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Project title	REVIEW OF KNOWLEDGE OF THE POTENTIAL IMPACTS OF GMOs ON ORGANIC AGRICULTURE
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Executive summary (maximum 2 sides A4)

1. Background

The organic movement believes that organic agriculture, by its nature, cannot involve the use of genetically modified organisms (GMOs). This has been incorporated into EU regulations which state that there is no place in organic agriculture for GMOs. The aim in this review is to consider the ways in which the use of GMOs in agriculture in the UK and internationally might impact on organic farming. It does not address the controversy about the rights or wrongs of GMO's *per se*. The subjects covered are based on a set of questions raised at the beginning of the study. The review is based primarily on evidence from peer-reviewed literature.

2. Fate of DNA in soil

- DNA can persist in soil long enough to be taken up by competent bacteria. However, its longevity and fate will be influenced by the environmental conditions.
- There is no evidence to suggest that DNA originating from transgenic organisms will persist or behave any differently in soil compared with that from non-GM organisms. In many cases, the DNA sequences that are used to genetically modify organisms originate from naturally occurring species and may thus be part of normal soil DNA, although the combinations of DNA sequences and their relative quantities might be altered.
- Although transformation of competent cells by free DNA has been observed in the laboratory under optimised conditions, the likelihood of uptake of free DNA from soil in the field, and its incorporation into soil microbes is less likely. This is due to the presence of soil nucleases and the requirement for naturally competent bacterial cells.
- The proportion of transgenic DNA (DNA recombined in the laboratory) in soil cropped with GM

plants is negligible compared with that from non-transgenic origins.

- Although DNA can persist in soil for several weeks or months, it declines in terms of its biological impact. The data available indicate that it would be undetectable following a 5-year conversion period (a period already set by some UK organic sector bodies) after a previous GM crop. The likelihood of transfer of even small amounts of soil from non-organic to organic sites is low. Even if this were to happen, the indications from the literature surveyed are that this would be unlikely to pose any problem to organic agriculture.

3. Fate of DNA in livestock feed and possible impact of GM feed

- Comparative feeding studies using feed from GM and non-GM origins have provided no convincing evidence that ingestion of the GM materials had any adverse impact on the health of animals or on any of their products, such as meat, eggs and milk.
- DNA probably does not survive feed processing conditions which involve heat treatment, although it can survive ensiling.
- Even if an animal is intentionally given a diet containing GM material, the proportion of DNA from transgenic origin (as a proportion of the whole diet) would be less than 0.0005%.
- Many of the genes (and other DNA sequences) that have been introduced into GM crops are taken from organisms which live in the same or similar environments as the unmodified crops. Therefore animals are likely to have ingested these genes from sources other than transgenic crops, although the combinations and quantities of the DNA in question may change under exploitation of GM crops.
- Horizontal gene transfer could theoretically occur between gut bacteria in the gastro-intestinal tract of animals, but would be unlikely, due to the unfavourable environmental conditions in the gut.
- Livestock feeds such as cereal grains, grain proteins, hay and other silages (grass/clover, whole crop cereal and cereal/bean) are used by UK organic farmers. It is understood that there are no GM crops of these types currently grown commercially in the UK, although some are grown on an experimental scale.
- If GM grain proteins, cereals, grass or clover were commercialized in the UK, procedures for organic production would need to be reviewed to ensure the minimization of non-organic, and particularly GM, feedstuffs in organic livestock production.

4. Fate of DNA in slurry, manure, compost and mulch

The scarcity of experimental data on the survival of free DNA in these environments makes it difficult to draw conclusions regarding its persistence and fate. The following indications were drawn from the available data.

- As with soil, both the persistence and fate of DNA will depend on the local environmental conditions, including temperature, which in compost heaps is likely to be considerably higher than ambient.
- It might also be expected that the high biological activity of bacteria and other organisms in these environments might contribute to more rapid DNA degradation than in soil.
- The implications of transgenic material in manures and slurries for organic production are likely to be small, since they are generally produced on-farm, with little export to other farms. Green waste composts produced at large central sites need separate consideration.
- There is no evidence to suggest that DNA originating from transgenic organisms will persist (or behave any differently) in these materials compared with that from non-GM organisms. In many cases the DNA sequences that are used to genetically modify organisms originate from naturally occurring organisms commonly found in agricultural environments.

5. Impact of herbicide tolerant crops

- The direct effects of GM herbicide tolerant crops on organic farming will be limited because chemical weed control is not practised by organic farmers.
- The possibility of producing more persistent crop or feral plants has been investigated in herbicide tolerant crops in Europe. These studies have been complemented by evidence from the use of conventional herbicide applications. There is no evidence from studies over a decade of research on GM crops that the addition of a herbicide tolerance gene causes any increase in ecological fitness (in the absence of herbicide applications), compared with equivalent non-GM crop plants or hybrids.
- The effect of changes in herbicide use associated with the introduction of GM herbicide tolerant crops and consequential impacts on plant and animal biodiversity in the UK is currently being investigated in the UK Farm Scale Evaluations due to report in 2003.
- Development of resistance may occur with the repeated use of a herbicide. This could be delayed or prevented by the use of appropriate crop rotations and other agronomic practices.

6. Impact of pest and disease resistant crops

- Pest resistant crops can contribute to the reduced application of broad-spectrum insecticides in agricultural fields and thus promote a more favourable crop environment for insect herbivores, predators and parasitoids.
- Most of the studies on the direct effects of GM Bt crops on non-target organisms indicate that impacts on natural populations are not likely to be significant. Some small but significant variation in the results of these studies suggests that careful selection of particular Bt transgenic lines is advisable in the development of pest resistant crops, and that further research is needed on the impact of Bt varieties on non-target species.
- The implementation of resistance management practices is important to delay or prevent adaptation of pest insects to the pest protection substances (in this case Bt toxins), and so avoid any threat to the effectiveness of, for example, Bt sprays used by organic farmers.
- Studies on the release of Bt toxin in soil by Bt transgenic crops show that the protein rapidly binds to soil particles, a condition that may limit any threat to non-target organisms. Field studies are needed to test the results obtained in soil microcosms. Extensive research has revealed little or no evidence of negative effects of Bt Cry proteins against terrestrial and aquatic invertebrates including earthworms, collembola, daphnids, insect predators and parasites, spiders, and honeybees.
- Most of the research results on recombination between transgenes containing viral sequences, and infecting viruses in crop plants were obtained in the laboratory, usually in experimental conditions where it was possible to provide intensive selection pressure for recombination events. The relevance of these findings for field-grown crops, therefore, needs to be interpreted with caution.

7. Safety of promoters

- Even though there has been active debate about use of the cauliflower mosaic virus 35S promoter, we could find no evidence of substance that the 35S promoter presents any hazard to health or the environment, and hence no evidence of any impact on organic agriculture.
- The impact of other promoters would need to be investigated on a case-by-case basis and this is part of the standard risk assessment procedure followed in considering the field use of a GM crop.

8. DNA transfer in pollen and seeds

- Cross-pollination between sexually compatible crops is for most practical purposes, inevitable.
- The level of cross-pollination between crops falls sharply with distance, but the distance at which it becomes zero is impossible to determine with certainty.

- The physical presence of GM pollen or other plant parts (whether dead or alive), transmitted in a variety of different ways, will complicate verification of GM free status in organic crops and produce.
- Seeds also provide a means by which GM plant material might be transmitted to organic crops, via soil, farm machinery, transmission by birds and other animals or by gene flow through GM hybrid 'weeds'.
- Transfer of GM material by seed may be easier to contain than that by pollen through appropriate hygiene, organic standards and the use of organic seed.
- Hybridisation between GM crops and weeds could potentially produce more persistent weeds, especially through the genetic modification of stress tolerances such as cold or salt tolerance. This possibility will need to be examined carefully in the GM crop regulatory approval process.

9. Horizontal gene transfer

- The potential impact of horizontal gene transfer (HGT) depends less on the frequency of transfer and more on the gene in question and the environment in which the organism is living.
- While the possibility of horizontal gene transfer in the field cannot be ruled out, the highly selective barriers to gene transfer, coupled with extensive dilution of the marker gene in the nuclear genome of transgenic plants, means that the potential for increased risk by gene flow from transgenic plants is likely to be extremely small.
- As long as the transformed cell does not enter the germline, incidental horizontal gene transfers are unlikely to become integrated and permanent parts of a genome.
- There is no experimental evidence demonstrating HGT from GM plants to naturally occurring soil bacteria associated with plants under field conditions. However this may be because the frequency of detection is too low due to the dilution of marker genes, or that investigations have generally focussed on transfer and expression of functional genes, instead of shorter gene fragments. It is also possible that the use of prokaryotic sequences in transgenic plants may provide sufficient homology for gene transfer between plants and bacteria.

10. Impact of scale

- Some impacts of GM crops will be scale dependent and this emphasises the need for extending impact assessments beyond small-scale field experiments to farm scale and eventually commercial scale impact monitoring. This is consistent with recent changes in EU regulations on GM crops, which makes post-commercialisation monitoring a statutory requirement.
- The scale up of GM crops will increase the difficulties of setting isolation barriers to achieve zero GM content in organic crops.

Recurrent migration of GM pollen (or pollen from any crops) could have an influence on natural populations even if the transferred crop characteristics make resultant individual hybrids less competitive than non-GM plants.

Scientific report (maximum 20 sides A4)

REVIEW OF KNOWLEDGE OF THE POTENTIAL IMPACTS OF GMOs ON ORGANIC AGRICULTURE

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CONTENTS

Acknowledgements

We thank the Department for Environment, Food and Rural Affairs (DEFRA) for their financial support (Project OF 0193). We also thank BBSRC, John Innes Centre and Elm Farm Research Centre for the use of their facilities, Ruth Peart for help with the literature search and Helen Ghirardello for preparation of the manuscript.

PART 1. INTRODUCTION

1.1 Genetically modified (GM) crops are now a reality in many parts of the world. They are grown extensively in the Americas and increasingly in Asia. In the year 2001 there were 52.6 million hectares grown world-wide, which is approximately twice the land area of Great Britain (James, 2001). There is an increasing move to commercialise GM crops in other parts of the world, including the UK where experimental field trials have been taking place with GM crops since 1987. It is in this context that DEFRA commissioned this report on the likely impacts of GM crops and other GM organisms on organic agriculture in the UK through a search of the scientific literature and interpretation of the findings of this search.

1.2 There is frequently the impression that supporters of GM crops are inevitably against organic agriculture and vice versa. This is far from the truth for many people who appreciate that both approaches have important merits. Even though we have different perspectives on some issues (outlined in Appendix 1) we agree that there should be an accommodation of different farming systems in UK agriculture. The objective of this report is to outline some of the underpinning information that will influence the potential impacts of GMOs (Genetically Modified Organisms) on organic agriculture in the UK.

1.3 The EU Council Regulation on organic farming (EC No 1804/1999 para 10 (supplementing regulation (EEC) No 2092/91)) states that “genetically modified organisms (GMOs) and products derived therefrom are not compatible with the organic production method”.

1.4 The recently published first report of the Agriculture and Environment Biotechnology Commission (AEBC) recognises the need for “adequate separation distances” between organic farms and the current Farm Scale Evaluation GM trials. By “adequate” it means “separation distances that allow current organic standards to continue to be maintained, but recognising that some flexibility will be required to ensure that the trial can be completed” (AEBC, 2001).

1.5 The concerns of the organic sector with GM crops arise from the unwanted presence of GM crops or material in organic land, produce and products. Organic standards (EU regulation, national and sector bodies) prohibit GM and GM products. Some organic sector bodies also require a longer conversion period for land that has been used to grow GM crops compared with land that has not (Anonymous, 2000). Therefore there is a need to understand the potential causes of the presence of GM organisms or materials and how they are likely to impact on organic agriculture.

1.6 The potential causes of the presence of GM material in organic production are likely to be:

- The movement of seed from GM crops into organic crops.
- Pollen flow from GM crops or plant hybrids into organic crops or other plants on organic land.
- Flow of DNA through soil, gut or other micro-organisms into organic systems

1.7 There are also issues associated with possible changes in farming practices that might accompany the introduction of GM crops. These include:

- If the introduction of GM crops is accompanied by an increasing trend towards monoculture, this could have a further impact on reducing biodiversity in the agricultural environment.
- Trends toward monoculture could be important generally, but also specifically in relation to crops such as sugar beet and oilseed rape, which may be grown increasingly on organic farms.
- A further impact may be in relation to the availability of new crop varieties. If GM plant breeding methods or GM plant lines are used increasingly to generate new varieties, there could be a

serious reduction in the supply of non-GM germplasm for general use and in particular for use in organic agriculture.

- The increased use of Bt pest resistance might diminish the effectiveness of Bt sprays available for use in organic agriculture.

1.8 There is a heated and not always constructive debate surrounding the use of GM crops in the UK. This review is not intended to cover all areas of the debate but will focus specifically on the impacts of GMOs on organic farming (in respect to the EU regulation). The subjects of the different parts in this report were prepared in response to a set of questions on the impact of GMOs on organic farming submitted to DEFRA by Elm Farm Research Centre. This report has drawn primarily on peer-reviewed scientific literature.

PART 2. FATE OF DNA IN SOIL

2.1 In this section we consider factors affecting the persistence and fate of DNA in soil. Data from experiments carried out in the field, as well as in laboratory-based microcosms are considered in order to assess the potential of free DNA in soil to act as a source of transforming DNA for soil bacteria. Finally, we compare the outcomes of microcosm and field-based approaches to study DNA in soil. The topic will be discussed under the following headings:

- Persistence of DNA in soil
- Fate of DNA in soil
- Ecological relevance

Persistence of DNA in soil

2.2 Questions have been asked about the persistence of transgenes in the soil, and their possible impact on organic systems. These include the potential for this genetic material to remain as a source of transforming DNA for competent bacteria in soil, or in the gut of soil invertebrates following ingestion. The real issue, however, is whether the nature of this potential impact is of biological significance to organic agriculture, or whether its primary impact will be in making it difficult to meet the regulatory standards set by certification bodies. For example, for some certifiers, land that has been used to grow a transgenic crop may not currently be registered as organic for 5 years.

