Dewar AM, Firbank LG, Perry JN, Rothery P, Scott RJ, Woiwod IP, Birchall C, Skellern MP, Walker JH, Baker P, Bell D, Browne EL, Dewar AJG, Fairfax CM, Garner BH, Haylock LA, Horne SL, Hulmes SE, Mason NS, Norton LR, Nuttall P, Randle Z, Rossall MJ, Sands RJN, Singer EJ & Walker MJ (2003) Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. I. Soil-surface-active invertebrates. *Phil. Trans, R. Soc. Lond. B* **358**, 1847-1862.

Burke C, Yu XB, Marchitelli L, Davis EA & Ackerman S (1990) Transcription factor IIA of wheat and human function similarly with plant and animal viral promoters. *Nucl. Acids Res.* **18**, 3611-3620.

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

Burke M (2003) GM crops: Effects on wildlife. ISBN 0-85521-035-4

Champion GT, May MJ, Bennett S, Brooks DR, Clark SJ, Daniels RE, Firbank LG, Haughton AJ, Hawes C, Heard MS, Perry JN, Randle Z, Rossall MJ, Rothery P, Skellern MP, Scott RJ, Squire GR & Thomas MR (2003) Crop management and agronomic context of the Farm Scale

Evaluations of genetically modified herbicide-tolerant crops. *Phil. Trans, R. Soc. Lond. B* **358**, 1801-1818.

Chen ZL, Gu H, Li Y, Su Y, Wu P, Jiang Z, Ming X, Tian J, Pan N & Qu LJ (2003) Safety assessment for genetically modified sweet pepper and tomato. *Toxicology,* **188**, 297-307.

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 *Bt*11. *J. Animal Sci.* **81**, 2546-2551.

Cooper M, Dultsev FN, Minson T, Ostanin VP, Abell C & Klenerman D (2001) Direct and sensitive detection of a human virus by rupture event scanning. *Nature Biotechnology,* **19**, 833-837.

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

Dewar AM, May MJ, Woiwood IP, Haylock LA, Champion GT, Garner BH, Sands RJN, Qi AM & Pigeon JD (2003) A novel approach to the use of genetically tolerant crops for environmental benefit. *Proc. Roy. Soc.* Lond. B. **270**, 335-340.

Dudek BR, Hammond BG, Nemeth MA, Lemen JK & Astwood JD (2002) 13-week feeding study in rats fed grain from YieldGard ® Event MON 810 corn. *Toxicologist* **66(**1S) Abstract # 930.

English L & Slatin SL (1992) Mode of action of delta-endotoxin from *Bacillus thuringiensis*: a comparison with other bacterial toxins. *Insect Biochem. Molec. Biol*. **22**, 1-7.

EPA (1998) EPA Registration Eligibility Decision (RED) *Bacillus thuringiensis*. EPA 738-R-98-004, March 1998.

Ewen S W B & Pusztai A (1999) The Lancet **354**, 1353-1354.

Falk BW & Bruening G (1994) Will transgenic crops generate new viruses and new diseases? *Science,* **263**, 1395-1396.

Fares NH & El-Sayed AK (1998) Fine structural changes in the ileum of mice fed on delta-endotoxin-treated potatoes and transgenic potatoes. *Natural. Toxins,* **6**, 219-233.

Federici BA (2002) Case study: *Bt* crops – a novel mode of insect control. In: Genetically Modified Crops. Assessing Safety. Ed: Atherton KT. Taylor and Francis New York, **22**, 164-200.

Firbank LG & Forcella F (2000) Genetically modified crops and farmland biodiversity. *Science*, **289**, 1481-1482.

Fraser P & Grosveld F (1998) Locus control regions, chromatin activation and transcription. *Curr. Opin.Cell Biol.* **10**, 361-365.

Freckleton RP, Stephens PA, Sutherland WJ & Watkinson AR (2003) Amelioration of biodiversity impacts of generically modified crops: predicting transient versus long-term effect. *Proc. Roy. Soc.* Lond. B. Published online 03pb0882.2.

Fu H & Dooner HK (2002) Intraspecific violation of genetic colinearity and its implications in maize. *Proc. Natl. Acad. Sci. USA,* **99**, 9573-9578.

Germolec DR, Kimber I, Goldman L & Selgrade MJ (2003) Key issues for the assessment of the allergenic potential of genetically modified foods: Breakout group reports. *Env. Health Perspectives*, **111**(8), 1131-1139.

Goff *et al.* (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). *Science,* **5**, 296: 79-92.

Gould F, Blair N, Reid M, Rennie TL, Lopes J & Micinski S (2002) *Bacillus thuringiensis* resistance management: Stable isotope assessment of alternate host use by *Helicoverpa zea*. *Proceedings of the National Academy of Sciences*, Published online, doi:10.1073/pnas.242382499 (2002).

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

Haughton AJ, Champion GT, Hawes C, Heard MS, Brooks DR, Bohan DA, Clark SJ, Dewar AM, Firbank LG, Osborne JL, Perry JN, Rothery P, Roy DB, Scott RJ, Woiwod IP, Birchall C, Skellern MP, Walker JH, Baker P, Browne EL, Dewar AJG, Garner BH, Haylock LA, Horne SL & Mason NS (2003) Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. II. Within-field epigeal and aerial arthropods. *Phil. Trans, R. Soc. Lond. B* **358**, 1863-1877.

Hawes C, Haughton AJ, Osborne JL, Roy DB, Clark SJ, Perry JN, Rothery P, Bohan DA, Brooks DR, Champion GT, Dewar AM, Heard MS, Woiwod IP, Daniels RE, Young MW, Parish AM, Scott RJ, Firbank LG & Squire GR (2003) Responses of plants and invertebrate trophic groups to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Phil. Trans, R. Soc. Lond. B* **358**, 1899-1913.

