February 2006

Friends of the Earth

Briefing

Could GM foods cause allergies?

A critique of current allergenicity testing in the light of new research on transgenic peas

Friends of the Earth, 26-28 Underwood Street, London N1 7JQ
Tel: 020 7490 1555 Fax: 020 7490 0881 Email: info@foe.co.uk Website: www.foe.co.uk

Friends of the Earth Limited company number 1012357
♻ Printed on paper made from 100 per cent post-consumer waste

Introduction

The safety of genetically modified foods (known as GM or GMOs) has been a controversial issue over the past decade. Despite major concerns by the European public very little independent research has been carried out to establish their long-term safety. The approvals system for new GM foods in Europe is centred on the arguments and research put forward by the very biotechnology companies who are trying to get approval for their products.

One of the main safety issues is the question of allergies. Will a new protein that a genetically modified food produces cause allergic reactions in people or animals? Will modified plants contain any other novel proteins as a result of dormant genes being switched on by genetic engineering events? Biotechnology companies argue that many of the individual proteins used in GM crops have been consumed over a long period in their natural host with no health effects seen, so simply creating the proteins in a new plant will surely be the same.

But this assumes both that the new protein in the GM plant is identical to the naturally produced protein, and that no unintended effects have occurred during the genetic modification process that could produce other proteins. This assumption can be seen in action in the USA, where after ten years of commercialisation of GM crops there is still no post-market surveillance for allergic reactions[1].

New research now challenges this assumption, and brings into question the safety of both new and previously approved GM foods.

GM pea research

Scientists in Australia at the CSIRO[2] halted commercialisation of a genetically modified pea after their research revealed some surprising and alarming results[3]. Scientists had developed the GM peas over a ten year period, with various studies carried out on nutritional quality and digestibility, field performance, likelihood of cross-pollination and effects on wildlife. But it was not until they investigated whether the GM peas caused immune responses that the problems with the GM pea emerged.

The GM pea contains a protein, found naturally in beans, which protects them from pea weevils, a common pest. The protein, called alpha-amylase inhibitor, inhibits an enzyme that the weevils need to digest starch, making them starve to death.

When researchers fed the GM peas to mice, the mice showed an immune response, producing antibodies to the GM protein. When they were exposed to the GM protein again, by injection or via the airway, they had allergic-type reactions. When the protein was injected, swelling occurred. When their airways were exposed to the protein, they suffered an asthmatic-type reaction with narrowing and inflammation of the airway, and inflammation of the lungs with excessive mucus secretion.

The research also found that when the mice were fed a common food allergen (egg white protein) at the same time as the GM peas, they developed an immune response to the egg white protein too, indicating that the new protein was priming the mice to react to other foods.

The protein had not been found to cause any allergic reactions when expressed naturally in beans. But after transferring the gene that produces the protein to peas using genetic modification, subtle changes were seen in the protein produced. These changes are thought to be due to post-translational modification - the way the plant produces the protein, with differences occurring in the way sugars are added to the protein. The issue of such changes and their potential for toxic or allergenic effects was highlighted by Schubert[4] in 2002, but regulators appear to have paid little attention to the issue to

date.

The results of this research are alarming because they are the opposite to the assumptions used in current testing of GM foods. Scientists developing GM foods and those serving on regulatory bodies as well as the GM industry itself assume that a protein produced in a GM plant behaves identically to the same protein that is produced naturally in another plant. Using this argument the GM industry has avoided the need to carry out thorough testing of GM foods. If this assumption is now wrong then it may be that GM foods currently on sale in shops could cause allergenic effects.

Current requirements for allergenicity testing of GMOs

Before any GMO or derived product can be marketed in the EU, it must pass through an approval system which is intended to assess its safety for humans, animals and the environment. The GMO Panel of the European Food Safety Authority (EFSA), which provides scientific advice and technical support for GM food safety issues, published guidance[5] for applicants seeking authorisation of GM food and/or feed.

A section of the guidance covers current requirements for assessment of allergenicity. The guidelines are based on the recommendations of the Codex Alimentarius Commission's *ad hoc* Intergovernmental Task Force on Foods Derived from Biotechnology[6] (Codex is an organisation that develops international standards for food standards).