2.3 Soil is a dynamic, complex, heterogeneous environment composed of solid particles, aqueous and gas phases, inorganic ions and low molecular weight organic molecules. This complexity makes experimental analysis of DNA persistence in soil problematic. Here we discuss published evidence of factors influencing the persistence and fate of DNA in soil, and consider whether DNA from transgenic organisms is likely to behave in any way differently in soil compared with DNA from non-transgenic organisms.

2.4 On average, one gram (of any soil type) contains in the region of 50-200 micrograms of high molecular weight extracellular DNA (Reany *et al.,* 1982) with an estimated 1 μg being “free” DNA (Torsvik and Gorsøyr, 1978). This material probably originates from lysed animal tissues (Widmer *et al.,* 1996), microbial cells (Lorenz *et al.,* 1992, Nielsen *et al.,* 2000a), and also plant cells. A useful indication of the scale of the natural contribution of DNA to the environment is that pollen, fruit and leaves alone contribute thousands of tonnes of DNA to the environment each year (Doerfler *et al.,* 1998).

2.5 Under suitable conditions, DNA can persist in fossilized plant material for up to 20 million years (Golenberg *et al.,* 1990; Soltis *et al.,* 1992), although the survival of naked DNA in soil is much less. There are two ways in which the persistence of DNA can be investigated; using laboratory-based microcosms, or sampling soils for specific genes following crop trials.

2.6 Using non-sterile soil microcosms, the former approach revealed that less than 0.1% of the target sequence DNA from transgenic tobacco plants was detectable after 40 days (Widmer *et al.,* 1996). In a separate study, when free plasmid DNA was introduced into a soil microcosm, the full-length polymer was undetectable after 5 days (Romanowski *et al.,* 1992). Similar microcosm experiments demonstrated that DNA from recombinant *E.coli* remained detectable for 40 days, but at very low levels and was not transferred to the indigenous microflora (Recorbet *et al.,* 1993). By contrast, transfer of DNA from a genetically engineered strain to recipient bacteria was observed in microcosms (Henschke and Schmidt, 1990).

2.7 Several studies suggest that on average, small quantities of transgenic plant DNA survive for a few months in the field. For example, trace amounts of DNA from transgenic tobacco were detectable for several months (Paget *et al.,* 1998), but none was detected after 3 years, and there was no evidence of gene transfer to indigenous soil bacteria. In addition, Widmer *et al. (*1997) found that the amount of target sequence persisting from a transgenic tobacco trial declined rapidly to less than 0.4% of the initial amount within 2 weeks. Small amounts of transgenic DNA from sugar beet plants have also been detected in soil for up to 2 years after a trial, although again, no transfer to soil bacteria was detected (Gebhard and Smalla, 1999). These results, however, give no indication as to how long the DNA retains sufficient physical integrity to act as a source of transforming DNA. Furthermore, they do not take into account the persistence of DNA sequences found naturally in soil which are the same (or very similar) to those found in transgenic organisms. These include antibiotic resistance genes, the gene encoding the Bt toxin, and certain viral gene promoters, such as the 35S promoter from the cauliflower mosaic virus.

2.8 Enzymes in soil which can degrade naked DNA (nucleases) could have a significant effect on DNA persistence. These are most likely to be microbial in origin (Blum *et al.,* 1997). The main factor affecting the impact of soil nucleases on DNA persistence is the adsorption of DNA to the soil particles, which appears to confer protection to the DNA against nuclease degradation (Khanna and Stotzky, 1992; Lorenz and Wackernagel, 1994).

2.9 In addition, soil type appears to affect the degree of protection conferred to the DNA by binding (Gallori *et al.,* 1994; Romanowski *et al.,* 1992; Blum *et al.,* 1997). For example, binding to sand made DNA a hundred times more resistant to nucleases, with just 1 gram of sand able to bind the equivalent of 10^9 prokaryotic genomes (Romanowski *et al.,* 1992). Clay particles also adsorb soil nucleases, thus reducing the enzymic activity (Demanèche *et al., 2*001). Analysis of the interaction between soil particles and free DNA by transmission electron microscopy revealed that some areas of the DNA molecule are also adsorbed into the soil particles, leaving some portions available for degradation (Paget and Simonet, 1994) or transformation (Lorenz *et al.,* 1988; Demanèche *et al., 2*001).

2.10 Other factors, both environmental and chemical, also influence the persistence of DNA in soil, such as temperature and humidity (with DNA persistence being greatest in microcosms at 4 ° C, and at 10% water holding capacity; Widmer *et al.,* 1996). Soil debris (such as cell membranes, polysaccharides etc) may also increase persistence by reducing accessibility of nucleases to DNA (Nielsen, 1998). The binding capacity of soil increases as the salt concentration increases, and adsorption is greatest at pH 1, and weakest at pH 9 (Ogram *et al.,* 1988; Lorenz and Wackernagel, 1987; Khanna and Stotzky, 1992). The presence of divalent metal ions also increase the extent of binding of DNA to soil particles, with the degree of adsorption being affected by the concentration of the cations. Calcium promotes binding most strongly, followed by magnesium (Paget *et al.,* 1992; Romanowski *et al.,* 1991; Khanna and Stotzky, 1992; Lorenz and Wackernagel, 1987).

2.11 Another factor which may influence the binding of DNA to soil particles is the length of the DNA fragment. Shorter DNA fragments (of around 2.7 kb) bind preferentially compared with fragments of 23 kb (Ogram *et al.,* 1994). There are, however, conflicting results regarding the potential influence of DNA conformation on binding. Some authors (Gallori *et al.,* 1994) present evidence that plasmid DNA can be differentially retained on a clay column depending on its conformation, while others (Romanowski *et al.*, 1991) found that the conformation of plasmid DNA did not appear to affect its persistence in microcosms.

2.12 It is, however, difficult to imagine how these lab-based observations can be extrapolated in a

meaningful way to crop husbandry or field management strategies in order to influence DNA persistence.

Fate of DNA in soil

2.13 A free DNA fragment in soil could be subject to one of several possible fates. If there is high nuclease activity in the soil region, it is likely to be degraded. Alternatively, it may be used by other soil microbes as a nutrient source, or it may persist in the soil. In this latter case, if suitably competent bacteria are in the vicinity, it is possible that the DNA could be taken up and potentially incorporated into the bacterial genetic information.

2.14 In sterile microcosms, soil type appears to potentially influence transformation frequencies. For example, Nielsen *et al. (*1997b) showed that competent cells of *A. calcoaceticus* were transformed at a higher rate and for a longer period in silt loam than in sandy loam. In a separate study using microcosms containing plants, transfer of a plasmid from an introduced bacterium to the indigenous soil bacterial population was higher in the rhizosphere than the bulk soil in loamy sand and silt loam (Richaume *et al.*, 1992).

2.15 DNA bound to soil particles may still be available for transformation. For example *P. stutzeri* competent cells can take up both plasmid and chromosomal DNA bound onto soil particles in non-sterile microcosms (Sikorski *et al.*, 1998). Chamier *et al. (*1993) found that sand-adsorbed chromosomal DNA transforms as efficiently as that in solution. Furthermore, clay-adsorbed DNA can transform competent *E.coli*, cells even after DNase treatment (Paget *et al.*, 1992; Khanna and Stotzky, 1992), and *B. subtilis* and *P. stutzeri* cells can be transformed by sand-adsorbed DNA (Lorenz *et al.*, 1988; Lorenz and Wackernagel, 1990), in some cases up to 25-50-fold more efficiently than in liquid culture. Furthermore, clay-DNA complexes persisted in non-sterile soil microcosms, and retained biological activity (i.e. transformed competent *B. subtilis* cells) for up to 15 days. These experiments demonstrate that by binding to soil particles, DNA is not only protected against nuclease attack, but also remains available as a source of transforming DNA. It would appear that these interactions occur rapidly - within 2-3 hr in non-sterile soil (Berg and Trevors, 1990; Bertolla *et al.*, 1997). Kinetic analysis of the rate of DNA entry into competent *Streptomyces pneumoniae* cells suggests a rate of around 100 nucleotides/sec (at 30^{o}C; Méjean and Claverys, 1993). While this rate would presumably be lower under field temperatures, together, these results indicate that DNA persists in soil long enough to act as a potential source of transforming DNA.

2.16 Soil-dwelling animals may affect the fate of free DNA in several ways. They may ingest the DNA, breaking it down and absorbing the constituents. Alternatively, the fragment may remain intact as it moves through the gastro-intestinal tract of the animal, thus providing the potential for transfer of a foreign gene to gut bacteria (Hoffman *et al.*, 1998).

2.17 The feeding activities of microarthropods such as collembolans and mites can alter the structure of microbial communities in soil, as well as potentially enhancing the spread of plasmids (Hoffman *et al.*, 1998). They play a role in redistributing bacteria within the soil matrix to regions where the nutrient availability to cells could alter the bacterial competence status. They may also affect the extent of conjugation between indigenous soil bacteria. In non-sterile soil microcosm studies, the presence of earthworms increased the number of transconjugants from inoculated donor to indigenous recipient microorganisms by 100-fold. The distribution and diversity of transconjugants and bacteria within the microcosm also increased with the presence of the earthworms (Daane *et al.*, 1996). Earthworm casts and developing cocoons also provide a location for bacterial conjugation to occur (Henschke *et al.*, 1989). By optimising conditions in the laboratory to favour mobility of recombinant DNA by microarthropods in microcosm studies, Armstrong *et al.* (1990) found

occasional transfer of DNA between bacteria. From this the authors conclude that transfer between donor and recipient bacterial strains may occur in the field situation, but is rare.

2.18 There are few reports about the effect of soil pH on transformation frequencies, but in a microcosm study, the transformation frequency increased as the pH of the DNA-clay complex increased (Khanna and Stotzky, 1992). No gene transfer was seen at pH 1.

Ecological relevance

2.19 Much of the work cited here was carried out in small-scale, sterile microcosms in the laboratory. The advantage of such an approach is that experimental conditions can be strictly controlled, and it is possible to monitor small changes very accurately. There are, however, limitations to this approach, and caution should be exercised when extrapolating this work to the field situation (Nielsen, 1998).

2.20 There are numerous barriers to natural transformation (Nielsen *et al.*, 1997b), which probably means that the actual frequency of transfer is lower than may be suggested by microcosm studies. In addition, there are concerns about the interpretation of the results from some microcosm experiments.

2.21 First, soil is a heterogeneous matrix, and while a certain soil type might predominate in particular regions, microcosms consisting entirely of pure sand, for example, may not yield data that are directly relevant to the field situation. In addition, soil samples in microcosm studies are sometimes sterilised prior to the experiment. There are reported cases where results from sterile and non-sterile microcosms differ considerably. For example, Nielsen *et al. (*1997b) showed that transformation of *A. calcoaceticus* occurred in a non-sterile soil microcosm only in the presence of added nutrients and even then at a reduced frequency. Sterilisation releases additional nutrients into the microcosm, suggesting that bacteria in sterile microcosms may be of a higher nutritional status than in the field.

2.22 Furthermore, the biological potential of DNA to act as a source of transforming DNA may not be consistent with its apparent long-term physical integrity (Nielsen *et al., 2*000b). In addition, experiments to investigate transformation frequencies usually involve the addition of competent cells to the microcosm. These cells will have been prepared in the laboratory, using bacteria grown under conditions for maximum competence. This could result in unrealistically high transformation frequencies in a microcosm.

2.23 In an attempt to maximize the possibility of DNA transfer occurring in the microcosm, the amount of DNA added may also be higher than might be expected in the field. For example, Paget *et al.* (1992) carried out experiments where they added at least 1000-fold more DNA to the microcosm than is likely to be found in soil.

2.24 Finally, the distribution of bacteria in a microcosm may not be a realistic reflection of that in bulk soil (Henschke and Schmidt, 1989). Soil is a dynamic environment, with spatial and temporal changes taking place in the solid, aqueous and gaseous phases. The presence of invertebrates can also affect the distribution of bacteria (and, one might assume) other organic and inorganic components of the soil. Therefore a static microcosm may not give an accurate representation of the *in vivo* situation.

2.25 **Conclusions**

- DNA can persist in soil long enough to be taken up by competent bacteria. However, its longevity and fate will be influenced by the environmental conditions.

- On the basis of our existing knowledge, there is no reason to suggest that DNA originating from transgenic organisms will persist (or behave any differently) in soil compared with that from non-GM organisms. In many cases, the DNA sequences that are used to genetically modify organisms originate from naturally occurring species and may thus be part of normal soil DNA, although the relative quantities of different DNA sequences might be altered.

- Although transformation of competent cells by free DNA has been observed in the laboratory under optimised conditions, the likelihood of uptake of free DNA from soil in the field, and its incorporation into soil microbes is probably much less likely. This is due at least in part to the presence of soil nucleases and the requirement for a naturally competent bacterial cell.

- Relative to the thousands of tonnes of DNA being released annually into the environment by natural means, the proportion of transgenic DNA (DNA recombined in the laboratory) entering the soil environment from GM crops would be very small, even if such crops were grown commercially.

- While DNA can persist in soil, it could be argued that the rapid observed decline in its biological impact makes the 5-year conversion period after a GM crop more than sufficient

- From the literature surveyed there is no evidence of residual transgenic DNA in soil being a threat to organic agriculture.

PART 3. FATE OF DNA IN LIVESTOCK FEED AND POSSIBLE IMPACT OF GM FEED

3.1 Ideally, organic farmers should produce all of their own livestock feed. Current organic standards, however, allow for 40% of feed to be imported onto the farm, with 10% of this being from non-organic sources. These figures are for ruminants: there are slight differences for other livestock. The potential for GM material to enter a farm through livestock feed makes this an important issue for organic producers. The key points to be considered are:

- The persistence of DNA fragments in different types of feed
- The fate of DNA in the digestive system
- Feeding studies using GM crops

The persistence of DNA fragments in different types of feed

3.2 If DNA acting as an intact, functional transgene does survive in livestock feed, the principal question is whether there are effects on organic produce due to incorporation of the transgene into animal tissues, and if there are implications for the meat, eggs and milk (Beever and Kemp, 2000). It is also important to consider the potential movement of the transgene into the wider environment, such as in manure, or by horizontal gene transfer to gut bacteria by transformation. These issues will be discussed in later sections of this report.

3.4 There are several factors influencing the persistence of DNA in animal feed, and whether this DNA could be a source of transforming DNA. Here we will consider unprocessed, processed, and ensiled feed.