Heard MS, Hawes C, Champion GT, Clark SJ, Firbank LG, Haughton AJ, Parish AM, Perry JN, Rothery P, Roy DB, Scott RJ, Skellern MP, Squire GR & Hill MO (2003b) Weeds in fields with contrasting conventional and genetically modified herbicide-tolerant crops. II. Effects on individual species. *Phil. Trans, R. Soc. Lond. B* **358**, 1833-1846.

Hin CJA, Schenkelaars P & Pak GA (2001) *Agronomic and environmental impacts of the commercial cultivation of glyphosate tolerant soybean in the USA.* Utrecht: Centre for Agriculture and Environment (CLM).

Ho MW & Cummins J (2002) What's wrong with GMOs? *Science in Society,* **16**, 11-27.

Ho M-W, Ryan A & Cummins J (1999) Cauliflower mosaic viral promoter – a recipe for disaster? *Microb. Ecol. Health Dis.* **11**, 194-197.

Hofmann C, Luthy P, Hutter R & Pliska V (1988) Binding of the delta endotoxin from *Bacillus thuringiensis* to brush-border membrane vesicles of the cabbage butterfly (*Pieris brassicae*). *Eur. J. Biochem.* **173**, 85-91.

IPCS (1999) IPCS International Programme on Chemical Safety - Environmental Health Criteria 217: Microbial pest control agent *Bacillus thuringiensis*. World Health Organization, Geneva.

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.

Jank B & Haslberger AG (2003) Improved evaluation of potential allergens in GM food *Trends in Biotech.* **21**(6), 249-250.

Joubert GD, Venter MJ, Theron GC, Swanepeol A, Eulitz EG, Schroder HF & Macaskill P (2001) South African experience with *Bt* cotton. *Integrated Crop Management*, Papers presented at a technical seminar at the 60th Plenary Meeting of the International Cotton Advisory Committee, September 16-21. http://www.icac.org/icac/cotton_info/tis/biotech/documents/techsem/SAexperience_tis01.pdf.

Kashkush K, Feldman M & Levy AA (2002) Gene loss, silencing and activation in a newly synthesized wheat allotetraploid. *Genetics,* **160**(4), 1651-1659.

Kleter GA & Peijnenburg AACM (2002) Screening of transgenic proteins expressed in transgenic food crops for the presence of short amino acid sequences identical to potential, IgE-binding linear epitopes of allergens. *BMC Structural Biology*, **2**, 8-19.

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.

Labra M *et al.* (2001) Genomic changes in transgenic rice (Oryza sativa L.) plants produced by infecting calli with Agrobacterium tumefaciens. *Plant Cell Rep.* **20**, 325-330.

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.

Lufkin T (1997) Transcriptional regulation of vertebrate Hox genes during embryogenesis. *Crit. Rev. Eukaryot. Gene Expr.* **7**(3), 195-213.

Lukens L, Zoub F, Lydiate D, Parkin I & Osborn T (2003) Comparison of a Brassica oleracea genetic map with the genome of Arabidopsis thaliana. *Genetics*, **164**, 359-372.

Mann RS (1997) Why are Hox genes clustered? *Bio.Essays,* **19**, 661-664.

Mayer K, Murphy G, Tarchini R, Wambutt R, Volckaert G, Pohl T, Dusterhoft A, Stiekema W, Entian K-D, Terryn N, Lemcke K, Haase D, Hall CR, van Dodeweerd A-M, Tingey SV, Mewes HW, Bevan MW & Bancroft I (2001) Conservation of microstructure between a sequenced region of the genome of rice and multiple segments of the genome of Arabidopsis thaliana. *Genome Res.* **11**(7), 1167-1174.

McClintock JT, Schaffer CR & Sjoblad RD (1995) A comparative review of the mammalian toxicity of *Bacillus thuringiensis*-based pesticides. *Pestic. Sci*. **45**, 95-105.

Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM & Tauxe RV (1999) Food-related illness and death in the United States. *Emerging Infectious Diseases*, **5**, 607-625.

Murphy DJ (2002) Biotechnology and the improvement of oil crops – genes, dreams and realities. *Pytochemistry Reviews,* **1,** 67-77.

Noteborn HPJM, Rienenmann-Ploum ME, van den Berg JHJ, Alink GM, Zolla L & Kuiper HA (1993) Food safety of transgenic tomatoes expressing the insecticidal crystal protein Cry1Ab from *Bacillus thuringiensis* and the marker enzyme APH(3')II. *Med. Fac. Landbouww.* Univ. Gent. 58/4b.

Noteborn HPJM, Rienenmann-Ploum ME, van den Berg JHJ, Alink GM, Zolla L Reynaerts A, Pensa M, & Kuiper HA (1995) Safety assessment of the *Bacillus thuringiensis* insecticidal crystal protein Cry1A(b) expressed in transgenic tomatoes. In: American Chemical Society Series 605. Eds: Engel KH, Takeoka GR & Teranishi R Washington, 134-147.

Perry JN, Rothery P, Clark SJ, Heard MS & Hawes C (2003) Design, analysis and statistical power of the Farm-Scale Evaluations of genetically modified herbicide-tolerant crops. *J. Appl. Ecol*. **40**, 17-31.

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

Pusztai A, Bardocz S & Ewen SWB (2003) GM Foods: Potential human health effects. 'Ch. 16, in *Food Safety: Contaminants and Toxicants*. Ed. D'Mello JPF, CABI publishing.