EFSA/Codex guidelines

EFSA's guidance for assessment of allergenicity states that "a cumulative body of evidence which minimises any uncertainty with regard to the protein(s) in question" is required. The steps required are:

1. A consideration of the source of the transgene as to whether or not it encodes an allergen
2. A search for sequence homologies and/or structural similarities between the expressed protein and known allergens.
3. In vitro tests that measure the capacity of specific IgE from serum of allergenic patients to bind the test protein, in order to assess potential that exposure to the protein might elicit an allergic reaction in individuals already sensitised to cross-reactive proteins:
    a. If the source of the introduced gene is considered allergenic, but no sequence homology of the protein to a known allergen is demonstrated, specific serum screening should be undertaken with sera from patients allergic to the source material.
    b. If the source is not known to be allergenic but there are consistent indications of sequence homology to a known allergen, specific serum screening should be conducted with sera from patients sensitised to this allergen.
4. Positive IgE responses in step 3 mean the newly expressed protein is considered allergenic. If no IgE binding is observed, the newly expressed protein should undergo additional testing:
5. Pepsin resistance test – stability to digestion by proteolytic enzymes is considered a characteristic of allergenic proteins – if a rapid and extensive degradation of a protein in the presence of pepsin is not confirmed under appropriate conditions, further analysis should be conducted to determine the likelihood of the newly expressed protein being allergenic.

6. Targeted serum screening to assess the capacity of the protein to bind to IgE in sera of individuals with clinically validated allergenic responses to categories of foods broadly related to the gene source. Models can substitute for and/or complement this process, including appropriate *in vivo* animal models.
7. Animal models are useful tools for the assessment of the sensitising potential of the newly expressed proteins, ie their capacity to induce an allergic immune response with the synthesis of specific IgE in individuals that have never been exposed to those proteins nor to proteins that cross react with them.

Assessment of allergenicity of the whole plant is only required where the host and/or source of the introduced gene is known to be allergenic.

The guidelines do not specify a requirement for testing beyond step 2 if the source of the protein is not considered allergenic and there are no consistent indications of sequence homology to known allergens.

FAO/WHO guidelines

The Codex guidelines were themselves based on a FAO/WHO Expert Consultation[7] that aimed to establish a reliable methodology to assess the allergenicity of GM foods. But there are some important differences between the guidelines the Expert Consultation drew up, and the Codex guidelines that emerged. Several key principles did not make it into the Codex guidelines.

The FAO/WHO guidelines involve the use of a decision tree which is split into two sides – one for food containing a gene from a source known to be allergenic, and one for food containing a gene from a source not known to be allergenic. In the latter case the recommended steps are:

1. Sequence homology to known allergens (food and environmental)
2. Targeted serum screening for cross-reactivity with sera from patients allergenic to materials that are broadly related to the source material for the gene
3. Pepsin resistance
4. Immunogenicity testing in animal models.

The key point that does not appear in the Codex/EFSA guidance is that **all** these steps should be followed, even for food containing a gene from a source not known to be allergenic. Progress through the steps should only cease if the expressed protein is found to be an allergenic risk. In the EFSA/Codex guidance, further testing is only carried out where the source is thought to be allergenic, and merely has to be considered for other sources. As the Codex guidelines state, "for proteins from sources not known to be allergenic, and which do not exhibit sequence homology to a known allergen, targeted serum screening may be considered where such tests are available".

It is interesting to note that the EU's Joint Working Group on Novel Foods and GMOs consisting of members of the Scientific Committees on Food, Plants and Animal Nutrition (essentially the predecessors to EFSA) produced guidelines[8] based on the FAO/WHO recommendations, which were superseded by EFSA's guidelines. They stated that where the source was not known to be commonly allergenic and no sequence homology to known allergens was demonstrated, or where specific serum screening results were equivocal, additional tests **should** be performed, including pepsin resistance or

targeted serum screening. But EFSA have ignored these recommendations, and opted for weaker guidelines.

In addition, the Codex/EFSA guidelines do not require immunogenicity testing in animal models. The use of animal models is only thought by EFSA to be "useful", but does not appear to be a specific requirement. Yet it is a clear part of the FAO/WHO decision tree, and as Spök et al[9] point out, allergenicity ("the likelihood of inducing *de novo* sensitisation in a nonallergenic individual", as opposed to the propensity of a substance to induce allergic reactions in sensitised allergic patients) cannot be determined by the first three steps of the Codex/EFSA guidelines, but requires models of allergic sensitisation, which are mostly conducted in animals.

By following EFSA's weak guidelines, effects such as the immune reaction seen in mice to the GM peas could never be identified. These weaker standards mean potentially allergenic foods are being allowed onto the market.