Unprocessed feed

3.5 The persistence of plant or microbial DNA in the feed presumably depends on the storage conditions and we were unable to find any reports where this has been investigated. Similarly, we were unable to find evidence on the persistence of DNA in material such as hay or dried grass (which has undergone desiccation prior to feeding), so cannot comment on the possible longevity of DNA in intact dried material. If, however, the plant cells remain unbroken, and there has been no heat treatment, it is reasonable to assume that the DNA is present and substantially intact until released from the plant tissues following ingestion (Duggan *et al., 2*000).

Processed feed

3.6 Animal feed may undergo a range of processing conditions which can affect the physical integrity of the DNA (Chiter *et al., 2*000; Forbes *et al.*, 1998). For the limited number of feed samples in which this has been analysed, grinding and milling did not affect DNA integrity, but heat treatment at 95°C resulted in fragmentation, rendering the DNA incapable of transforming competent bacterial cells. Chemical expulsion and extrusion also completely degraded genomic DNA from oilseeds (Chiter *et al., 2*000). Treatment of plant tissues at temperatures of 95°C or above for a few minutes were found to be adequate to render them incapable of transferring genetic information.

Ensiled feed

3.7 Silage (from corn, grass, alfalfa clover etc) is an important winter feed for ruminants (Daeschel *et al.*, 1987). Ensilation involves mechanical chopping of plant material which disrupts the cells, releasing DNA and nucleases. Lactic acid bacteria then ferment the water-soluble carbohydrate to produce lactic acid (Rixon *et al.*, 1995); the resulting decrease in pH prevents the growth of spoilage microorganisms. Lactic acid bacteria may also be added to stimulate rapid fermentation.

3.8 The two routes by which GM crops could have an impact on organic silage production are the

presence of transgenic plants in the crop to be ensiled, and mixing of a lactic acid bacterial inoculum with a recombinant strain.

Silage from transgenic plants

3.9 Fresh maize and maize silage contains high molecular weight plant DNA, some of which undoubtedly contains intact plant genes (Chiter *et al., 2*000). In silage made from Bt maize, fragments of transgenic DNA persisted (in declining amounts) for up to 106 days (Hupfer *et al.*, 1999). Shorter, non-functional regions of the gene persisted up to 7 months after ensilage, as did other, non-transgenic plant sequences. In separate laboratory microcosm experiments, a 1914 bp fragment of the Cry1A gene was only detectable in Bt maize silage for 5 days. While a non-functional smaller fragment was detectable for up to 100 days, the Cry1A protein was undetectable in Bt maize silage when examined after 4 months (Fearing *et al.*, 1997).

3.10 Another consideration is the potential survival of DNA in the effluent from silage fermentation. Plasmid DNA incubated for 30 seconds in silage effluent was undetectable by PCR, and the physical integrity of non-transgenic maize chromosomal DNA was destroyed after incubation for 1 minute in silage effluent at ambient temperature (Duggan *et al., 2*000).

3.11 Furthermore, *E.coli* cells lost viability after incubation for 4 hours in silage effluent (Duggan *et al., 2*000), suggesting that the conditions are unlikely to be conducive to the long-term persistence of bacterial competence, and the uptake of free DNA in this environment.

Recombinant lactic acid inoculum

3.12 Carbohydrate-degrading enzymes (such as amylases and cellulases) have been introduced into lactic acid bacteria, with the aim of increasing substrate levels to enhance lactic acid production during ensilation. This could improve digestability and nutritional content of the feed.

3.13 There are two pieces of evidence which indicate that recombinant lactic acid bacteria can survive and proliferate in silage. A recombinant strain proliferated and dominated the indigenous microflora (Sharp *et al.*, 1992), while a different *Lactobacillus* strain survived and stably expressed high levels of the recombinant protein *in situ* (Rixon *et al.,* 1995). No evidence was found, however, describing the development of bacterial competence in silage, making it impossible to speculate about the possibility of gene transfer in this environment.

Fate of DNA in the digestive system

3.14 If DNA fragments encoding intact genes persist in some types of unprocessed feed long enough to be ingested, their fate depends mainly on the digestive system of the animal. The question for organic herds is whether ingestion of a functional transgene could lead to incorporation of the gene into tissues (meat) or presence of the gene (or its product) in animal products, such as milk or eggs. Here we consider the potential for degradation of the gene by nucleases, as well as the possibility of DNA uptake by competent bacteria in the digestive system.

3.15 In considering the fate of DNA ingested by cattle and other ruminants, the regions of the gastro-intestinal tract on which we have focused here are the mouth and the rumen, which both precede a degradative acidic barrier in the digestive system. While microbes are present in other regions of the ruminant digestive system, these are far less abundant than in the mouth and rumen. It is not known whether the absence of a rumen in monogastric animals (such as chickens) will influence the likelihood of DNA transfer to gut bacteria. Here we summarise the published evidence regarding the effect of passage through a ruminant and monogastric digestive system on DNA integrity and transformation capability.

3.16 Chewing causes mechanical breakdown of cells and begins cleavage of the DNA molecules. Release of degradative enzymes in the mouth (McAllan, 1980), as well as some acid hydrolysis, cause further fragmentation of the DNA. There are some data on the survival of DNA and the possibility of horizontal gene transfer in the mouth; for example, DNA survives for a matter of hours in sheep saliva (Duggan *et al., 2*000). Maize chromosomal DNA and plasmid DNA was incompletely degraded after 1 hour at body temperature in sheep saliva, but chromosomal sequences were amplifiable after 24 hr incubation, and plasmid sequences after incubation for 2 hours. The DNA was capable of transforming competent *E.coli* cells even after incubation for 24 hours in the saliva.

3.17 By contrast, up to 25% of a target sequence (from a recombinant plasmid) persisted following incubation for 1 hour in human saliva. The transforming capability of the DNA, however, declined rapidly, with a half-life of just 50 seconds (Mercer *et al.*, 1999a). Unfortunately there are no data available regarding the incidence of competent bacteria in the mouth. We therefore cannot comment on the likelihood of uptake of free DNA by oral bacteria in this environment.

3.18 The rumen is mainly involved in fermentation and mixing of the food, aiding digestion by breaking down the plant material. The conditions are warm and moist, with a high microbial density (10^{11} microbes / ml), so it is reasonable to expect frequent and rapid encounters between bacteria. These conditions could, in theory, at least, be conducive to horizontal gene transfer (HGT) between rumen bacteria, or uptake of any surviving free DNA (Salyers, 1993; Forano and Flint, 2000).

3.19 HGT has been observed between cultured rumen bacteria in the laboratory (Morrison, 1996), by both transformation (Mercer *et al.*, 1999b) and conjugation (McConnell *et al.*, 1991). The rumen, however, contains nucleases (Flint and Thomson, 1990; McAllan and Smith, 1973), which degrade free DNA, making transformation less likely.

3.20 Experiments to investigate the persistence and biological activity of DNA in rumen fluid from sheep have revealed that while a target sequence could still be detected by PCR after a 30 minute incubation, biological activity (as judged by the ability to transform competent bacterial cells) lasted less than 60 seconds. Similar work demonstrated the degradation of DNA within 10 minutes in ruminal fluid (Russell and Wilson, 1988). Furthermore, the competence of rumen bacteria is inhibited by rumen fluid (Mercer *et al.*, 1999b), leading to the conclusion that gene transfer between rumen bacteria *in vivo* is likely to be a rare event (Folmer *et al.,* 2000).

3.21 It has been estimated (McAllan, 1982) that over 85% of plant DNA consumed by ruminants is broken down into nucleotides before entering the duodenum, which restricts the possibility of transformation by free DNA encoding genes.

3.22 Studies to estimate the rate of degradation of transgenic DNA in mice revealed that simulated gastric fluid resulted in complete degradation of DNA from glyphosate tolerant soybeans within 15 seconds, and within 100 minutes in simulated intestinal fluid (Harrison *et al.*, 1996).

3.23 There is, however, some evidence that foreign DNA may be exchanged between bacteria inhabiting the gastro-intestinal tract of mice (McConnell *et al.*, 1991), and that this DNA may not be completely degraded to mononucleotides (Doerfler and Schubbert, 1998). Following ingestion of viral DNA (from the M13 phage), pregnant mice produced foetuses containing the foreign DNA in some, but not all of their cells. This suggests a transplacental route for transfer, rather than incorporation into the parental germline. Other studies (Schubbert *et al.*, 1994; 1997; 1998), revealed viral DNA in the spleen, liver and intestines of mice who had also been fed M13 DNA.

3.24 There have, however, been some queries about the validity of extrapolating this work to the impact of foreign DNA on other, larger animals. For example, viral DNA behaves in a different way to plasmid or chromosomal DNA, and the amounts of DNA fed to the animals might have been excessively high. Also, the ratio of animal body weight to amount of DNA supplied in feed in these experiments means that the results might not be suitable for extrapolation to other animals (Beever and Kemp, 2000).

The results of feeding studies using GM crops

3.25 There are many examples of potential improvements to animal feed which could be made by genetic modification (Phipps and Beever, 2000), including enhancing rumen digestability of fibrous material, increasing availability of essential amino acids, and use of recombinant enzymes to improve palatability. Here we review feeding studies of existing GM crops to animals and consider the possible impact on animal or human health.

Glyphosate tolerant soybeans (GTS)
3.26 There are several studies where GTS have been fed to animals. In these studies, the feed performance when given to rats, chickens and dairy cattle is comparable with that of conventional soybeans (Hammond *et al.*, 1*996;* Harrison *et al.*, 1996). Furthermore, Padgette *et al. (*1996) produced evidence that GM (glyphosate tolerant) and conventional soybean were compositionally equivalent, while other authors demonstrated that milk production and composition was unaffected in cattle fed with GTS (Hammond *et al.*, 1996).

Bt maize
3.27 Comparative feeding studies have been carried out using Bt maize (Brake and Vlanchos, 1998). Hens fed either mash or pelleted maize showed improved feed performance compared with conventional maize (such as improved feed conversion ratios and increased yield of breast muscle). No adverse effects or differences in survival rate or body weight were observed.

3.28 Plant-derived non-transgenic DNA has been detected in the meat from chickens fed unprocessed whole Bt maize, but no evidence of the Cry1A transgene or its product in the meat was reported (Einspanier *et al., 2*001). No plant-derived DNA was found in eggs, and while short DNA fragments from plant chloroplasts were detected in the blood, none were found in the liver, spleen or kidney.

3.27 No Bt signal was detected in any tissue samples from cattle fed on Bt maize, including those from milk (Einspanier *et al., 2*001).

Plants containing snowdrop lectins
3.28 Studies on rats fed potatoes containing a gene encoding a snowdrop lectin have been reported by Ewan and Pustai (1999). These data remain somewhat contentious, as the experiments have been criticized for poor experimental design, making the results difficult to interpret in a statistically meaningful way. In addition, it has been suggested that the symptoms observed in the rats (damage to internal organs) were most likely to be associated with long-term protein deprivation.

3.29 It is important to put the findings of all the studies described here in a context which is relevant to the field situation. The two main points to consider are:

- The amount of DNA originating from transgenic plant material, as a proportion of the total intake of DNA in the diet.
- The fact that detection of the presence of a transgene does not imply actual or potential biological activity.

3.30 The first point has been considered by Beever and Kemp (2000), who calculated that if one assumes that DNA comprises less than 0.02% (dry matter) of plant material, then a 600 kg dairy cow fed GM maize as 60% of its total daily ration will ingest 2.6 μg transgenic DNA. Compared with the estimated 608 mg DNA ingested per day, this approximates to a transgenic dietary component of 0.00042% of total dietary DNA. This is an extremely low proportion of transgenic material which would be ingested. In addition, if homologues of the transgene gene carried by the plant are present in the environment anyway, it is difficult to see how this could be deemed to have an impact on organic animals. The second point is that detection of DNA in feed or the digestive system of an animal (by PCR) doesn't necessarily imply biological functionality, or capacity for transfer. The ability of bacteria in the digestive system to become competent *in vitro* doesn't necessarily mean that this will happen *in vivo*. We conclude that more data are needed on the incidence of bacterial competence to be able to assess meaningfully the potential for gene transfer in different environments.

3.31 **Conclusions**

- None of the published feeding trials provide unequivocal evidence of the harmful effects of feed containing GM material.
- DNA probably does not survive feed processing conditions which involve heat treatment, although it can survive ensiling.
- The proportion of GM DNA taken up as part of the whole diet will be extremely small.
- Many of the genes that have been introduced into GM crops are taken from organisms which live in the same or similar environments as unmodified crops. Therefore, the animal is likely to have ingested these genes from sources other than transgenic crops, although the combinations and quantities of the DNA in question may change under exploitation of GM crops.
- Horizontal gene transfer could theoretically occur between gut bacteria in the GI tract of animals, but would be highly unlikely, due to the unfavourable environmental conditions in the gut.
- Livestock feeds such as cereal grains, grain proteins, hay and other silages (grass/clover, whole crop cereal and cereal/bean) are used by UK organic farmers. It is understood that there are no GM crops of these types currently being grown commercially in the UK, although some GM crops are currently being grown experimentally.
- Should GM grain protein crops, cereals, grasses and clovers be introduced commercially in the UK, procedures for organic production would need to be reviewed to ensure the minimization of non-organic feedstuffs, and particularly GM feedstuffs, in organic livestock production.

PART 4. THE FATE OF DNA IN SLURRY, MANURE, COMPOST AND MULCH

4.1 Manures and composted materials are central to enhancing soil quality and fertility in organic systems. DNA could theoretically be present in manure and slurry as a result of incomplete digestion of food by animals, and in compost and mulch by release from plant cells as they break down. In addition, it is likely that these materials will also contain other micro-organisms or invertebrates, which could provide additional sources of transforming DNA. The presence of transgenic DNA in these materials could have an impact on organic systems if free DNA encoding a functional gene became incorporated into other species, such as bacteria. Here we consider three possible routes via which transgenic DNA might come to be present in manure, slurries, composts and mulches: (i) using animal feeds containing transgenic ingredients, resulting in manure and slurry containing GM material; (ii) composting or mulching of transgenic plant material; (iii) lysis of, or DNA release from, recombinant bacteria used to facilitate breakdown of material in compost or sludge units.