Quemada H, L'Hostis B, Gonsalves D, Reardon IM, Heinrikson R, Hiebert EL, Sieu LC & Slightom JL (1990) The nucleotide sequences of the 3'-terminal regions of papaya ringspot strains W and P. *Jounral of General Virology,* **71**, 203-210.

Quist D & Chapela IH (2001) Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature,* **414**, 541-543.

Read MA & Ball JG (1999a) *The control of weeds with glufosinate ammonium in genetically modified crops of forage maize in the UK.* Proceedings of BCPC Conference, Weeds 1999, 847-852, British Crop Protection Council.

Read MA & Ball JG (1999b) Control of weeds in genetically modified crops of winter and spring oilseed rape with glufosinate ammonium in the UK. *Aspects of Applied Biology*, **55***,* 27-33.

Read MA & Bush MN (1998) Control of weeds in genetically modified sugar beet with glufosinate ammonium in the UK. *Aspects of Applied Biology*, **52**, 401-406.

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.

Roy DB, Bohan DA, Haughton AJ, Hill MO, Osborne JL, Clark SJ, Perry JN, Rothery P, Scott RJ, Brooks DR, Champion GT, Hawes C, Heard MS & Firbank LG (2003) Invertebrates and vegetation of field margins adjacent to crops subject to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Phil. Trans, R. Soc. Lond. B* **358**, 1879-1898.

Royal Society (1999) *Review of data on possible toxicity of GM potatoes.* The Royal Society. Document 11/99. http://www.royalsoc.ac.uk/gmplants/

Sayyed AH, Cerda H & Wright DJ (2003) Could *Bt* transgenic crops have nutritionally favourable effects on resistant insects? *Ecology Letters,* **6,** 167-169.

Schnepf E, Crickmore N, Van Rie J, Lereclus D, Baum J, Feitelson J, Zeigler DR & Dean DH (1998) *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol. Molec. Biol. Rev.* **62**, 775-806.

Shimada N, Kim YS, Miyamoto K, Yoshioka M & Murata H (2003) Effects of Bacillus thuringiensis Cry1Ab toxic on mammalian cells. *J. Vet. Med. Sci.* **65**(2), 187-191.

Siegel JP (2001) Minireview: The mammalian safety of *Bacillus thuringiensis*-based insecticides. *J. Invert. Pathol.* **77**, 13-21.

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

Spok A, Hofer H, Valenta R, Kienzl-Plochberger K, Lehner P & Gaugitsch H (2002) *Toxikologie und allergologie von GVO-produkten: Empfehlungen zur Standardisierung der Sicherheitsbewwerung von gentechnisch veranderten Pflanzen auf Basis der Richtlinie 90/220/EWG (2001/18/EG)* Federal Environment Agency Monographien Band 109 Wien, Austria. http://www.umweltbundesamt.at/publikationsdetail.html?&pub_id=1066

Squire GR, Brooks DR, Bohan DA, Champion GT, Daniels RE, Haughton AJ, Hawes C, Heard MS, Hill MO, May MJ, Osborne JL, Perry JN, Roy DB, Woiwod IP & Firbank LG (2003) On the rationale and interpretation of the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Phil. Trans, R. Soc. Lond. B* **358**, 1779-1799.

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

Strandberg B & Pedersen, MB (2002) Biodiversity in glyphosate tolerant fodder beet fields - timing of herbicide application. Silkeborg: National Environmental Research Institute.

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.

Thomas WE & Ellar DJ (1983) Mechanism of action of *Bacillus thuringiensis* var. *israelensis* insecticidal □ -endotoxin. *FEBS Lett*. **154**, 362-368.

Toivanen P, Vaahtovuo J & Eerola E (2001) Influence of major histocompatibility complex on bacterial composition fecal flora. *Infection and Immunity*, **69**, 2372-2377.

Van Mellaert H, Botterman J, Van Rie J & Joos H (1999) Recombinant plant expressing non-competitievely binding insecticidal crystal proteins. U.S. Patent 5,866,784.

Van Slageren MW (1994) Wild wheats: a monograpgh of *Aegilops L.* and *Amblyopyrum* (Jaub and Spach) Eig (Poaceae). Wageningen Agriculture University Papers, (7), 513pp.

Vazquez-Padron RI, Gonzales-Cabrera J, Garcia-Tovar CB, Neri-Bazan L, Lopez-Revilla R, Hernandez M, Moreno-Fierro L & de la Riva GA (2000) Cry1Ac protoxin from *Bacillus thuringiensis* sp. *kurstaki* HD-73 binds to surface proteins in the mouse small intestine. *Biochem. Biophys. Res. Commun.* **271**, 54-58.

Vazquez-Padron RI, Moreno-Fierros L, Neri-Bazan L, de la Riva GA & Lopez-Revilla R (1999) Intragastric and intraperitoneal administration of Cry1Ac protoxin from *Bacillus thuringiensis* induces systemic and mucosal antibody responses in mice. *Life Sci.* **64**, 1897-1912.

Veraksa A, Del Campo M & McGinnis W (2000) Developmental patterning genes and their conserved functions: From model organisms to humans. *Mol. Genet. Metab*. **69**, 85-100.

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.

Wang G *et al.* (1996) Poplar *(Populus nigra* L.) plants transformed with a *Bacillus thuringiensis* toxin gene: insecticidal activity and genomic analysis. *Transgenic Res.* **5**, 289-301.

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

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

Wevers JDA (1998) Agronomic and environmental aspects of herbicide-resistant sugar beet in the Netherlands. *Aspects of Applied Biology*, **52**, 393-399.