Other problems with current allergenicity testing

Several tests currently used to demonstrate a lack of allergenic risk have also been criticised:

Digestibility studies/Pepsin resistance

Rapid degradation of the protein in simulated gastric conditions is also used in applications to demonstrate lack of allergenic risk. But a recent study by Spök et al[10] casts doubt on the suitability of such studies to address the allergenic potential of a protein, and Freese & Schubert[11] found that industry procedures used to measure digestive stability often use highly acidic conditions with a very large excess of pepsin, favouring rapid digestion but failing to simulate the gastric fluid content. Chowdhury et al[12] found that rapid degradation of Cry1Ab does not occur, as suggested by industry, and can in fact pass through the digestive tract and be detected in the faeces of farm animals. Yet in their recent Opinion for Bt11 maize, which expresses the Cry1Ab protein, EFSA state that part of the evidence for low allergenicity risk is the fact that there is "rapid and extensive degradation by pepsin"[13].

Surrogate proteins

'Surrogate' proteins produced by bacteria, rather than the protein produced by the GM plant, are usually used in the testing processes because biotechnology companies find it difficult to extract sufficient quantities of the proteins from the GM plant. Freese & Schubert[14] raised serious concerns about this practice, as surrogate proteins may not reflect the toxicity or allergenicity of the plant-produced protein to which people are actually exposed. It is a particular concern in the light of the findings for the GM pea, because the researchers suggested that differences in glycosylation (the way sugars are added to proteins) were likely to have led to the altered antigenicity of the alpha-amylase inhibitor protein when transferred from beans to peas. Yet the process of glycosylation in bacteria is not well understood[15] – until recently they were not thought to glycosylate proteins at all. Had the researchers used a bacterial surrogate protein for their immunogenicity testing, it is possible that they would not have seen the same immunogenic reaction in mice.

Low level of protein

This is used in several applications for GM foods that have been approved for use in the EU, on the basis that most allergenic proteins are present in foods in large quantities. But this concept was not

deemed suitable for assessment of allergenicity by the FAO/WHO consultation, as allergens can sensitise susceptible individuals at possibly less than microgram levels. It is not therefore possible to define a level below which a protein can be considered safe.

Assumptions of historic safe use

Most applications for GMOs expressing Bt toxins, such as MON863, 1507, MON810 and Bt176 in part base their assessment of lack of significant allergenic risk on the history of safe use of products containing Bt toxins. Yet a 1999 study by Bernstein et al[16] found exposure to Bt pesticide spray led to immune reactions in some farm workers, including skin sensitivity to the protein. Lack of reported allergy to microbial pesticides containing similar proteins is not acceptable evidence for lack of significant allergenic risk, particularly when evidence exists to the contrary.

Gaps in current allergenicity testing

There are also several areas that are not covered by current allergenicity testing guidelines.

Lack of consideration on a case by case basis

The Opinions of EFSA's GMO Panel are used by the European Commission as evidence of the safety of the GMO when placing it on the market. But many of the evaluations of allergenicity risk in the Opinions are mostly based on previous evaluations, sometimes for different GM crops or even different proteins. Yet according to EU law, every application should be considered on a case by case basis.

- The CP4 EPSPS protein confers tolerance to glyphosate. EFSA's evaluation of allergenicity risk for this protein in GT73 oilseed rape[17] was mainly based on evaluation of the same protein, produced by a different plant, in their Opinion for NK603 maize[18]. And the evaluation for NK603 was itself mainly based on allergy risk evaluations for "previous applications evaluated by the EC Scientific Committees and the national competent authorities" – none of which were referenced.

- Similarly, for the PAT protein, which confers resistance to glufosinate ammonium, EFSA's evaluation of allergenicity of this protein in Bt11 maize[19] was mainly based on previous evaluations in their Opinion for 1507 maize[20]. The evaluation for 1507 maize simply stated "the PAT protein has been previously evaluated for its safety in the frame of other applications for the placing on the market of PAT-expressing GM crops" – again these other applications were not referenced.

- For the Cry1Ab protein, which confers resistance to lepidopteran pests, EFSA's evaluation for this protein in Bt11 maize[21] deems the strategy for allergy risk evaluation sufficient because the same strategy was used in previous applications for Bt11 and MON810. Yet in the cited Scientific Committee on Plants Opinion for MON810[22], and two of the cited Bt11 references[23] [24], it is stated that "the often applied in vitro methodology used to study the survival of Btk toxin can be improved. In particular, the use of the isolated protein in toxicity studies does not adequately model degradation of the same protein when fed as an integral component of the diet". The other cited Bt11 Opinion [25] makes no specific mention of allergenicity testing.

- Similarly, for the Cry3Bb1 protein, which confers resistance to coleopteran pests, EFSA's evaluation for this protein in MON863[26] states that the "indirect evidence for an allergenicity risk being very low" is deemed acceptable based on previous applications for Cry1Ab – a different toxin – citing the MON810[27] and Bt11[28] Opinions which again criticise the methodology used.