4.2 As we described in the section of this report on soil (Part 2), the key issues are the persistence and fate of free DNA in these materials, and whether the behaviour of DNA from transgenic organisms is different from non-transgenic organisms. Unfortunately, we have little or no knowledge of the persistence (or otherwise) of DNA in these materials, nor of the potential for bacteria in these environments to become competent to take up free DNA fragments. Here we review the published evidence under the following headings:

- Manure and slurries
- Composts and mulches
- Recombinant bacterial inocula
- but advise caution when extrapolating to field situations.

Manure and slurries

4.3 We were unable to find data relating to the persistence of free, non-transgenic DNA in manure. However, no transgenic DNA has been detected in manure from chickens or cattle fed grain or silage from Bt maize (Einspanier *et al., 2*001). This suggests that, in this case at least, the DNA was degraded to such an extent as to render the DNA smaller than the required length for a functional gene.

4.4 There is more information about the survival of plasmid-containing bacteria which have been isolated from manure (Smalla *et al., 2*000; Gotz *et al.*, 1996; 1997). These are important data to consider, as conjugation could be a potential method for DNA transfer. In some cases, the actual transfer of plasmids between these bacteria when cultured *in vitro* is reported (McClure *et al.*, 1989). In other studies, the bacteria were found to be carrying broad host range, or self-transmissible, mobilisable or self-conjugable plasmids, demonstrating the potential for conjugal DNA between bacteria in manure (Smalla *et al., 2*000).

4.5 This suggests that in the event of a competent bacterial cell in manure becoming transformed by a transgene, the DNA could potentially be transferred to other bacteria. There are, however, no data on the availability of competent bacteria which could take up free DNA in these environments. This makes it difficult to judge the likelihood of transformation of free-living bacteria in manure by a transgenic DNA fragment.

Composts and mulches

4.6 Again, little or no data are available on the survival of free DNA in composts and mulches, but many bacteria are present, which could release sources of transforming DNA as they lyse. Up to

10^{10} colony-forming-units per gram of compost have been detected in mushroom compost, (Amner *et al.*, 1988), with representatives of at least 5 different genera of bacteria identified in compost samples at phase 2 of the composting process (during which the compost is pasteurised).

4.7 Plasmid-bearing bacteria have also been identified in compost (McDonald *et al.*, 1998). In this case, there was no evidence of plasmid transfer, despite survival of the bacteria for up to 13 weeks. Other authors have also reported a low frequency (10^{-5}) of plasmid transfer between *B. subtilis* donor cells carrying a naturally occurring tetracycline resistance gene in mushroom compost and a non-plasmid bearing recipient strain (Amner *et al.*, 1991).

4.9 Again, there are no data on the development of natural competence in compost so it is difficult to draw conclusions regarding the possible uptake of free DNA by transformation.

Recombinant bacterial inocula

4.10 We can only speculate on the possible impact of using GM bacterial inocula. The best indication comes from results from McClure *et al.* (1989) who introduced a recombinant bacterial population into an activated sludge unit. The population survived for over 8 weeks, but gradually declined (which is characteristic of populations introduced in environments containing competitive indigenous bacterial populations). This suggests that recombinant strains could potentially survive in these environments.

4.11 **Conclusions**

The scarcity of experimental data on the survival of free DNA in these environments makes it difficult to draw conclusions regarding its persistence and fate. The following indications were drawn from the available data:

- As with soil, both the persistence and fate of DNA will depend on the local environmental conditions, including temperature, which in compost heaps is likely to be considerably higher than ambient.

- It might also be expected that the high biological activity of bacteria and other organisms in these environments might contribute to more rapid DNA degradation than in soil.

- The implications of transgenic material in manures and slurries for organic production are likely to be small, since they are generally produced on-farm, with little export to other farms. Green waste composts produced at large central sites need separate consideration.

- There is no evidence to suggest that DNA originating from transgenic organisms will persist (or behave any differently) in these materials compared with that from non-GM organisms. In many cases the DNA sequences used to genetically modify organisms originate from naturally-occurring organisms found in agricultural environments.

PART 5. IMPACT OF HERBICIDE TOLERANT CROPS

5.1 Four issues relevant to the potential impact of GM crops on organic agriculture will be discussed:

- Direct effects of the use of GM herbicide tolerant crops.
- The production of more invasive (weedy) GM crop plants or feral plants.
- The potential impact of a change in use of herbicides.
- Development of weeds resistant to particular herbicides.

Direct effects of the use of GM herbicide tolerant crops

5.2 The issues surrounding the direct impact of GM herbicide tolerant crops on organic farming will be limited, because organic farmers do not use herbicides. Furthermore because of the use of physical weed control measures by organic farmers, a GM herbicide tolerant weed that has no increased ecological fitness over its non-GM neighbours would be unlikely to impact directly on organic farming.

The production of more invasive (weedy) GM crop plants or feral plants

5.3 The important issue is whether certain GM plants might themselves become more persistent in agricultural habitats (weedy) than their non-GM counterparts or the GM plants might hybridise with wild relatives and the progeny become more persistent volunteers or weeds on organic farms.

5.4 The definition of invasive plants, weeds and colonisers, together with their ecological characteristics, were discussed by Williamson (1993b). The characters that define a plant as an invader, a coloniser or a weed, remain obscure. Luby and McNichol (1995) and Baker (1974) stated that weediness arises from many different characters and that the addition of one gene is unlikely to cause a crop to become a weed. This statement is opposed by Fitter *et al.* (1990) and Williamson *et al.* (1990) who suggest that small genetic change can cause large ecological alterations. But, there is consensus, however, that if a crop species has very few weedy characteristics, the addition of one or a few genes would be unlikely to cause the crop to become a weed problem. Special attention must be given to crops that already have weedy characteristics or when the added genes might be expected to improve the crop competitiveness in agricultural or natural systems (Ammann *et al.,* 2000).

5.5 It is important to note that many plant species can be found both as a crop and as a weed (Keeler *et al.,* 1996). A change in habit may potentially result in the evolution of a weed from a cultivated plant, the selection of cultivated forms from weed species or a change in habitat of a wild form that is closely related to cultivated species (Boudry *et al.,* 1993). Different authors (cited by NRC, 1989 and 2000; Boudry *et al.,* 1993, and Ammann, 2000) have observed each of the above examples in and around agricultural environments.

5.6 The currently available scientific evidence indicates that GM tolerant crops in the absence of herbicide applications are no more likely to be invasive in agricultural fields or in natural habitats than their non-GM counterparts (Lavigne *et al.,* 1995; Keeler *et al.*, 1996; Hails *et al.,* 1997; Sweet and Shepperson, 1997; Snow *et al.,* 1999 and Crawley, 2001). Squire *et al.* (1999) are of the opinion that GM “superweeds” are unlikely to arise in the UK, based on the following reasoning: (a) their sexually compatible wild relatives are not particularly aggressive or competitive as weeds, (b) the presence of

herbicide tolerance will not alter this invasiveness in the absence of the particular herbicide and (c) other herbicides or weed control practices could be used to control them.

5.7 Introgression of herbicide tolerance genes has been investigated by some authors. It has been shown that hybrids between oilseed rape and field mustard (*Brassica rapa*), when backcrossed with one of the parent lines, may have significantly higher seed yield than the weed species parent (*B. rapa*) line in some circumstances (Brown *et al.,* 1996), but not in others (Hauser *et al.,* 1998 a, b). The characteristics of hybrid plants can vary considerably so this is unlikely to be of general relevance in assessing the impact of other hybrid combinations.

The potential impact of a change in the use of herbicides

Effects on biodiversity

5.8 The widespread introduction of GM herbicide tolerant crops may cause a shift in weed populations and thus reduce weed species diversity and ecosystem complexity in the GM field and on neighbouring farms. Herbicides are widely used in conventional agriculture and few weeds are normally observed in many non-GM crops following standard herbicide treatments. It is argued, however, that because the currently available GM herbicide tolerant crops confer tolerance to broad spectrum herbicides such as glufosinate and glyphosate, their extensive use may reduce the diversity of weeds in agricultural habitats. It is debatable how broad spectrum these herbicides are in practice and it is unclear what their precise impact will be on weed populations in comparison with the range of herbicides (some relatively persistent) currently used in conventional agriculture. The Farm Scale Evaluations currently being carried out in the UK and due to report in 2003, are designed specifically to address these issues (AEBC, 2001).

5.9 The precise impact of the introduction of herbicide tolerant crops will depend on the rotational and agronomic practices adopted to manage them. The adoption of different herbicide use programmes may have different effects on plant and animal biodiversity in fields and field margins (Burnside, 1992; Giaquinta, 1992; Altieri, 2000). Radosevich *et al.* (1992) predicted that shifts in weed species composition and abundance would be exacerbated with the consecutive use of the same herbicide, providing favourable conditions for growth of particular weeds, insects and diseases (see also Altieri, 2000). Weeds exhibit considerable plasticity and thus are able to adapt to a wide range of cultivation practices. Experience with conventional agriculture has shown that weed species composition will vary within the same crop, among different fields and at different times of year (Burnside, 1996). Thus, weed population shifts are natural ecological phenomenon in crop management and should not be viewed as exclusive to GM crops. The effect of changes in herbicide programmes on plant and animal biodiversity in field and field margins are being investigated by NIAB, Cambridge (Sweet and Shepperson, 1997).

Effects on soil and water

5.10 It is sometimes argued that the use of herbicides will increase with the widespread introduction of GM herbicide tolerant crops and this will contribute to the contamination of soil and groundwater and have undesirable effects on plant and animal diversity generally. The pattern of herbicide use in the UK following the introduction of GM herbicide tolerant crops remains to be seen. However, no significant change in the overall amount of herbicide use has been observed with the adoption of herbicide tolerant crops in the United States (Heimlich *et al.,* 2000). A small but significant decrease in herbicide use has been reported in the US, after the introduction of GM soybeans (USDA, 2000; AgBiotech, 2001). An analysis by US soybean growers reported by Trewavas and Leaver (2001) shows that 7.2 million pounds of other herbicides have been replaced by 5.4 million pounds of glyphosate. This substitution resulted in the replacement of herbicides that are at least three times

more toxic, and that persist nearly twice as long as glyphosate (Heimlich *et al.,* 2000). Furthermore, the Canola Council of Canada (2001) reported the results of a study that shows that herbicide-tolerant oilseed rape eliminated the use of >6000 tones of herbicide in the 2000 growing season. Another important benefit argued from the use of herbicide tolerant GM crops in the US is that they facilitate zero till agronomic systems, which contributes to a reduction in soil erosion. There are however still active discussions on the significance of these kinds of data and on the long term impact of the use of broad spectrum herbicides (AEBC, 2001)

Development of weeds resistant to particular herbicides

5.11 Weed species have developed resistance to particular herbicides, through the long-term use of specific herbicides in many countries (Heap, 1997; Preston and Rieger, 2000). Typically, resistance to particular herbicides has appeared in areas where only one or a few herbicides are used consistently to manage weeds (Preston and Rieger, 2000). The regular use of a herbicide, including post-sowing application, will increase the likelihood of development of resistance. As discussed by Heap (1997), development of resistance will be of greatest concern for those herbicides to which some weeds have already developed resistance. Development of resistance to particular herbicides following the introduction of particular GM herbicide tolerant crops should be delayed, provided rotations and weed control measures are correctly managed (Preston and Rieger, 2000).

5.12 **Conclusions**

- The direct effects of GM herbicide tolerant crops on organic farming will be limited because chemical weed control is not practised by organic farmers.

- The possibility of producing more persistent crop or feral plants has been investigated in herbicide tolerant crops in Europe. These studies have been complemented by evidence from the use of conventional herbicide applications. There is no evidence from studies over a decade of research on GM crops that the addition of a herbicide tolerance gene causes any increase in the ecological fitness (in the absence of herbicide applications), compared with equivalent non-GM crop plants or hybrids.

- The effect of changes in herbicide use associated with the introduction of GM herbicide tolerant crops and consequential impacts on plant and animal biodiversity in the UK is currently being investigated by NIAB in Cambridge and the Farm Scale Evaluations due to report in 2003.

- Development of resistance may occur with the repeated use of a herbicide. This could be delayed or prevented by the use of appropriate crop rotations and other appropriate agronomic practices.

PART 6. IMPACT OF PEST AND DISEASE RESISTANT CROPS

6.1 Some GM pest and disease resistant crops have been grown very extensively (mostly in the Americas) so there is considerable experience in their management. The relevance of GM pest and disease resistance to organic agriculture will be considered under the following headings:

- Effect on agricultural biodiversity
- Impact on non-target organisms
- Development of resistance to Bt toxins in pests.
- Fate of Bt toxins in the soil
- Virus resistant crops

Effect on agricultural biodiversity

6.2 Experience with traditional breeding indicates that pest resistant crops may affect other organisms in the food chain, both positively and negatively (Hoy *et al.,* 1998). It is possible that the high expression of an insect toxin could affect other organisms in addition to the target herbivores (the crop pest), thereby reducing species diversity and simplifying food webs. The abundance of predatory insects, spiders and mites will decrease if their herbivore prey populations are reduced. Bats and birds are known to prey on larvae and adults of several lepidopteran corn pests. If pest insects and their predators and parasitoids are significantly reduced in crop fields and adjacent margins, it is likely that there will be a decrease in the insect prey available for birds, rodents and amphibians. This could potentially interfere with the diversity within food chains including those on neighbouring organic farms.

6.3 This type of ecological effect has been observed following the overuse of insecticides or regional planting of highly resistant traditional crop varieties (Gould *et al.,* 1991; Gould, 1998; Hoy *et al.,* 1998). Widespread use of chemical pesticides to control primary pests often disrupts the natural controls that prevent the outbreak of secondary pests by destroying natural insect enemies. If the planting of GM pest resistant crop varieties eliminate the need for broad-spectrum insecticidal control of primary pests, naturally occurring control agents are more likely to suppress secondary pest populations, maintaining the diversity and abundance of prey for birds, rodents and amphibians.