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

Willamson M (1996). Biological Invasions. Chapman & Hall.

Yu *et al.* (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). *Science,* **5**, 296: 92-100.

GLOSSARY OF TERMS AND ABBREVIATIONS

This glossary includes the glossary of scientific and technical terms for our First Report, which was posted on the GM Science Review website shortly after its publication, and abbreviations used in both the First and Second Reports.

Abiotic	Non-biological; abiotic factors include frost, heat, drought, cold and salt.
ACAF	Advisory Committee on Animal Feedingstuffs (for the UK)
ACNFP	Advisory Committee on Novel Foods and Processes (for the UK)
ACRE	Advisory Committee on Releases to the Environment (for the UK)
AEBC	Agriculture and Environment Biotechnology Commission
Agrobacterium tumefaciens	A naturally occurring soil bacterium that is capable of inserting DNA (genetic information) into plants. Used in agricultural biotechnology to carry transgenes into plants.
Alien species	Species introduced intentionally or unintentionally to locations beyond the native range of the species (usually taken as post-1500). Also known as non-indigenous, non-native, exotic or introduced species. See also invasiveness.
Allele	Any one of a number of alternative forms of the same gene occupying a given position on a chromosome.
Allelopathy	Interaction between two different species involving chemicals produced by one, which suppresses the growth or reproduction of the other.
Allergenic/allergens	Substances that cause an allergic reaction.
Anaphylaxis	A severe and rapid allergic systemic reaction to contact with an allergenic trigger substance. The classic form involves prior sensitisation with later re-exposure, producing symptoms via an immunological mechanism.
Antigen	A macromolecule that is recognized by antibodies or immune cells and can trigger an immune response.

Antisense techniques	Method for down regulating the expression of particular genes by putting a ‘reverse’ version or ‘mirror image’ version of the gene, or part of the gene, into a cell.
Apomixis	Biological reproduction by seeds without fertilisation, meiosis or production of gametes. The asexual production of seeds.
ARM	Antibiotic resistance marker
Atrazine	See Triazines.
BA	British Association for the Advancement of Science
Back-crossing	Crossing an individual with one of its parents or with a genetically equivalent organism. The offspring of such a cross are referred to as the backcross generation or backcross progeny.
Bacteriophage	A virus that infects bacteria. Altered forms are used as cloning vectors.
Base pairs (bp)	Two nucleotides, one on each of the opposite complementary strands of DNA or RNA, connected via hydrogen bonds are called a base pair. In DNA, adenine and thymine, as well as guanine and cytosine, can form a base pair. The length of a nucleic acid molecule is often given in terms of the number of base pairs it contains. Modified to kbp (kilobase pairs, often used to measure transgene sizes) and Mbp (megabase pairs, used to measure total genome sizes).
BBSRC	Biotechnology and Biological Sciences Research Council
Biodiversity	A measure of the range and abundance of living organisms, this includes diversity within species and between species.
Bioinformatics	The science of managing and analysing biological data using advanced computing techniques. Especially important in analysing genomic research data. See also *in silico*.
Biolistics	Method for introducing genetic material into plant cells. DNA is coated onto micro-particles (gold or tungsten) that are fired into plant tissue.
Bolters	Plants that flower prematurely; most commonly sugar beet bolters which flower in the first year after planting rather than the second year.
bp	See base pairs

Break crop	A crop grown to benefit the soil and reduce pests and pathogens.
Bromoxynil	A nitrile herbicide that inhibits photosynthesis (in photosystem II).
Bt	*Bacillus thuringiensis*
Canola	Oilseed rape (used in North America).
CEC	Commission for Environmental Cooperation
Chimaera	(or chimera) An organism made up of cells containing different genetic information.
Chloroplast	Specialised plastid (general term for plant cell organelles which carry non-nuclear DNA) that contains chlorophyll. They are the site of solar energy transfer and some important reactions involved in starch and sugar synthesis. Chloroplasts have their own DNA genome and this is generally inherited through the female parent.
Clearfield	A commercial herbicide tolerant system using a group of conventionally bred crops with resistance to the broad-spectrum herbicide imidizole.
Cleistogamy	Relating to a flower that does not open and is self-pollinated in the bud.
Codon	A group of three consecutive nucleotides that represent the unit of genetic coding by specifying a particular amino acid during the synthesis of polypeptides in a cell. Each codon is recognised by a transfer RNA carrying a specific amino acid, which is incorporated into a polypeptide chain during protein synthesis.
Codon usage	As there are 64 possible triplet combinations of the 4 nucleotides (4x4x4) that are present in mRNA and DNA and only 20 amino acids, there is approximately a 3-fold excess of triplet combinations available to encode these amino acids. This means that codons can be changed without altering the amino acid sequence of the protein they encode. For some amino acids the codons most commonly used to encode them differ between eukaryotes (e.g. plants) and prokaryotes (e.g. bacteria). Therefore, the codons of a transgene derived from a prokaryote may be changed so that expression of the transgene in plant cells is more efficient.