One of the key principles in risk assessment of GMOs is assessment on a case by case basis. But from the evidence above, it can be seen that many of the currently approved GM crops have not been considered on a case by case basis for allergenicity testing. Instead, many refer to prior assessments, which in turn refer to either unstated references or assessments that have raised concerns or not specifically considered allergenicity at all. This is a clear violation of the case by case principle.

Lack of consideration of the whole plant

Almost all of the approved GM foods in the EU have their approval based on lack of significant allergenic risk from the newly produced protein only – the whole plant is not considered. EFSA's guidelines for allergenicity testing[29] only consider this to be necessary where the host of the introduced gene is known to be allergenic.

But this ignores the potential for unintended effects arising from the general mutagenic nature of the GM transformation process[30]. EFSA's Opinion for NK603[31] acknowledges the issue only in terms of the possibility of overexpression of native proteins, and so dismisses the issue because maize is not considered a major allergenic food. But unintended effects can also include creation of novel fusion proteins with unknown properties[32].

For GT73, concerns were raised by a Member State about possible unintended effects which could alter the allergenicity of oilseed rape in relation to inhaled dust/flour from oilseed rape seeds. EFSA's response was simply that "there is no information whether the genetic modification might alter the allergenicity of the GM oilseed rape", and that "assessing such possible change would be extremely difficult due to the low number of patients"[33].

Lack of consideration of animal allergenicity

EFSA's guidelines for allergenicity testing[34] simply state "regarding animal health, allergenicity is not a significant issue that needs to be specifically addressed". No further rationale is given for this statement. Similarly, in the EFSA Opinion on GT73 oilseed rape, it is stated that "in the case of feed use only, the GMO Panel considers that additional experimental data on possible allergenicity is not required".

Presumably this is because livestock is generally slaughtered before the harmful effects of allergenicity have a major impact on animal health. But clearly there is an animal welfare aspect to consider, as well as production losses for farmers.

Summary of testing for currently approved GM foods/feed

The following table illustrates the testing that has been carried out on GM plants that have approval for use in food or animal feed in a form that contains protein in the European Union. For the sake of brevity, the following approvals which are on the EC existing products register[35] for feed materials and/or highly processed foods only (and are therefore unlikely to contain protein) have been excluded:

Maize: NK603xMON810, GA21xMON810, MON863xNK603
Cotton: MON1445, MON531, MON1445xMON531, MON15985, MON15985xMON1445
Oilseed rape: MS8xRF3, MS1xRF1, MS1xRF2, Topas 19/2, T45.

Event and crop type	Biotech company	Introduced trait	Approval for food/feed?	Proteins expressed	Tests carried out/ justification for lack of testing	Surrogate protein used? (Source)	Whole plant considered?
GA21 maize	Monsanto	Glyphosate tolerant	Food approval January 2006, prior approval for feed materials.	mEPSPS	* Analysis of sequence homology * Pepsin resistance * Not derived from allergenic source * Low level of protein expressed	No	No
MON863 x MON810 maize	Monsanto	Insect resistant	Import/processing approval January 2006, prior feed approval.	Cry3Bb1, Cry1Ab and NPTII	* Analysis of sequence homology * Pepsin resistance * Not derived from allergenic source	Yes (*E. coli*)	No
MON863 maize	Monsanto	Insect resistant	Feed approval August 2005, food approval January 2006	Cry3Bb1 and NPTII	* Analysis of sequence homology * Pepsin resistance * Low level of protein expressed * Lack of reported allergy to microbial pesticides containing similar proteins	Yes (*E. coli*)	No
1507 maize	Pioneer/ Mycogen	Glufosinate tolerant/insect resistant	Feed only, approved November 2005	Cry1F and PAT	* Analysis of sequence homology * Pepsin resistance * History of safe use of Bt toxin products	Yes (*P. fluorescens*)	No
GT73 oilseed rape	Monsanto	Glyphosate tolerant	Feed only, approved August 2005	CP4 EPSPS and GOX	* Analysis of sequence homology * Pepsin resistance * Feed use only so no further requirements	Yes (*E. coli*)	No
NK603 maize	Monsanto	Glyphosate tolerant	Food and feed, approved October 2004	CP4 EPSPS	* Analysis of sequence homology * Pepsin resistance * Not derived from allergenic source * No characteristics of known allergens * Similar proteins already consumed	Yes (*E. coli*)	No
Bt11 maize	Syngenta	Glufosinate tolerant/insect resistant	Food and feed approved 1998, sweetcorn approved May 2004	Cry1Ab and PAT	* Analysis of sequence homology * Pepsin resistance * Not derived from allergenic source	Yes (*E. coli*)	No
MON810 maize	Monsanto	Insect resistant	Food and feed, approved 1998	Cry1Ab	* Analysis of sequence homology * Pepsin resistance * Low level of protein expressed * History of safe use of Bt toxin products	Yes (*E. coli*)	No