6.4 A five year period of intensive sampling of arthropods in Bt potato fields in the United States showed the presence of an abundant and diverse community of more than 200 species of arthropods, including biological control agents (parasitoids and predators) and potential insect prey (work reported by Hoy *et al.*, 1998). However, this did not involve a direct comparison with non-GM potato fields. Riddick *et al.* (2000) examined the effect of Cry3A in transgenic potatoes on the abundance of naturally occurring generalist predators throughout the growing season. They concluded that there were no deleterious effects on the populations of heteropterous predators, ladybirds and ground foraging carnivorous carabids, ants and spiders. The predator *Orius insidiosus* and the spiders were significantly more abundant in the transgenic potato fields compared with the non-transgenic potato fields. Lozzia (1999) looked at biodiversity and structure of ground beetle populations in Bt corn and concluded that there was no detrimental effect of Bt corn on beetle species biodiversity.

6.5 A reduction in pesticide use to control insect pests has been reported with the planting of GM insect resistant cotton in the south-east region of the United States (Benedict and Altman, 2000 - cited by Perlak *et al.,* 2001; Fernandez-Carnejo *et al.,* 2000). This resulted in an increased number and diversity of other arthropods in the crop. Populations of predatory arthropods that help to control secondary pests like aphids were found to be more abundant in Bt cotton fields compared with sprayed conventional cotton crops (Trewavas and Leaver, 2001).

6.6 Before the commercial introduction of Bt maize in 1996, the European corn borer was only partially controlled using chemical insecticides. Timing of applications for control was difficult, so farmers either accepted yield losses or used more spray applications. Since Bt corn was introduced, the use of pesticides recommended for European corn borer control decreased from 6 million acres to slightly over 4 million acre sprayed in 1999, a drop of about one-third, according to the Environmental Protection Agency (USDA, 2001).

6.7 Moreover, the regulatory systems require that susceptible pest populations are maintained by having to grow non-transgenic crop refuges (discussed below). Such refuge areas can be managed to maintain and augment populations of natural enemies close to Bt crops. Indeed, it can be argued that the maintenance of refuges ought also to be part of the strategy for the conventional use of broad-spectrum insecticides.

Impact on non-target organisms

6.8 Some research has shown that microbial insecticide formulations of Bt (permitted in organic farming) can have negative effects on natural enemy species (Croft, 1990; Laird *et al.,* 1990; Glare and O'Callaghan, 2000). This experience shows that it is important to determine the impact of Bt crops on populations of insect predators and parasitoids in the agroecosystem. A reduction in natural enemy populations would result in the kind of undesirable effect discussed above (6.2) on neighbouring organic farms. Bt crops can potentially affect natural enemies in the following ways: (i) their host populations (food source) may be reduced to the extent that their development will be limited (as discussed in the above paragraphs), (ii) the natural enemy species may feed directly on the Bt plant tissues (e.g. pollen), and (iii) they may feed on pest herbivores that have sequestered Bt toxin from the crop.

Effect on predators and parasitoids

6.9 A number of laboratory studies have looked at the direct effects of GM Bt crops on pest-predators or parasites and found no negative impacts. Other laboratory studies presented contrasting results (reviewed by Hoy *et al.,* 1998; and Schuler *et al.,* 1999 and Schuler, 2000). In field studies, however, there are no reported negative effects of Bt crops on natural enemies (Schuler *et al.,* 1999).

6.10 Caution must be taken when drawing conclusions from results of small sets of laboratory studies that test non-target species under unnatural conditions or focus on hazard alone, without considering the level of exposure that will occur under natural conditions (Trewavas and Leaver, 2001). The type of effect on natural enemies depends as much on the specific attributes of the resistance mechanism as it does on the details of the interaction with the natural enemies, their host/prey interaction, and the plants on which this interaction occurs (Hoy *et al.,* 1998).

Effect on non-target herbivores

6.11 Many species of Lepidoptera or Coleoptera (depending on the type of Bt toxin expressed), both target and non-target, are likely to be directly susceptible to the Bt toxins produced by transgenic crops. Losey *et al.* (1999) showed evidence that the larvae of monarch butterflies (*Danaus plexippus*) living in weeds near maize fields could potentially be affected adversely by Bt maize pollen drifting on to the foliage of plant species favoured by the butterfly. These results have been questioned on the basis that they came from small-scale laboratory assays with high levels of toxin expressed in no-choice tests. Indeed, six recent studies (Hellmich *et al.,* 2001; Oberhauser *et al.,* 2001; Pleasants *et al., 2*001; Sears *et al., 2*001; Stanley-Horn *et al.,* 2001; Zangerl *et al., 2*001) when taken together, suggest that the risks to monarch butterflies posed by current Bt maize are not likely

to be significant. These studies show that, while Bt pollen does have some toxic effects when fed to butterfly larvae, the pollen densities likely to be encountered in the field are too low to pose a risk to monarch larvae. However, another study showed that in field tests low concentrations of pollen from event 176 Bt maize (a particular line of Bt maize that is being phased out of production) dramatically reduced growth rates among black swallowtail caterpillars, *Papilio polyxenes* (Zangerl *et al., 2*001). An earlier study (Wraight *et al., 2*000) noted that a widely used Bt maize-containing event 810 (another line of Bt maize) had no adverse effect on black swallowtails living on weeds near cornfields. From these results it is reasonable to infer that a careful selection of particular Bt transgenic lines is advisable in the development of pest resistant crops, and that research is needed on the impact of Bt varieties on non-target species.

Development of resistance to Bt toxins in pests

6.12 Farmers and plant breeders have for many years struggled to keep ahead of a pest's ability to adapt to the techniques used to control them. Experience with chemical pesticides has proven to be challenging, as pests quickly evolve resistance to intensively used pesticides (Raymond *et al.,* 1991; Gould, 1991). Pests have also historically overcome resistance introduced into crop plants in traditional breeding programmes (Gould, 1998). More recently, several studies have shown that pests can also adapt to Bt toxins produced by the biopesticide bacterium *Bacillus thuringiensis* under field and laboratory conditions (Gould, 2000). Pest resistance to Bt transgenic crops has been observed under laboratory conditions (reviewed by Tabashnik 1994; Obrycki *et al.,* 2001) but not, to our knowledge, under field conditions to date.

6.13 One of the greatest concerns is that the widespread use of Bt crops could lead to the evolution of a number of important insect pests that are resistant to the Bt pesticide. This is of particular concern to organic farmers because they use the *B. thuringiensis* bacterium as a permitted pesticide. Several strategies for resistance management have been proposed to delay adaptation to Bt crops by pest populations (Gould 1998). The most widely used is the high-dose-refuge strategy, which has been implemented in North America (Alstad and Andow, 1995). The implementation of effective resistance management practices is crucial to obtain the greatest benefits from pest resistant transgenic crops and to allow the continued effective use of *B. thuringiensis* biopesticides.

6.14 It must be pointed out that a recent survey conducted by United States maize growers (Dove, 2001) has shown that, in 2000, almost 30% of the farmers failed to comply with the refuge protocols designed to prevent or delay the emergence of insects resistant to Bt toxins. This rate of non-compliance could enhance the risk of plant resistance breakdown. There is no reported evidence of insect resistance to Bt crops under field conditions in the United States, although Bt-resistant insects have been observed in areas where Bt biopesticides are sprayed on crops (Dove, 2001).

Fate of Bt toxins in the soil

6.15 It has been shown that Bt plants exude Bt toxins from their roots during their entire life cycle, and the toxin is also released from dead plant material incorporated into the soil after harvest (Saxena and Stotzky, 2000). This could potentially result in additional exposure of soil organisms to these toxins, with potential effects on non-target organisms, both negative or positive. It is important to emphasise that *B. thuringiensis* is a natural soil-borne bacterium that normally sheds its toxin into soils. A body of work has been reported on the issue of Bt toxins on soil, with differing conclusions. Sims and Holden (1996) and Palm *et al.* (1996) observed a rapid decrease of free Bt toxin on the surfaces of the plant and in soil. Others have found that the toxin can be bound to elements of the soil (clay particles, humic acids) and become stabilised (Tapp and Stotzky, 1995, 1998; Koskella and Stotzky, 1997; Crechio and Stotzky 1998; Saxena and Stotzky 2000; Stotzky 2000).

6.16 The work on Bt toxin bound in soils has shown that the toxin can be active (Tapp and Stotzky, 1995, 1998, Koskella and Stotzky, 1997, Crechio and Stotzky, 1998, Saxena and Stotzky, 2000, Saxena *et al*., 1999, Stotzky, 2000) for possibly many hundreds of days (Saxena and Stotzky, 2000). As the toxin needs to be ingested to be active against insects, only those organisms that feed on soil, such as earthworms, would be potentially threatened by toxins bound on soil particles. Bt Cry proteins have been tested against a wide variety of terrestrial and aquatic invertebrates including earthworms, collembola, daphnids, insect predators and parasites, spiders, and honeybees. In most cases, no adverse effects were observed even though test populations were exposed to levels of toxin in excess of 500-1,000-fold higher concentrations than they would be expected to encounter under field conditions (EPA, 2000).

6.17 The studies determining rates of degradation of Cry proteins in soil were essentially carried out in soil microcosms. The fate of Bt toxins in soil under field conditions still remains to be investigated.

Virus resistant crops

6.18 Organic farming is likely only to be indirectly affected by issues surrounding the use of virus resistant GM crops. The main questions surround the possibility of transcapsidation, recombination and synergism among viral genomes present in a GM plant. These may potentially have undesirable consequences such as: (1) disease transmission in a novel way or by a novel vector (Osbourn *et al.,* 1990; Farinelli *et al.,* 1992; Candelier-Harvey and Hull, 1993; Lecoq *et al.,* 1993), (2) the creation of a virus with novel combination of properties (Lommel and Xiong, 1991; Gal *et al.,* 1992; Greene and Allison, 1994), or (3) the production of a more severe disease symptom (Valkonen, 1992).

6.19 According to Falk and Bruening (1994), the probability of recombination between transgene RNA and viral genomic RNA is unlikely to be any greater in GM plants carrying viral DNA sequences compared with natural viral infections. Furthermore, it also is unlikely that any virus resulting from recombination will be more competitive than wild type viruses throughout the full infection cycle.

6.20 Most of the research results available in this area were obtained under laboratory conditions, usually under intensive selection pressure. The occurrence and frequency of the above events in field conditions are yet to be determined. The significance of recombination between viruses in the same taxonomic group is unclear at this time. Furthermore, potential interactions among different species of pathogens are important and should be tested before a new cultivar is marketed.

6.21 It has been proposed (Hoy *et al.,* 1998) that the incorporation of resistance to potato leaf roll would avoid the need to spray against the aphid vector of the virus. As a consequence of allowing the number of aphids to increase, the expectation is that the number of the natural control beetles (ladybirds) would also increase. This kind of application of disease resistance may have a direct benefit to increasing the numbers of natural pest predators in the agroecosystems.

6.22 **Conclusions**

- Pest resistant crops can contribute to the reduced application of broad-spectrum insecticides in agricultural fields and thus promote a more favourable crop environment for insect herbivores, predators and parasitoids.

- Most of the studies on the direct effects of GM Bt crops on non-target organisms indicate that the impact on natural populations are not likely to be significant. Some small but significant variation in the results of these studies suggests that careful selection of particular Bt transgenic lines is

advisable in the development of pest resistant crops, and that further research is needed on the impact of Bt varieties on non-target species.

- The implementation of resistance management practices is important to delay or prevent adaptation of pest insects to the pest protection substances (in this case Bt toxins), and so avoid any threat to the effectiveness of, for example, Bt sprays used by organic farmers.

- Studies on the release of Bt toxin in soil by Bt transgenic crops show that the protein immediately binds to soil particles, a condition that limits any threat to non-target organisms. Field studies are needed to test the results obtained in soil microcosms. Extensive research has revealed little or no evidence of negative effects of Bt Cry proteins on terrestrial and aquatic invertebrates including earthworms, collembola, daphnids, insect predators and parasites, spiders, and honeybees.

- Most of the research results available on virus resistant crops were obtained under laboratory conditions, usually under intensive selection pressure. The occurrence and frequency of the phenomena observed in these experiments are yet to be tested under field conditions and thus should be interpreted with caution.

PART 7. SAFETY OF PROMOTERS.

7.1 A question was raised about the safety of gene promoters used to regulate the expression of transgenes in GM plants. The most common promoter used in GM plants is the cauliflower mosaic virus 35S promoter. The issues raised were whether this promoter:

- Can produce toxic affects
- Can recombine with other micro-organisms and
- Can produce potentially damaging organisms.

Can the 35S promoter produce toxic effects?

7.2 The 35S promoter is the most widely used promoter in GM plants and is present in a substantial area of transgenic plants grown worldwide. Cauliflower mosaic virus and its promoters have been the subject of extensive research over many decades and the 35S promoter has been used widely in research programmes to study the expression and stability of transgenes in a range of plant (including crop) species. Cauliflower mosaic virus is a pathogen that infects plants (mainly *Brassica* species) in many parts of the world, is frequently present as several thousands of viral copies (with its 35S promoter) per cell and is found in *Brassica* plants bought from shops and markets. Indeed, these are often the sources of viral isolates used in research on the virus. From this extensive experience over many decades, there is no evidence that the 35S promoter is toxic or in any way harmful to people, animals or the environment.

Can the 35S promoter recombine with other micro-organisms?

7.3 A substantial amount of work has been undertaken to test whether horizontal transfer of the 35S promoter (and other promoters) can occur. Robinson (1996) showed that it was possible to move transgene DNA into 'wild type' virus that infected GM plants. Others showed that recombination could occur between plants and virus under experimental conditions designed to favour recombination or its detection. (Vaden and Melcher, 1990; Schoelz and Wintermantel, 1993; Greene and Alison, 1994; Wintermantel and Schoelz, 1996). Recombination between viruses is known to occur naturally in mixed viral infections in plants.

7.4 Recombination between infecting viruses and transgene viral sequences could cause a change in an infecting virus with the possibility of widening the viral host range, modifying symptom expression and the mode of viral movement and dispersal. Recombination between the transcript of a virus-derived transgene and genome of another virus has been observed, but only under conditions of strong selection and when the two virus were closely related (Lommel and Xiong, 1991; Gal *et al.,* 1992; Greene and Allison, 1994). No cases of natural recombination leading to new viruses have been documented, even after intensive research on particular virus groups. There is accumulated evidence of rearrangements of transgenic DNA during transformation. In most cases, these rearrangements result in the non-functioning of the transgene and are selected out (i.e. the transgenic lines are discarded) in the early stages of analysis and testing of the transformed lines (Hull *et al.,* 2000).