Co-existence	In this Review, co-existence refers to the simultaneous but separate cultivation of crops by different agricultural methods (e.g. conventional non-GM, GM, organic, non-food industrial and certified seed crops).
Coleaoptera	Insect order that includes beetles.
CMV	Cucumber mosaic virus
Conjugation	Unidirectional transfer of plasmid DNA from one bacterium to another, involving cell-to-cell contact. The plasmid usually encodes the majority of functions necessary for its own transfer.
Conservation headlands	Crops at the edges of fields that are not treated with agrochemicals (part of qv Countryside stewardship scheme).
Containment	Growth conditions for organisms including bacteria and plants where they are not freely released in the local environment.
COT	**Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment**
Countryside Stewardship scheme	A Government-run scheme that makes payments to farmers and other land managers to enhance and conserve English landscapes, their wildlife and history and to help people to enjoy them.
CP	Coat protein
Crop rotation	Crops on a specific area of land are changed year by year in a planned sequence.
Cry	Cryptochrome
cv	Cultivar
Defective interfering (DI) RNA	Small RNA molecules derived from viral RNA by extensive deletion. DI RNA depends on the original virus for replication and usually reduces the level of replication of the host (helper) virus.

Defective virus	A virus that, by itself, is unable to reproduce at all, or at the level of the wild type virus when infecting its host cell, but that can grow in the presence of another virus. This other virus provides the necessary molecular machinery that the first virus lacks.
Diploid	Having two complete sets of chromosomes, most commonly one set of paternal origin and the other of maternal origin.
DNA	Deoxyribonucleic acid. The molecule that encodes genetic information. DNA is a double-stranded molecule held together by weak bonds between base pairs of nucleotides. The four nucleotides in DNA contain the bases adenine (A), guanine (G), cytosine (C), and thymine (T). In nature, base pairs form only between A and T and between G and C; thus the base sequence of each single strand can be deduced from that of its partner. The structure of DNA (double helix) was published in 1953 by Crick and Watson.
Ecosystem	The complex of a living community and its physical environment, functioning as an ecological unit in nature.
EEA	European Environment Agency
EFSA	European Food Safety Authority
Embryo rescue	A sequence of tissue culture techniques used to enable a fertilised immature embryo, resulting from an interspecific cross (between plants from different species), to continue growth and development (when it may not otherwise), until it can be regenerated into an adult plant.
Encapsidation	Packaging of viral genetic material (RNA or DNA) in a regular protective, geometric array (shell) or coat (capsid) protein molecules.
ENTRANSFOOD	European Network for Safety Assessment of Genetically Modified Food Crops
EPA	Environmental Protection Agency (for the USA)
Epidemiological monitoring	See Post-marketing monitoring
Epidemiological surveillance	See Post-marketing surveillance
Epitopes	The individual surface feature of an antigen that elicits the production of a specific antibody (monoclonal) in the course of the immune response. Each antigenic determinant, typically a

	few amino acids in size, causes the synthesis of a different antibody and thus exposure to a single antigen may result in the expression of a number of antibodies (polyclonal).
ESTO	Earth Science Technology Office
EU	European Union
Eukaryote/ eukaryotic	Cell or organism with membrane-bound, structurally discrete nucleus and other well-developed subcellular compartments. Eukaryotes include all organisms except viruses, bacteria, and bluegreen bacteria (algae).
Exon	Protein-coding DNA sequence of a gene**.** Many eukaryotic genes are composed of a mosaic of exons and introns.
F1 hybrids	The initial hybrid generation resulting from a cross between two genetically unlike parents.
FAO	Food and Agriculture Organisation (of the United Nations)
Farm-saved seed	Retaining some seed from a harvest to sow in following years on the same farm, rather than buying in new seed produced commercially.
FDA	Food and Drug Administration (for the USA)
Fertilisation (self and cross)	Union of two gametes from opposite sexes to form a zygote. There are different categories: 1. Self-fertilisation (selfing): fusion of male and female gametes from the same individual. 2. Cross-fertilisation (crossing): fusion of male and female gametes from different individuals.
FISH	Fluorescence *in-situ* hybridisation. A method for locating particular sections of DNA on chromosomes.
Fitness	The survival value and the reproductive capability of an individual, compared to that of competitor individuals of the same or other species within a population or an environment.
Fixation	Situation in which only one allele for a given gene/locus is present in a population. This can occur as a result of direct selection where the allele delivers a greater level of fitness; because of indirect selection, where the locus is linked to a gene that is subject to direct selection; or because of genetic drift. Also: Nitrogen fixation: the process of conversion of gaseous nitrogen in the atmosphere to compounds which can be metabolized by plants, a process which can be carried out by some bacterial species.

FOE	Friends of the Earth
FSA	Foods Standards Agency (for the UK)
FSEs	GM Crop Farm-Scale Evaluations
Fusion protein	A polypeptide synthesised from a chimeric gene. The different fragments of DNA are joined so that their protein coding sequences are in the same reading frame. The resulting construct is transcribed and translated as a single gene, producing a single protein.
Gamete	Mature male or female reproductive cell.
Gene	The unit of heredity transmitted from generation to generation during sexual or asexual reproduction. The simplest gene consists of a segment of nucleic acid that encodes an individual protein or a length of RNA.
Gene construct	The DNA unit, usually including transgenes, promoters and selectable markers, which is used to make a GM plant.
Gene flow	The transfer of genes between different individuals e.g. pollen-mediated gene transfer between sexually compatible plants. Also refers to the transfer of genes from one plant population to another through seed dispersal or the movement of regenerative plant parts, (e.g. tubers), or whole plants. This Review also considers the possibility of plant genes being transferred and stably integrated into the genomes of soil and gut microbes and into viruses that infect plants (see horizontal gene flow).
Gene product	RNA and proteins.
Gene stacking	Accumulation of genes conferring different traits in one plant resulting from cross-fertilisation or transformation with several gene constructs. Also, see transgene stacking.
Genotype	The genetic constitution of an organism, as distinguished from its physical characteristics (its phenotype).
GLP	Good Laboratory Practice
Glufosinate ammonium	Used to provide post-emergence, broad spectrum control of annual grasses and broad-leaved weeds. Glufosinate ammonium can be sprayed after emergence if the crop is tolerant to it. This herbicide acts by inhibiting an enzyme that is responsible for ammonia detoxification ultimately leading to the cessation of photosynthesis. The trade names of herbicides

