GM SCIENCE REVIEW

FIRST REPORT

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

PREPARED BY THE GM SCIENCE REVIEW PANEL (JULY 2003)

Chapter 5

GM DERIVED FOOD AND ANIMAL FEED SAFETY

5.1 INTRODUCTION

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

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

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

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

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

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

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

5.5 The effect of GM derived feed in the food chain

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

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

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

5.2 POSSIBLE NUTRITIONAL AND TOXICOLOGICAL DIFFERENCES IN GM FOOD

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

5.2.1 Summary

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

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

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

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

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

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

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

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

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

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

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

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

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

5.2.2 Background

EU regulatory classification for GM food safety assessment

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

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

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

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

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

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

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

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

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

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

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

The ‘parent’ traditional crop or substance

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

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

The gene donor, new gene or transformation process

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

The primary gene product(s) or metabolites

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

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

The new (transformed) crop

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

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

5.2.3 Range of views and quality of evidence

The issues and concerns

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

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

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

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

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

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

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

Two overarching issues are considered in this Section:

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

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

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

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

The scientific evidence in relation to questions raised

Is the new gene itself toxic?

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

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

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

Could the new gene be transferred to a human?

This is considered in Section 5.4.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

5.2.4 Is there general scientific agreement?

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

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

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

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

- toxicological/carcinogenic/allergenic;

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

- nutritional; or
- gene transfer.

5.2.5 Is the issue unique to GM?

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

5.2.7 Likely future developments

Detection of unintended effects

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

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

Safer, nutritionally enhanced, foods?

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

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

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

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

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

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

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

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

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

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

Removing detrimental (antinutrient) substances

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

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

Enhancing health-promoting substances

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

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

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

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

Vitamins and micronutrients

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

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

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

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

Research

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

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

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

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

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

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

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

- Bioinformatics for improved safety assessment.

Technological approaches

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

Regulatory approach

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

5.3 FOOD ALLERGIES FROM GM CROPS

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

5.3.1 Summary

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

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

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

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

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

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

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

5.3.2 Background

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

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

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

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

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

5.3.3 Range of views and quality of evidence

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

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

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

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

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

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

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

5.3.4 Is there general scientific agreement?

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

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

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

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

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

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

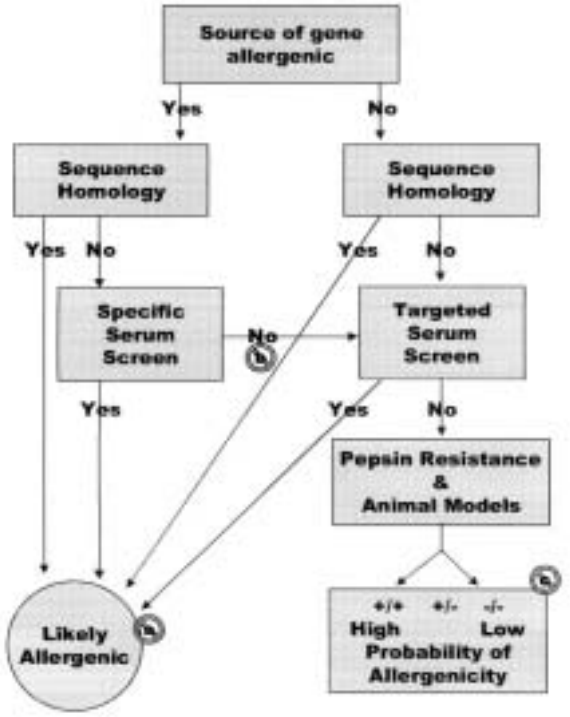
In 2001 the FAO/WHO (2001) developed and proposed a new decision tree approach for the assessment of the allergic potential of proteins (Figure 5.1). This refined previous decision trees (Metcalfe, 1996; FAO/WHO 2000). Whilst important information can be gained using this decision tree, there are divergent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

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

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

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

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



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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

5.3.5 Is the issue unique to GM?

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

5.3.7 Likely future developments

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

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

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

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

Research

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

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

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

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

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

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

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

Regulatory approach

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

5.4 THE FATE OF TRANSGENIC DNA

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

5.4.1 Summary

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

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

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

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

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

5.4.2 Background

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

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

5.4.3 Range of views and quality of evidence

What is the effect of food processing on transgenic DNA?

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Antibiotic resistance marker genes

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

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

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

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

5.4.4 Is there general scientific agreement?

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

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

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

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

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

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

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

5.4.5 Is the issue unique to GM?

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

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

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

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

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

5.4.7 Likely future developments

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

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

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

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

5.4.9 Concluding remarks

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

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

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

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

5.5 THE EFFECT OF GM DERIVED FEED IN THE FOOD CHAIN

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

5.5.1 Summary

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

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

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

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

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

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

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

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

5.5.2 Background

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

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

5.5.3 Range of views and quality of evidence

The Issues

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

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

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

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

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

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

The effects of feed processing

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

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

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

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

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

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

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

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

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

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

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

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

Effects on animal and human health

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

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

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

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

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

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

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

5.5.4 Is there general scientific agreement?

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

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

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

5.5.5 Is the issue unique to GM?

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

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

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

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

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

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

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

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

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

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

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

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

5.5.7 Likely future developments

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

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

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

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

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

Research

The following research issues have been identified:

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

Regulatory approach

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

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