Can the 35S promoter produce potentially damaging organisms?

7.5 Kohli *et al.* (1999) believed he had identified 35S recombination hot spots. In a review of much of the above literature Ho *et al.* (1999) believed there was a danger of horizontal transfer into new virus with possible new host ranges. However, these views are not universally held. Hull *et al.* (2000) responding to Ho *et al.* (1999) saw minimal risk: even though recombination could occur, its probability is extremely low. A review by Morel and Tepfer (2000) supports this view and suggests

that the 35S promoter is not particularly mobile, that there is no evidence of activity of the promoter in mammalian cells and that we are already continually exposed to the promoter through our normal intake of food.

7.6 Therefore, after several decades of research on the virus, extensive exposure of people and the environment to the virus in infected food crops and considerable experience of the use of transgenic plants containing the 35S promoter, there is no evidence of any direct danger to humans or the environment from the use of the 35S promoter.

7.7 **Conclusions**

- Even though there has been active debate about use of the 35S promoter, we could find no evidence of substance that the 35S promoter presents any hazard to health or the environment, and hence no evidence of any impact on organic agriculture.

- The impact of other promoters would need to be investigated on a case-by-case basis. This is part of the standard risk assessment procedure followed in the EU regulations when considering the field use of a GM crop.

PART 8. DNA TRANSFER IN POLLEN AND SEEDS

8.1 The transfer of DNA from GM crops by pollen and seeds could potentially have implications for organic farming in three ways:

- DNA transfer by pollen, including:
  (i) The pollination of organic crops by GM pollen
  (ii) The physical presence of GM pollen on an organic crop
- DNA transfer by seeds
- The transfer of GM traits associated with invasiveness into weeds and the exacerbation of weed problems.

DNA transfer to organic crops by pollen

The pollination of an organic crop by GM pollen

8.2 The pollination of organic crops by GM crops might occur between related, sexually compatible crop species, growing physically close enough for viable pollen to move between them and where there is synchronous flowering. There is substantial evidence collected over many decades of crop plant breeding and genetics, that cross-pollination occurs in agricultural fields. There are reports on pollination from a range of crops such as maize (Doebley, 1990; Garcia *et al.,* 1998; Hall *et al.,* 2000), cucurbit (Wilson, 1990; Hokanson *et al.,* 1997a and b), millet (Till-Bottraud *et al.*, 1992), sunflower (Arias and Rieseberg, 1994), raspberry (Luby and McNichol, 1995) and oilseed rape (Lefol *et al.,* 1995, Lefol and Darmency, 1996; Darmency *et al.,* 1998; Lavigne *et al.,* 1998; Snow *et al.,* 1999).

8.3 It is clear from this evidence that there has been and will continue to be pollination between sexually compatible crops in agriculture, including between non-organic and organic crops. In a UK context the main GM crops of significance to organic agriculture, in terms of gene flow, are likely to be cereals (wheat, barley, oats etc), maize, oilseed rape and sugar beet. Neither oilseed rape nor sugar beet is currently widely grown in organic systems, but there is increasing interest and demand for organic production in these crops. Maize is a potential problem but the pollen is generally heavy and does not travel far; sugar beet is biennial and flowers only to a minor extent during agricultural production.

8.4 The factors influencing cross pollination between GM crops and organic crops has been considered in detail in a previous report (Moyes and Dale, 1999) where background knowledge of the distance of pollination in different crops was considered. This report drew extensively on the experience gained over many decades of the production of high genetic purity Breeders' Seed and Certified Seed for supplying the agricultural industry. The conclusion from that report was that pollination falls rapidly with distance, but the distance at which pollination is zero is impossible to determine with accuracy.

8.5 It has been argued that the potential to limit transgene movement by the modification of the chloroplast genome might reduce the possibility of gene transfer by pollen (Daniell *et al.*, 1998). Tobacco plants with the EPSP synthase gene stably incorporated into the chloroplast genome were glyphosate tolerant. The transgene is, it is argued, less likely to transfer by pollen to an adjacent crop, because the transgene is encoded on the plastid genome. Due to their maternal inheritance, plastid genes are rarely transmitted by pollen, and hence this may reduce the potential for hybridization of GM crops with their crop or feral relatives. While this is an important scientific development it has some limitations in application; (a) It is likely that some transgenes will not

function adequately in the plastid genome; (b) The transfer of pollen from a feral species or non-GM crop to a GM crop would still produce hybrid seeds carrying the transgene, and (c) Although crops generally do not transfer plastids in pollen, some plants do have a detectable level of pollen transmission (paternal inheritance).

The physical presence of GM pollen on an organic crop

8.6 The mere presence of GM pollen (or for that matter any plant material or debris of GM plant origin), whether dead or alive may make it difficult to achieve a zero transgenic DNA content in an organic crop or its product. Modern methods of DNA analysis (the polymerase chain reaction or PCR) are capable of detecting minute amounts of pollen from a GM crop whether it is from the same crop type or different. This will make zero GM content, as verified by PCR methods, difficult to achieve in practice. The other difficulty with zero GM content is that many of the transgene DNA sequences that are used for GM molecular detection are already present in nature and are likely to complicate and confuse analysis for the detection of minute levels of transgene DNA. The consequence of these practical agricultural and analytical constraints will present major difficulties for the verification and policing of zero transgenic DNA content in organic crops and produce.

8.7 Several studies have looked at pollen movement and distances needed to minimise GM pollen drift to organic crops (Moyes and Dale, 1999; Sweet and Shepperson, 1997; Tufto *et al.,* 1997; Assuncao and Jacobi, 1999; Giddings, 2000). From these reports it is clear that it is probably impossible accurately to predict the spread of pollen and to conclude with any certainty how far a GM crop needs to be from an organic crop to ensure that zero GM pollen (even if dead) arrives on the organic crop. Bees, other animals and methods of human transportation may also contribute to long-distance pollen transfer.

DNA transfer by seeds

8.8 Seeds are also an important means by which transgenic DNA might be transferred to organic crops. This subject was also considered in the earlier report by Moyes and Dale (1999). Seeds can remain viable in soil for many years (depending on the plant species and soil conditions) and are usually dispersed over shorter distances than pollen, although they can occasionally be moved over long distances by birds and other animals (Hancock *et al.,* 1996; Gressel and Kleifeld, 1994). Another major method of movement of seeds is on farm machinery, including seed drills, combine harvesters etc. It can be very difficult to remove all traces of seeds following previous machinery use. Another means of dispersal is through the establishment of a plant containing a transgene followed by seed dispersal from that plant, and in this instance both time and selection pressures are crucial factors (Raybould and Gray, 1993). Pessel *et al.* (2001) studied volunteer oilseed rape on road verges showing that DNA transfer by seed is possible, if seeds escape from cultivated areas into crop borders or roadsides.

The transfer of GM traits associated with invasiveness into weeds and the exacerbation of weed problems.

8.9 The impact of hybridisation with a GM crop on weed (including volunteer) flora will be based on the ability of weed plants to pollinate (or be pollinated by) GM plants. A review by Ellstrand *et al.* (1999) gives examples of oilseed rape barley, wheat and beans that hybridised with weeds. In the UK the probability of hybridisation with weeds is considered minimal for wheat, low for oilseed rape and barley, and high for sugar beet (Raybould and Gray, 1993). Hybridisation with maize is not an issue in the UK as there are no sexually compatible wild relatives.

8.10 The possibilities of hybridisation between oilseed rape and related species have been reviewed by Scheffler *et al.* (1993, 1995). There have also been several detailed studies of hybridisation between oilseed rape and particular weed species. Lefol *et al.* (1995) and Lefol and Darmency (1996) showed that oilseed rape could hybridise with hoary mustard, Darmency *et al.* (1998) with wild radish, and Jorgensen and Andersen (1994), Brown *et al.* (1996), Mikkelsen *et al.* (1996), Snow *et al.* (1999) and Halfhill *et al.* (2001), with wild brassicas.

8.11 The consequences of the transfer of transgenes from GM crops to weeds will depend on the nature of the transgene and the biology and ecology of the recipient weed species. The consequences of the transfer of herbicide tolerance has already been discussed in an earlier section (Part 5) and is unlikely to confer any competitive advantage to hybrids outside agricultural areas, and almost certainly not on organic farms where herbicides are not used for weed control. The transfer to weed species of characters such as resistance to particular pests and diseases or tolerance to stressful conditions (e.g. drought or salt tolerance) could potentially give weed species a selective advantage. In this context, Ramachandran *et al.* (2000) investigated the competitive ability of an insect-resistant transgenic oilseed rape variety with non-transgenic oilseed rape in seed mixtures. The transgenic variety was competitively superior when the two varieties were subject to diamondback moth selection pressure in green house experiments and in field plots. In a similar study, Stewart *et al.* (1997) showed that under certain conditions there is a likelihood of an increased fitness in oilseed rape varieties expressing Bt transgenes.

8.12 These kinds of impacts on agriculture and on the wider environment following hybridisation are issues that are examined in the regulatory approval process. Some of the modifications made by GM plant breeding may have parallels in conventional plant breeding. Therefore experience of the impact of hybridisation between conventional crop varieties and weed species on organic agriculture would have important lessons for the interpretation of the impact of hybridisation between GM crops and weed species. However some of the genetic modifications that can be envisaged for the future in GM plant breeding, such as salt tolerance or cold tolerance, could potentially produce novel crop types that will need to be examined extra carefully when interpreting their impact on organic and other farming systems and on the environment as a whole.

8.13 Conclusions

- Cross-pollination between sexually compatible crops is for most practical purposes, inevitable.
- The level of cross-pollination between crops falls sharply with distance, but the distance at which it becomes zero is impossible to determine with certainty.
- The physical presence of GM pollen or other plant parts (whether dead or alive), transmitted in a variety of ways, will complicate analytical verification of GM free status in organic crops and produce.
- Seeds also provide a means by which GM plant material might be transmitted to organic crops, via soil, farm machinery, transmission by birds and other animals.
- Transfer of GM material by seed may be easier to contain than that by pollen through appropriate hygiene, organic standards and the use of organic seed.
- Hybridisation between GM crops and weeds could potentially produce more persistent weeds, especially through the genetic modification of stress tolerances such as cold or salt tolerance.

This possibility will need to be examined very carefully in the GM crop regulatory approval process.

PART 9. HORIZONTAL GENE TRANSFER

9.1 Horizontal gene transfer (HGT) is the non-sexual transfer of genetic material between organisms (Prins and Zadoks, 1994). It is an important issue, as it might provide a means by which a transgene could, in theory at least, become incorporated and expressed in the DNA of a non-target species. Horizontal gene transfer events are difficult to observe directly, indicating perhaps that our technical ability to search for and determine horizontal gene transfer may prevent us from properly monitoring and quantifying this phenomenon in the field. There are, however, examples where apparently natural gene transfers between species, or even across kingdoms appear to have taken place, which we consider in this section.

9.2 The key issues to address are the extent, frequency and impact of natural horizontal gene transfer between species. This will give some indication as to the potential impact of gene flow from a genetically modified organism. It is, however, impossible to draw generalised conclusions for all transgenic species. This means, therefore that it is important that risk assessment studies are carried out on a case-by-case basis to ascertain whether gene transfer from a particular genetically-modified organism would pose an increased risk to human or animal health (such as by exposure to new allergens or toxins), or whether the transfer would present an increased risk to the environment by transfer of a novel gene to a different species.

9.3 Non-sexual gene transfer can potentially occur between microbial species, (via transmissible DNA molecules), and between plants and microbes (during pathogenesis and symbiosis). For the purposes of this report, we will consider only the evidence relating to the potential impact on organic systems as a result of gene transfer from recombinant bacteria or genetically modified plants.

9.4 Gene transfer between bacteria is thought to be important in maintaining the genetic plasticity and adaptability of a bacterial population (Bertolla and Simonet, 1999), and the “mosaic” nature of apparently acquired genes in microbes suggests that horizontal gene transfer has been an evolutionary driving force (Forano and Flint, 2000). There is a large body of evidence to suggest that this occurs naturally in the environment.

9.5 Horizontal gene transfer will be discussed under the following headings:

- Methods of gene transfer
- Factors affecting successful HGT
- Experimental analyses of HGT
- HGT involving transgenes

Methods of gene transfer

9.6 There are three possible methods by which foreign genes could become incorporated into a bacterial genome (Dröge *et al.*, 1998; Paul, 1999). Conjugation is the most frequent method of natural transfer between bacteria in the environment (Clerc and Simonet, 1998), but has the strictest requirements. Cell-to cell contact is needed, at least one partner must contain a transmissible plasmid, with genes enabling DNA mobilisation being present and active to permit transfer of DNA.

9.7 The second method, transduction, results in gene transfer due to host DNA becoming packaged by a bacteriophage (by a virus present in the bacterium), then passing this DNA during infection of a new host bacterium by the bacteriophage. Even when there is contact between bacterial cells, phages contained within them tend to display a narrow host range of infection, so gene transfer by transduction is likely to occur only between closely related organisms (Dröge *et al.*,

1998; Lorenz and Wackernagel, 1994).

9.8 Transformation is a DNase (enzyme able to break down DNA) mediated process by which competent bacteria can take up free DNA (Smalla *et al., 2*000). It has several criteria which must be fulfilled simultaneously, the first of which is the availability of free DNA encoding a functional gene. In addition, this DNA must encounter bacterial cells which are "competent" to be transformed. The state of competence is generally transient, and in the laboratory only a small proportion of the cells in a culture may be competent at any one time (Lorenz and Wackernegel, 1994). Although we have little knowledge of the incidence of competence in the field, over 40 species have been shown to develop natural competence (Nielsen *et al.*, 1997b: Lorenz *et al.,* 1992; Lorenz and Wackernegel, 1994; Smalla *et al., 2*000) including bacteria present in plants (Bertolla *et al.*, 1999).

9.9 Generally bacteria become competent when under stress conditions, enabling the possible uptake of novel genes which could contribute to bacterial survival. Environmental factors such as freezing and thawing, pH changes and low nutrient levels may induce competence. Additional factors (such cations to act as cofactors for the transport and uptake of DNA) may also be required for transformation (Lorenz and Wackernagel, 1994), with the requirements being specific to the bacterial species and even the strain.