	containing glufosinate ammonium include: Basta, Liberty, Ignite, and HOE 39866.
Glyphosate	Systemic herbicide that is used for post-emergence, broad spectrum control of annual and perennial broad-leaved and grass weeds. Can be sprayed after emergence if the crop is glyphosate tolerant. Acts by inhibiting an amino acid metabolism pathway that exists in higher plants and microorganisms, but not in animals. Inactivated on contact with clay particles in soil, and requires no hazard warning symbols on packaging. The trade names of some herbicides in which glyphosate is the active ingredient are: Roundup, Rodeo, Touchdown, and MON-0573.
GM	Genetically modified/ Genetic modification. Altering the genetic material of an organism in a way that does not occur naturally by mating and / or natural recombination.
GM derived	Products that are derived from genetically modified organisms, including products (e.g. some vegetable oils or enzymes used for making cheese) in which it is not possible to detect any DNA or protein.
GMHT	Genetically Modified Herbicide Tolerance
GMIR	Genetically Modified Insect Resistant
GMO	Genetically modified organism. An organism in which the genetic material has been altered in a way that does not occur naturally by mating and / or natural recombination.
GFP	Green fluorescence protein
GI	Gastro-intestinal
GURT	Genetic use restriction technologies. Mechanisms that prevent or restrict gene flow from GM crops, e.g. GM male sterility.
Haploid	Possessing only one copy of each chromosome. Within higher organisms, only the reproductive cells are haploid, whereas the somatic (body) cells are diploid (two copies of each chromosome) or polyploid (three or more copies of each chromosome - often found in plants).
Homologous recombination	See Recombination.
Homology	Similarity between sequences of DNA or amino acids (that make up proteins) in individuals of the same, or different species.

Horizontal gene flow	See Horizontal gene transfer.
Horizontal gene transfer (HGT)	Non-sexual, non-parental-to-offspring processes by which genetic material can sometimes transfer between organisms with distant genetic relationships.
Hybrids/hybridisation	Offspring of two genetically unlike parents. Crossing of two sexually compatible but genetically different plants.
ICSU	International Council of Scientific Unions
IFPRI	International Food Policy Research Institute
IgE	Immunoglobulin E
ILGRA	Interdepartmental Liaison Group on Risk Assessment (for the UK)
ILSI	International Life Sciences Institute
In silico	In a computer. In the present context, the use of data bases of DNA and protein sequence to help answer biological questions. This is a growing area of biology, as the amount of genomic and proteomic data continue to grow. See bio-informatics.
Interspecific plant competition	Competition between one plant species and another.
Introgression	Introduction of new allele(s) or gene(s) into a population from an exotic source, usually another species. This is achieved by repeated backcrossing of the initial hybrid in order to eliminate all genetic changes except for the desired new gene(s).
Intron	DNA sequence that interrupts the protein-coding sequence of a gene. An intron is transcribed into RNA but is cut out of the message before it is translated into protein.
Invasiveness (or invasive species)	Ability of an organism, particularly an alien species (qv), to spread beyond its presently established site, and become established in new locations.
in vitro	Outside the organism, or in an artificial environment. Applied, for example, to cells, tissues or organs cultured in glass or plastic containers.
in vivo	The natural conditions in which organisms reside. Refers to biological processes that take place within a living organism or cell under normal conditions.

JRC	Joint Regulatory Commission (for the European Union)
Late successional closed vegetation	The endpoint of natural change following disturbance (e.g. forest or heath).
Lepidoptera	Order of insects that includes butterflies and moths.
Linkage drag	When the selection pressure operating for, or against, a trait encoded by a gene(s) is affected by the co-inheritance of linked genes.
Macrophage	Large white blood cells that ingest foreign substances and display on their surfaces antigens that are recognised by other cells of the immune system.
Marker genes	A gene of known function, sequence and/or location, used for marker-assisted selection or for genetic studies. Marker genes conferring traits that are novel to plants are used to identify plants into which transgenic DNA has been successfully introduced e.g. genes encoding resistance to certain antibiotics.
Marker rescue	Restoration of gene function by replacement of a defective gene with a normal one through recombination.
Meiosis	Process of two consecutive cell divisions in the formation of sex cells (gametes).
Metabolomics	The large-scale study of the full complement of secondary metabolites produced by a given species in all its cells, tissues and/ or growth stages.
Microarrays	A large set of DNA molecules immobilized as a compact and orderly pattern of sub-microlitre spots onto a solid matrix (e.g. a glass slide). Used to analyse patterns of gene expression, presence of markers, or nucleotide sequences. The major advantage of microarrays is the extent to which the process of genotyping can be automated, thereby enabling large numbers of individuals to be genetically typed at many loci simultaneously. A similar approach may be used with other immobilised components (e.g. proteins) for other purposes.
Micronutrients	Components of nutrition required in relatively small quantities by organisms, e.g. vitamins or minerals.
Millieumeetlat system	Scoring system is used to evaluate toxicity, mobility and persistence of pesticides.
mRNA	Messenger RNA. The RNA molecule resulting from transcription of a protein-encoding gene, following any