Factors affecting successful HGT

9.10 Assuming that a DNA fragment encoding a foreign gene enters a host cell, there are several barriers which may impede or prevent integration into the host genome and expression of the transgene (Nielsen *et al.,* 1998). These include restriction and modification processes within the recipient cell which prevent the incorporation of foreign DNA into the host's own genetic information (Bertolla and Simonet, 1999).

9.11 In order to integrate into the host chromosome, there must be homology between the foreign and the host DNA (de Vries *et al., 2*001). This is an important issue when DNA sequences from soil bacteria are used in the development of transgenic crops; genetic homology could increase the chance of recombination (Forano and Flint, 2000).

9.12 The environmental conditions also influence the likelihood of successful horizontal gene transfer. For example, nutrient availability seems to play a role in the likelihood of transfer between bacteria. So proximity to plants may increase the potential for transfer (Dröge *et al.,* 1998). For example, the leaf surface in bean (Bjorklof *et al.,* 1995; Normander *et al.,* 1998), the sugar beet rhizosphere (Lilley *et al.,* 1994), and the residuesphere (between decaying plant material and soil) may be hot-spots for intraspecific gene transfer between bacteria, by either conjugation or transformation (Sengelov *et al., 2*001). This may be due to increased nutrients, provision of a solid surface, or simply a greater biomass (or closer contact) between donors and recipients (de Lipthay *et al., 2*001). On the phylloplane, increased conjugal transfer between bacteria is related to bacterial metabolic activity (Normander *et al.,* 1998). Perhaps surprisingly, however, the same does not always apply for the rhizosphere. Root exudates stimulate metabolic activity in rhizosphere (van Elsas *et al.,* 1988), but increased bacterial conjugation was not always related to this increased metabolic activity (Kroer *et al.,* 1998).

Experimental analysis of HGT

9.13 There are three ways of evaluating the occurrence and frequency of horizontal gene transfer (Dröge *et al.,* 1998; Nielsen *et al.,* 1998): (i) retrospective analysis by sequence homology (Lorenz and Wackernegel, 1994; Doolittle *et al.,* 1990; Smith *et al.,* 1992); (ii) short term retrospective

analysis based on screening fields for putative transformants (Smalla *et al.,* 2000); (iii) an experimental approach under optimised lab conditions to maximise the likelihood of transfer occurring and being observed (Nielsen *et al.,* 1997a; Paget and Simonet, 1994; Gebhard and Smalla, 1998).

9.14 None of these are ideal, however, in that they are not directly relevant to the field situation and there are experimental limitations to each approach. First, only a small proportion of total soil bacteria can be cultured (ca. 1%), which means that experimental analyses of this type may not give a true picture of the microbial ecology of the soil. Thus, *in vitro* studies using microcosms may not yield ecologically relevant results. Also, the fact that in culture, bacteria have long periods of exponential growth, often in the presence of unlimited, balanced nutrients, means that these studies may have little relevance to the field situation (Nielsen *et al.*, 1998). Furthermore, it is impossible to know whether the limit of detection of the experimental approach is enabling the true situation to be revealed. Finally, there is a lack of selectable markers by which donor and recipient bacterial strains can be distinguished (Clerc and Simonet, 1998).

9.15 It is difficult to extrapolate laboratory-based studies to the field situation, as the requirements for bacterial competence in vitro are often specific to the species, and even the strain. Here we discuss the available evidence surrounding the occurrence of natural HGT.

9.16 Pathogenesis and parasitism provide the potential for gene transfer, where the DNA of the two species might be transferred or exchanged. One of the best examples of this is the transfer of DNA from the plant pathogen *Agrobacterium* to its host during infection (Chilton *et al.,* 1977). There is also DNA sequence evidence suggesting that incorporation of *Agrobacterium* DNA into the plant genome took place at some point in the evolutionary history of tobacco (Furner *et al.,* 1986).

9.17 During infection of its tomato host, strains of *Ralstonia solanacearum* naturally become competent and exchange genetic material, as shown by co-inoculation of two cell populations into a plant (Bertolla *et al.,* 1999). In addition, *Plasmodiophora brassicae* takes up host DNA during each infection cycle in oilseed rape (Bryngelsson *et al.,* 1988). This DNA has been detected in resting spores and zoospores but there is no evidence of its stable incorporation into the genome (Buhariwalla and Mithen, 1995).

9.18 Another question is whether symbiotic relationships offer the potential for HGT (Lorenz and Wackernegel, 1994). Under optimized laboratory conditions, gene transfer has been detected between bacteria in the alfalfa nodule, but not between the plant host and bacterial genomes (Pretorius-Güth *et al.,* 1993).

9.19 Circumstantial evidence for trans-kingdom gene transfer, based on nuclear sequence analysis has been presented following several studies. The plant pathogen *Agrobacterium* has been shown to transfer portions of its DNA to the fusion yeast, *S. cerevisiae*, in a process that resembles bacterial conjugation (Bundock *et al.,* 1995). In addition, transfer between *E.coli* and the fission yeast, *S. pombe* (Sikorski *et al.,* 1990), and between *E.coli* and *S. cerevisiae* (Heinemann and Sprague, 1989) has been reported. In the latter case, broad host range and limited host range plasmids can transfer DNA from bacteria to yeast.

HGT involving transgenes

9.20 As with any DNA molecule, intact transgenes can potentially act as a source of transforming DNA. Under optimised laboratory conditions, there are examples of HGT between transgenic and non-transgenic organisms. For example, chromosomal DNA extracted from transgenic plants (potato, tobacco, sugar beet, oil seed rape and tomato) carrying a gene for kanamycin resistance

was able to “rescue” or repair deletions in the homologous gene in bacteria by transformation (de Vries and Wackernegel, 1998; Gebhard and Smalla, 1998).

9.21 Furthermore, purified plant DNA (or a plant homogenate) from transgenic sugar beet enabled transformation of competent bacteria under optimal lab conditions (Gebhard and Smalla, 1998), and total transgenic genomic DNA from plants containing the hygromycin resistance gene transformed *B. napus* protoplasts (Golz *et al.*, 1990). In addition, *Aspergillus niger* took up transgenic sequences from genetically modified brassica hosts expressing the hygromycin resistance gene. These transformants, however, were generally unstable (Hoffman *et al.,* 1994).

9.22 In all the cases described above, strict homology was necessary for transformation, which may account for the observation that horizontal gene transfer involving transgenic material in the field is highly unlikely (de Vries *et al., 2*001; Gebhard and Smalla, 1998). We know of no examples of gene transfer in the absence of homology between a foreign gene and the host genome. For example, Paget and Simonet *(*1994) detected no transfer from transgenic tobacco to indigenous soil bacteria in the field. Furthermore, under optimised laboratory conditions, Nielsen *et al. (*1997b) saw no transfer of DNA from transgenic sugar beet to the soil bacterium *A. calcoaceticus.* In a different study, competent *A. calcoaceticus* cells exposed to DNA from transgenic potato and sugar beet DNA containing the *nptII* gene (encoding kanamycin / neomycin resistance) were transformed at a frequency of 10^{-11} *in vitro* and at the equivalent of less than 10^{-16} in soil (Nielsen *et al.,* 2000b). Several authors have attempted to maximize the possibility of HGT in the laboratory, with little success. For example, HGT from a transgenic potato line to the bacterial plant pathogen *E. chrysanthemi* was not observed, despite optimising conditions to maximise the possibility of transfer. This led the authors to conclude that gene flow between transgenic potatoes and the pathogen occurs at an extremely low frequency (2×10^{-17}), if at all (Schlüter *et al.,* 1995).

9.23 **Conclusions**

- The potential impact of HGT depends less on the frequency of transfer and more on the gene in question and the environment in which the organism is living.

- While the possibility of horizontal gene transfer in the field cannot be ruled out, the highly selective barriers to gene transfer, coupled with extensive dilution of the marker gene in the nuclear genome of transgenic plants, means that the potential for increased risk by gene flow from transgenic plants is likely to be extremely small.

- As long as the transformed cell does not enter the germline, incidental horizontal gene transfers are unlikely to become integrated and permanent parts of a genome (Prins and Zadoks, 1994).

- There is no experimental evidence demonstrating HGT from GMOs to naturally occurring soil bacteria associated with plants under field conditions (Nielsen *et al.,* 1997b; Syvanen, 1999). However this may be because the frequency of detection is too low due to the dilution of marker genes (Bertolla and Simonet, 1999), or that investigations have generally focussed on transfer and expression of functional genes, instead of shorter gene fragments (Nielsen *et al.,* 1998). It is also possible that the use of prokaryotic sequences in transgenic plants may provide sufficient homology for gene transfer between these plants and bacteria (Gebhard and Smalla, 1998).

PART 10. IMPACT OF SCALE

10.1 The area of GM crops worldwide for the year 2001 is projected to reach 50 million hectares (125 million acres; James, 2001). As the production of GM crops increases world-wide and novel traits are introduced into a range of crop species, it is important to question how large plantings of GM crops might affect organic farming. The following issues will be discussed:

- Long term ecological impacts
- Impact of scale on the movement of pollen and other genetic material and its effect on the requirement for zero GM content in organic crops.
- Scale of migration and selection coefficients

Long term ecological impacts

10.2 Ecological or environmental impacts of new crop traits can be assessed by laboratory experiments and experimental field releases with GM crops, and these kinds of experiments have been in progress in the UK since 1987. However, this involves assessments that are often small scale and short term (Pool and Esnayra, 2001) rather than evaluating the impact of large scale, long term commercial use of GM crops in agriculture. Low probability events become more likely to occur when very large numbers of plants are cultivated and small effects could potentially accumulate over time (Regal, 1993; Pool and Esnayra, 2001). Rare hybridisation between crops and weeds could be undetectable in a one hectare experiment, but could be of potential significance in one hundred thousands hectares of a crop. Furthermore, ecological performance is highly context specific; the same genotype could give rise to phenotypes with different levels of fitness in different environments (e.g. Crawley *et al.,* 1993; Rees *et al.,* 1991). Existing studies emphasise that scale effects can vary spatially, temporarily, and according to the trait and cultivar modified (Pool and Esnayra, 2001).

10.3 Extensive experience has accumulated in some crops internationally (Kessler and Economidis, 2001), such as for herbicide tolerance and Bt insect resistance in several crops. Post-commercial environmental monitoring is an important requirement for assessing performance and environmental impact. Large farm scale trials (Squire *et al.,* 1999), or a system of scientific monitoring (Pool and Esnayra, 2001) are initiatives that are being implemented in order to examine ecological processes or possible unexpected environmental impacts of the commercial release of transgenic crops. The new EU Directive (EU, 2001) governing the release and commercialisation of GM crops in the European Union now includes a statutory requirement for environmental monitoring to deal with scale related impacts of GM crops. Monitoring will frequently need to be carried out for several years and subsequent commercial use will depend on satisfactory results of that monitoring.

10.4 The ecological consequences of conventional agricultural practices will need to be used as a baseline against which to assess the significance of the environmental impacts of GM crops. The cultivation of conventional crops and widely accepted farming practices can potentially have damaging effects by the creation of troublesome weeds, diminishing genetic diversity in crops and feral species, the selection of weeds resistant to particular herbicides and the use of pesticides that harm beneficial insects (Altieri, 2000; Pool and Esnayra, 2001). What kind of farming systems are set as a baseline against which GM crops are compared is a matter of active debate and involves important value judgements as well as scientific assessments.

Impact of scale on the movement of pollen and other genetic material and its effect on the requirement for zero GM content in organic crops

10.5 Organic farming is being actively encouraged by the UK government through the Organic Farming Scheme (and other initiatives) and currently the area under organic production is between 2 and 3 *per cent* of UK agricultural land. The area of organic production has continued to grow at a rate of about 25% per annum for the last few years (Anonymous, 2000) and the Minister for DEFRA recently stated that she wished to see this triple by 2006. Assuming that commercialisation of GM crops occurs in the UK, then it is likely that eventually much more agricultural land will be farmed using GM approaches than that using organic. This possible difference in size of the two sectors is not only based on land use in a given area, but generally organic farms are likely to have smaller fields than their conventional (and possibly GM neighbours). It is therefore important to have an understanding of the impact of a potentially large supply (source) of GM pollen, seeds and other plant material on a very much smaller area of organic crops (sink).

10.6 There is disagreement on what is the best model to be used when examining the impact of scale on the movement of pollen and other genetic material. Shaw (1995) argues that the use of a power law for predicting long distance dispersal of small biological particles (pollen, spores), rather than an exponential model, is more realistic as it allows for long distribution tails. The model of Giddings (2000) includes consideration of a Gaussian plume model of wind dispersal to ensure more accurate understanding of the influence of wind direction. The Shaw model was favoured by Timmons *et al.* (1996) working on herbicide tolerant oilseed rape. These authors proposed that a separation distance of 4km was necessary in order to minimise pollination levels.

10.7 Current thinking suggests a fixed separation distance of organic farms from transgenic crop sources. This does not consider the question of whether or not that static separation will remain equally effective from year to year or from location to location. If we consider the arguments included in the foregoing paragraphs, it seems likely that the effectiveness of any separation barrier will decrease with the scale up of production of GM crops in the UK.

Scale of migration and selection coefficients

10.8 The general discussion on potential distances of pollen transmission often omits any consideration of the scale of migration. Giddings (2000), modelling the spread of pollen from *Lolium perenne,* produced a model showing that, even at a distance of one kilometre from a source, a large-scale production of transgenic ryegrass pollen could swamp small natural con-specific (plants of same species) populations. This is because the small con-specific populations could actually receive more transgenic pollen than ‘own’ pollen. This has important implications for seed of non-transgenic crops being produced in areas where there is large-scale use of transgenic crops. Separation distance may need to be modified in areas where there is massive transgenic pollen production.

10.9 If pollination occurs from a GM crop, it is often argued that the stray transgenic pollen would often (depending of course on the nature of the genetic modification) lead to production of seed with a lower selection coefficient (less competitive) than the surrounding ‘normal’ seed. However, this again does not take into account the scale of pollen migration involved. If there is a very large source of migrant pollen then the amount involved may be sufficient to diminish or overcome any effect of a lower selection coefficient (Ellstrand and Marshall, 1985). Under such circumstances, transgenic plants might become established in another cropping area.