	splicing (removal of introns). The information encoded in the mRNA molecule is translated into a polypeptide (protein) by the ribosomes.
mRNA fingerprinting	Pattern of mRNAs present in an organism under specific conditions.
Mutation breeding	Induction of heritable change(s) in the genetic constitution of a cell through alterations to its DNA, using mutagenic chemicals or radiation. Used in breeding programmes to introduce genetic differences from which new crop phenotypes with desirable traits can be selected. Can cause gross and unpredictable changes to whole chromosomes as well as to specific genetic loci. These smallest changes can involve the substitution, deletion or insertion of a single nucleotide.
Naked DNA	DNA from any source (whether or not created by recombinant DNA techniques) that has been purified and separated from the proteins that normally surround the DNA in a living organism.
NGOs	Non-Governmental Organisations
Nuclear DNA	DNA organised into chromosomes and which contains most of the genes (typically 25 000 – 50 000) that are largely responsible for the differentiation and activity of the cell. Plastids and mitochondria contain non-nuclear DNA.
Nucleotides	A subunit of DNA or RNA consisting of a nitrogenous base (adenine, guanine, thymine, or cytosine in DNA; adenine, guanine, uracil or cytosine in RNA), a phosphate molecule and a sugar molecule (deoxyribose in DNA and ribose in RNA). Thousands of nucleotides are linked through repeating sugar-phosphate bonds to form a DNA or RNA molecule.
OECD	Organisation for Economic Cooperation and Development
Open reading frames	Abbreviation: ORF. A sequence of nucleotides in a DNA or RNA molecule that has the potential to encode a peptide or protein: comprises a start triplet (ATG), followed by a series of triplets (each of which encodes an amino acid), and ending with a stop codon (TAA, TAG or TGA). The number of ORFs provides an estimate of the number of genes that could be transcribed from the DNA sequence.
ORFs	Open reading frames
Origin of replication	See Replication origin.
Pathogen	Disease-causing organism (generally microbial: bacterial, fungal or viral; but can extend to other organisms, e.g.

	nematodes).
Pest	An organism that reduces the productivity of a crop e.g. certain insects, birds and nematodes.
PCR	See Polymerase chain reaction.
Phytochemicals	Molecules characteristically found in plants.
Phytoremediation	Biological remediation (restoration) of the environment using plants.
Plasmid	Autonomously replicating extra-chromosomal circular DNA molecule, distinct from the normal bacterial genome and non-essential for cell survival under non-selective conditions. Some plasmids are capable of integrating into the host genome. A number of artificially constructed plasmids are used as gene cloning vectors (vehicles).
Plastid	Plant-specific organelles, such as chloroplasts, which carry their own DNA.
Pleiotropy	The simultaneous effect of a gene on more than one apparently unrelated trait.
PLRV	Potato leafroll virus
Pollination	Part of the process of fertilisation, in which pollen is transferred from an anther (male part) to the stigma (female part) of the same (self-pollination) or a different (cross-pollination) sexually compatible plant.
Polymerase chain reaction	Abbreviation: PCR. A method for dramatically increasing the number of copies of a specific fragment of DNA *in vitro*.
Polyploid	Three or more copies of each chromosome in a cell.
Post-marketing monitoring	The hypothesis-driven, routine collection of information after a product is on the market (i.e. widely available). For example, epidemiological monitoring involves looking for a disease condition, characteristic or state in a population.
Post-marketing surveillance	Surveillance takes a general look at trends. e.g. epidemiological surveillance is the systematic collection, collation, analysis and interpretation of health-related events occurring in populations.
Prokaryotes	Unicellular organism lacking a membrane-bound, structurally discrete nucleus and other subcellular compartments. Bacteria are examples of prokaryotes.

Promoter	A DNA sequence at the start of a gene to which RNA polymerase (an enzyme) will bind and initiates transcription/expression of a gene into messenger (or other) RNA. Genomic and subgenomic promoters also exist in RNA viruses where they initiate copying of RNA into RNA.
PROSAMO	Planned release of selected and modified organisms
Protists	A single-celled eukaryote.
Recombinant DNA technology	Set of techniques for manipulating DNA, including: the identification, modification and cloning of genes; the study of the expression of cloned genes; and the production of large quantities of gene products.
Recombinase	Class of enzymes able to alter the arrangement of DNA sequences in a site-specific way.
Recombination	Process by which progeny derive a combination of genes different from that of either parent. In higher organisms such as plants, this can occur by crossing over (breaking during meiosis of one maternal and one paternal chromosome, the exchange of corresponding sections of DNA, and the rejoining of the chromosomes). In lower organisms such as bacteria and viruses, it describes the cutting and rejoining or RNA/DNA template switching, which results in the exchange of fragments of genetic material or information between different organisms. It also describes the transfer of transgenic material between lower organisms and GM plants containing homologous sequences. **Homologous recombination** mediates the transfer or exchange of genetic information between homologous sections of DNA.
Refugia	Area of crops or adjacent land where there is no control of weeds or, more usually, insects, and thus provide a safe haven for them. Refugia can be placed adjacent to conventional crops sprayed with herbicides or insecticides or near GM herbicide-tolerant or insect-resistant crops to reduce the selection pressure on the insects or weeds to evolve resistance and increase local biodiversity. May be accomplished by qv: Countryside Stewardship Schemes, Conservation Headlands and Wildlife strips.
Replication origin	The nucleotide position on a DNA sequence from which DNA synthesis (replication) is initiated.
Reverse transcriptase PCR	Abbreviation: RT-PCR. RNA (mRNA) molecule(s) reverse transcribed into a DNA copy are then amplified using PCR. RT-PCR can be used to identify which genes are being

	expressed in a cell (i.e. the mRNA population), whereas PCR identifies all of the genes (DNA) that are present.
RNAi	RNA interference.
RSPB	Royal Society for the Protection of Birds
Satellite RNA	Plant viruses often contain parasites of their own, referred to as satellites. Satellite RNAs are dependent on their associated (helper) virus for both replication and encapsidation.
SCIMAC	Supply chain initiative on modified agricultural crops
Secondary metabolite profiling	See Metabolomics.
Silencing	Mechanisms in a genome that repress the expression of genes; can be achieved using transgenes. Recent work involves qv RNAi.
siRNA	Small interfering RNA
SSCP	Single-strand (RNA) conformation polymorphism
SSSI	Site of Special Scientific Interest
Substantial equivalence	Used to structure the comparison of a novel food with its conventional counterpart to identify any compositional differences that then become part of a more focussed safety assessment.
Sulfonyl urea	Herbicides that block the synthesis of essential branched chain amino acids (leucine, isoleucine and valine) by inhibiting the enzyme acetolactate synthase (ALS). For this reason, these chemicals are sometimes referred to as SU/ALS herbicides or ALS herbicides.
Synergism	Phenomenon in which one virus may facilitate replication and/or increase the symptom severity of another co-infecting virus, resulting in more severe disease.
Terminator	DNA sequence just downstream of the coding segment of a gene, which is recognized by RNA polymerase as a signal to stop synthesizing mRNA.
Terminator technology	A type of GURT. Transgenic method that genetically sterilises the progeny of a planted seed.
Tillage	Ploughing or harrowing. Zero-tillage or low-till agricultural practices may be implemented.

Trait	One of the many characteristics that define an organism. The phenotype is a description of one or more traits.
Trans	Spatially separated, e.g. on opposite chromosomes.
Transencapsidation	Complete or partial encapsidation (packaging), of the genome of one virus with the coat protein of another virus.
Transformation	Uptake and integration of DNA in a cell.
Transgene stacking	Accumulation of transgenes conferring different traits in one plant. This can arise intentionally or unintentionally through cross-fertilisation or by the introduction of different traits into a GM plant variety through one or a number of successive transformation events.
Transgenic DNA/ transgene	Isolated sequence of DNA stably inserted into the genome of a recipient organism.
Translation initiation signal/factor	The RNA codon (AUG) that specifies the first amino acid of a polypeptide chain. An assemblage of proteins necessary for the initiation of polypeptide synthesis from mRNA.
Transposon	DNA element that can move from one location in the genome to another, or through an RNA intermediate.
Triazines	Herbicides that inhibit photosynthesis (in particular photosystem II) e.g. atrazine.
UK	United Kingdom
UNCED	United Nations Conference on Environment and Development
Unencapsidated	Viral DNA or RNA not enclosed by a coat protein shell or capsid.
USDA	US Department of Agriculture
Vector	Small DNA molecule (plasmid, virus, bacteriophage, artificial or cut DNA molecule) that can be used to deliver DNA into a cell. Vectors must be capable of being replicated and contain cloning sites for the introduction of foreign DNA. Vector can also refer to an organism, usually an insect, which carries and transmits pathogens. Also refers to an organism, usually an insect that carries and transmits pathogens/ disease.
Vernalisation	Chilling juvenile plants for a minimum period in order to induce flowering. Some plants (e.g. sugar beet) require vernalisation to flower, but others have no such requirement.

Viroids/virusoids	Unique plant pathogenic agents, composed of infectious single-stranded low molecular weight RNAs, and no coat protein.
Volunteer	Crop plant self-propagated from a previous year's crop (e.g. from seed or tubers).
Wild type	The most frequent allele or genotype found in nature.
Wildlife strips	Edges of fields that are not planted or treated with agrochemicals (part of qv Countryside stewardship scheme).
WHO	World Health Organisation
Zygote	The result of fertilisation between two gametes. It undergoes a cycle of multiple divisions to become an embryo.

Annex I

Responses to the First Report of the GM Science Review

The following organisations and individuals responded to our invitation to comment on the First Report. The great majority of responses were e-mailed[1] to us and these can be viewed on the GM Science Review website[2].

T Addiscott

Agriculture and Biotechnology Council

M H Arnold

M Berwyn-Jones

J Bond

Bristol Area SERA Group

Bristol Genetix Group[3]*

British Crop Protection Council

British Society of Plant Breeders Limited

British Statutory Nature Conservation Agencies: Countryside Council for Wales, English Nature, Joint Nature Conservation Committee and Scottish Natural Heritage

British Trust for Ornithology

T Brown

D Burke

M Cantley

A M Claparols

J Clarke

S Cooke

C Creighton*

E Cross

J Davison

A Dean

P Dupont

J Dutton

R Eburne

Econexus, the Five Year Freeze, Friends of the Earth, Genewatch UK, Greenpeace, the Soil Association and Dr M Antoniou

P Elliot

G Finch

M Fuller

H Gatt

F A Gibson*

D Gledhill

Green Party

N Grice

S C Harris*

[1] An asterisk against a name denotes a written response.
[2] http://www.gmsciencedebate.org.uk/report/comment/default.htm
[3] Similar letters from several individuals.

G Humphreys

Independent Science Panel (ISP)

Institute of Biology on behalf of the Biosciences Federation

Institute of Science in Society (ISIS)

S James

John Innes Centre

A Kerry-Bedell

I Langford*

U Loening

N Maclean

R Marsh

N Mullan

Munlochy Vigil

S Neil

T Nichells

E Overton

R Patterson

Penwith Organic Gardeners and Growers*

Prospect

D Reid

O Richardson*

The Scientific Alliance

Scientists for Global Responsibility

R Scullion

Swindon Friends of the Earth

Syngenta

J Tipper*

I Wade*

G White

M White*