10.10 The likelihood of GM plants becoming established in natural or agricultural habitats will depend largely on the nature of the genetic modification. As with the introduction of new plant characters by conventional plant breeding methods, the impact of a new plant variety on agriculture and its environment will depend on the nature of that change. Herbicide tolerant crop varieties are produced

by conventional and GM plant breeding, so it is important to draw on the experiences of conventional plant breeding in assessing the possible impacts of GM crops.

10.11 Potential changes in the competitive ability of GM crops are in general likely to impact on organic agriculture and on conventional non-GM agriculture, in a similar way. However, pest and disease resistance may have some unique features for organic farmers because the transfer of resistance to a crop or weed on an organic farm, could potentially produce more competitive volunteer crop plants or weeds, which organic farmers would not be able to control by herbicides. Similar arguments about increased competitive ability could of course be made for the transfer of pest and disease resistance from conventionally bred crops, but this would not have the added complication of requiring a GM free status. Some characters may have ecological advantage, together with important effects of population size, founder effects, genetic drift (e.g. Abbott, 1994) and shifts in dominance. Such considerations have been noted by Williamson (1993a and b, 1996), Parker and Bartsch (1996) and Hancock *et al.* (1996).

10.12 Potential changes in competitive ability in different environments are considered in the UK and EU Regulatory Process while a GM plant is being consi**d**ered for commercial use (Directive 2001-18-EU). The statutory post commercialisation environmental monitoring would also provide an opportunity to assess the extent of any agronomic impact during the early phases of scale-up in agriculture.

10.13 These considerations also have a bearing on the question of persistence of transgenes in volunteers and weeds. This subject has been discussed in another part of this report, with the conclusion that the impact will be case specific and will depend on the nature of the genetic modification. Recent work by Crawley *et al*. (2001) who planted transgenic plants into natural habitats and observed them over a ten year period, concluded that the particular transgenic plants tested were no more persistent than comparable non-GM plants. Given the interplay of the many factors suggested above, such conclusions must be evaluated against a wider view of use in agriculture.

10.14 **Conclusions**

- Some impacts of GM crops will be scale dependent and this emphasises the need for extending impact assessments beyond small-scale field experiments to farm scale and eventually commercial scale impact monitoring. This is consistent with recent changes in EU regulations on GM crops, which makes post-commercialisation monitoring a statutory requirement.

- The scale up of GM crops will increase the difficulties of setting isolation barriers to achieve zero GM content in organic crops.

- Recurrent migration of GM pollen (or pollen from any crops) could have an influence on natural populations even if the transferred crop characteristics make the resultant individual hybrids less competitive than non-GM plants.

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APPENDIX - OUR PERSPECTIVES

We have endeavoured in this report to avoid the polarisation of argument prevalent in the GM/organic debate. As was said in the Introduction, there is frequently the impression that supporters of GM crops are inevitably against organic agriculture and vice versa. This is certainly not the case for many people who see important benefits in both organic and GM farming systems. The views represented are those of the authors based on their independent analysis of the peer-reviewed scientific literature and not necessarily of the organisations they are associated with. We agreed that it would be informative for readers if we outlined our perspectives to the subject along with our background experience and expertise.

Perspectives of the authors associated with the John Innes Centre

1. The organic farming sector is an important component of British agriculture that needs to be supported. Conventional agriculture has important lessons to learn from organic agriculture about balancing competing and complementing biologies in agriculture (weeds, pests, diseases, wildlife).

2. GM methods of plant breeding provide a valuable novel approach to plant breeding, and complement the range of methods used in conventional plant breeding (including marker-assisted breeding, hybrid embryo rescue, induced mutagenesis and in-vitro cell selection). Responsibly handled, GM crops have the potential to reduce the inevitable impact of agriculture on the environment, particularly through improved resistance to pests and diseases and more targeted weed control.

3. Rigorous safety assessment is required for the approval of GM crops (and other GM organisms) for use in agriculture. The assessment methods employed are considerably more rigorous than those adopted for conventionally bred crops, and are designed to produce GM crops as safe for human health, animal health and the environment, as conventionally bred crop varieties

4. GM crops need not be associated with monoculture. If GM crops are incorporated into sound crop rotations and agronomic practices which support wildlife, soil fertility, water quality and other environmental criteria, they have the potential to make a significant effect on reducing the impact of agriculture on the UK environment.

5. Agriculture needs to be competitive in world markets and transgenic crops will provide important opportunities for UK agriculture in coming decades as crops are diversified for the production of novel crops for food, biofuels, industrial processing and pharmaceutical products. It will be vitally be important to have the capacity to adapt to meet the new demands of crop production.

6. The significant challenges that lie before us for agriculture in the coming decades cannot be left to market forces alone, but need incentives for farmers to practice in ways that provide adequate financial returns and contribute to improving the environment and supporting rural communities.

7. All forms of agriculture should be subject to the same degree of scrutiny in terms of food health, environmental impact and consumer benefits. Claims made for different farming systems and their produce need careful examination if consumers are to be informed in an open, transparent and balanced way.

8. GM crops have the potential to impact on organic agriculture in two respects:

- On the agricultural environment. Although these are often assumed to be entirely negative for organic farming, GM crops have potential benefits to organic agriculture.
  - Admixture of organic crops with GM crops. The organic sector has declared that "there is no place for GM crops in organic agriculture" and this has been embodied in an EU directive, so there is a potential impact on organic agriculture in maintaining organic crops and their produce "GM free".

9. The criterion of "GM free" is arguably driven by a desire for the production and marketing of a distinctive product and what is perceived by some as consumer expectation. However, the difficulty of providing absolute segregation of any crop product in agriculture has not been fully appreciated.

10. There are two primary methods of mixing between GM and organic crop material: (a) pollination, and (b) seed mixing. Both of these are virtually impossible to control with 100% accuracy. Also, no analytical procedure will prove conclusively that a crop sample is 100% free of GM plant material. The long-distance movement of pollen and other parts of crops carried inadvertently by air movement, living things or transportation may also complicate analysis. The only practical solution to providing "non-GM crops" is to set tolerance limits that are workable in practice. These will need to allow for the limits of resolution of analytical methods, and variations in the sampling of thousands of tonnes of crop produce shipped internationally.

11. We trust that this report will make a valuable contribution to understanding, and hopefully help to navigate through the current difficulties in accommodating both organic and GM crops in agriculture.

About the authors associated with the John Innes Centre:

Professor Philip J Dale. Leader of the Genetic Modification and Biosafety Research Group at the John Innes Centre. Plant Scientist with 29 years experience in plant breeding and research, working at the Welsh Plant Breeding Station (now IGER), the Plant Breeding Institute Cambridge and the John Innes Centre, Norwich. Member of the Advisory Committee on Novel Foods and Processes, the Agricultural and Environment Biotechnology Commission, and a former member of the Advisory Committee on Releases to the Environment.

Dr Belinda Clarke. An independent scientific consultant to the project. Currently Science Liaison Manager at the Norwich Research Park. Formerly a research scientist the John Innes Centre, with over 10 years' experience in a range of research areas, including plant-microbe interactions, gene expression in soil bacteria and starch biosynthesis in plants.

Dr Eliana Fontes. A Research Group Leader with 16 years' post-doctoral experience in the biological control of insects and weeds, concentrating on insect/plant interactions and population dynamics at EMBRAPA, Brasilia (Brazilian Enterprise for Agricultural Research). Formerly Secretary and member of the Brazilian National Biosafety Commission. Currently a visiting scientist at the John Innes Centre.

Perspectives of the authors from the Initiative on Organic Research at Elm Farm Research Centre.

1. EFRC was established as an educational charity in August 1980. It is a national charity based in Berkshire, near Newbury, and has a strong relationship with the local community. This food and farming organisation takes an authoritative and independent approach to the research and development of organic agriculture and production. It undertakes applied and policy research, development, extension, advisory, demonstration and educational work in the fields of sustainable organic agriculture and appropriate technology.

2. Our approach to GMO and the technology is based on the organic principles and standards. EU regulations have stated that there is no place in organic agriculture for GMOs or their products and much of the organic philosophy does not fit easily with GMOs. We believe there is limited information on the effects and impacts of GMOs on the environment and food safety and hence their impact on organic farms and food production. We believe these issues need to be resolved before widespread introduction or commercialisation of GM crops in the UK.

3. This project and report form part of our ongoing commitment to investigating the issues and informing the debate on GMOs in the UK

About the authors from the Initiative on Organic Research at Elm Farm Research Centre:

Prof. Martin Wolfe, Research Director: Plant scientist with 28 years experience at the Plant Breeding Institute, Cambridge and nine years in the Chair of Phytopathology at the Swiss Federal Institute of Technology, Zurich, Switzerland. Research interests primarily include the development of cereal mixtures for the management of disease and yield stability and inter-cropping systems. Prof. Wolfe was President of the British Society for Plant Pathology in 1983 and is now an Honorary Member of that Society. In 1998, he was awarded the Silver Medal of the British Crop Protection

Dr. Bruce Pearce, Deputy Research Director and Head of Operations: Experience of research in a range of plant sciences and over 10 years experience in research and contract management for both government and the private/charitable sectors

Dr. James Welsh, Senior Researcher: Plant scientist with 9 years research experience (3 years at post-doc level). Principal research interests include weed science and cereal agronomy. Dr. Welsh is an active member of the Association of Applied Biologists and serves on the Weeds and Agronomy Group committee. Dr Welsh is also a member of the UK Weed Liaison Group.

GLOSSARY

AEBC	Agriculture and Environment Biotechnology Commission
Bacteriophage	A virus that infects and replicates within a bacterium
Cation	An ion with a positive electrical charge e.g. Na^+
Cellulase	An enzyme found in certain fungi and lower animals, such as insects and bacteria, that catalyzes the breakdown of cellulose into glucose
Chloroplast	A cellular organelle in plants that contains chlorophyll and is a site of photosynthesis
Collembola	The springtails, an order of tiny, primitive, wingless apterygote insects with biting mouthparts, short antennae, and abdomens fused in six segments; living in damp places, they jump by releasing an abdominal spring held in place by a hook
Competence	The state of bacterial cells in which they are able to take up free DNA fragments from the surrounding media
Compost	A mixture of decaying organic matter, such as rotting leaves or manure, used as a fertilizer or soil conditioner
Conjugation	In bacteria, a step in the sexual cycle involving the transfer of genetic material from a donor cell to a recipient cell through direct cell-to-cell contact.
Conspecific	Belonging to the same species
Conventional plant breeding	Crop improvement by various methods, including: sexual hybridisation (pollination), induced mutation (chemical or radiation), induced polyploidy, molecular marker assisted methods and in vitro cell selection
DEFRA	Department for Environment, Food and Rural Affairs
EC	European Community
EEC	European Economic Community
Ensilation	The preservation of green fodder in a silo or pit
EU	European Union
Germ line	A lineage of cells that contain the complete genome of the individual and produce the reproductive cells that transfer the genome to the next generation.

Glyphosate	Organic molecule used as herbicide; $C_3H_8NO_5P$; a white solid; soluble in water
GM	Genetic modification
GMO	Genetically modified organism
Heterologous	Derived from a different species
Homology	Where two gene sequences are identical
Horizontal Gene Transfer (HGT)	The non-sexual transfer of genes between organisms
Hybridisation	The formation of a sexual hybrid between plants (in the context of this report) by pollination
Inoculum	The small quantity of material containing microorganisms that is introduced into a medium for culture
Introgression	The incorporation of a gene into an organism from another organism as a result of sexual hybridization
Lectin	A generic term for proteins extracted from plants that exhibit antibody activity in animals
Lysis	The processes of breaking open a cell by disruption of the plasma membrane
Micro-arthropods	Micro-invertebrates of the phylum Arthropoda, the largest animal phylum, including insects, arachnids, and crustaceans, typified by segmented bodies and paired, jointed antennae, wings, or legs
Microcosm	A very small and localised ecosystem used as closed systems in laboratory studies
Mononucleotide	The hydrolysis product of a nucleic acid
Mulch	A protective covering that is spread on the ground around plants to inhibit evaporation and control weeds, usually organic matter such as leaves, peat, or wood chips
Non-target species	A species that is not the main species targeted by a particular action such as a pest control measure
Organic farming	A production system which avoids or largely excludes the use of synthetically compounded fertilisers, pesticides, growth regulators and livestock feed additives. To the maximum extent feasible, organic farming systems rely on crop rotations, crop residues, animal manures, legumes, green manures, off-farm organic

	wastes, and aspects of biological pest control to maintain soil productivity and tilth, to supply plant nutrients and to control insects, weeds and other pests
Phylloplane	The surface of a leaf, used especially in reference to a leaf as a habitat for micro-organisms.
Plasmid	A small, circle of double-stranded DNA forming an extrachromosomal self-replicating genetic element in many bacteria and some eukaryotes; widely used in genetic engineering as a cloning vector
Plastid	An organelle that occurs in plants a variety of forms, including chloroplasts, chromoplasts and leucoplasts, which are capable of being interchangeable
Polymerase Chain Reaction (PCR)	Process for the rapid amplifying a specific DNA molecule
Progeny	The offspring of a plant or animal; descendants
Recombinant DNA	DNA molecules in which sequences, not normally connected, have been placed next to each other by molecular methods
Residuesphere	The region between decaying plant material and soil
Rhizosphere	The area of soil immediately surrounding plant roots
Rubisco	The enzyme, ribulose-1, 3-diphosphate carboxylase involved in photosynthesis. The most common protein on earth
Slurry	Animal manure in liquid or semi-liquid form, without straw, sawdust and other animal bedding material
Symbiont	An organism that forms a close association with another organism; see symbiosis
Symbiosis	A relationship in which two dissimilar organisms live in close association with each other. e.g. Rhizobium in association with a leguminous plants
Synthase	One of the six primary classes of enzymes
Transconjugant	A cell that is the product of conjugation
Transduction	The transfer of a bacterial gene from one bacterium to another by a bacteriophage (a virus that lives in a bacterium)
Transformation	A genetic change involving the incorporation into a cell of free DNA

Transgene	Gene introduced into an organism by modern genetic modification methods
Transgenic DNA	DNA introduced into an organism by the process of transformation
Transgenic organism	An organism that is genetically modified by the process of transformation
Volunteers	A plant originating from germinated seeds from previous crop harvests.
Wild Relative	A wild plant relative of a crop. Frequently from a different species