T25 maize	Bayer (previously AgrEvo)	Glufosinate tolerant	Food and feed, approved 1998	PAT	* Analysis of sequence homology * Pepsin resistance * Unlikely to be glycosylated in plants	Protein derived from canola and different maize event	No
Bt176 maize	Syngenta (previously Ciba-Geigy)	Glufosinate tolerant/insect resistant	Food and feed, approved 1997	Cry1Ab and PAT	* Analysis of sequence homology * Pepsin resistance * History of safe use of Bt toxin products * No allergies in workers developing plant	No	No
MON 40-3-2 soybean	Monsanto	Glyphosate tolerant	Food and feed, approved April 1996	CP4 EPSPS	* Immuno-blot assay to demonstrate equivalence to endogenous allergenic proteins * Analysis of sequence homology * Pepsin resistance * Low level of protein expressed * Protein not stable to processing * Protein not glycosylated	Unclear from dossier – some tests use seed-derived protein	For demonstration of equivalence to endogenous allergenic proteins only

Source: Application dossiers and EFSA/SCP/SCF Opinions[36]

Other comments relevant to testing:

GA21 – Member states raised concerns about the use of gastric and post-gastric digestibility studies to discount allergenicity and question whether analysis of sequence homology is sufficient to provide reassurance as to the safety of GA21[37].
MON863xMON810 – Member states suggested extended testing for allergenicity including immunogenicity of transgenic proteins or the unintended alteration of intrinsic allergenicity of maize. These concerns were not thought relevant by EFSA[38].
GT73 - EFSA note that as cross reactivity between the GOX protein and tropomyosin (a muscle protein associated with seafood allergy) is not ruled out completely, "persons allergic to shrimp meal should be aware of the possibility of hypersensitivity reaction when working with GT73 oilseed rape"[39].
NK603 - Several member states raised concerns about the approach to allergenicity testing, but these were dismissed by EFSA[40].
MON 810 - The Scientific Committee on Plant's Opinion agreed that there is no significant risk, but they raised concerns about the in vitro methodology used to study the survival of Bt toxin and the use of isolated protein in toxicity studies[41].
Bt176 - The Scientific Committee on Food's Opinion agreed that it was unlikely that the potential for allergenicity had changed, but did not "exclude the possibility that there will be individuals allergic to this variant of maize, just as there are individuals who are allergic to traditionally produced variants of maize"[42].
T25 - The Scientific Committee on Plant's Opinion agreed that there is not significant risk, but stated that the applied in vitro methodology to study the survival of the PAT can be improved[43].

In almost all of the above cases:

- Conclusions of no significant allergenic risk were based solely on comparison to known/putative allergens, speed of breakdown in simulated gastric fluids, and in some cases on historical safety of the source and/or host of the transferred gene.
- The tested protein was produced by bacteria, not the GM plant for which approval was sought
- Only the proteins the plant was genetically modified to produce were considered, not the whole plant

No human serum screens or immunogenicity tests in animals were carried out for any of the GMOs. Only for MON863xMON810 was there any consideration of immunological cross priming, as was discovered in the transgenic pea research. This concern was dismissed by EFSA because although the Cry1Ab protein has been shown to act as an adjuvant, enhancing responses to co-administered proteins, maize is not a common allergenic food[44], apparently discounting the possibility that the GM maize could be eaten in combination with other, more common, food allergens.

Conclusion

Even small crosses within a plant family can change the properties of a protein from neutral to immunogenic, as demonstrated by the transgenic pea. But none of the testing protocols used for the GMOs discussed above would have detected such immunogenic changes. In essence, the allergenic and immunogenic potential of currently approved crops is not known.

Allergic reactions affect only a small proportion of the population, but the consequences can be severe, even deadly. Once allergies have developed, there may be no safe level of exposure for the affected person. As the UK's GM Science Review Panel noted following their review of science relative to GM crops and food[45], "our relative lack of knowledge about factors that are important in sensitisation and the elicitation of an allergic response suggest that we should continue to exercise caution when assessing all new foods, including foods and animal feeds derived from GM crops."

It should be noted that there is no validated and widely accepted animal model for allergenicity testing available[46]. Although present animal models provide additional information on potential allergenicity of novel proteins, they do not reflect all aspects of food allergies in humans[47]. But there is a clear need for further research in the whole area of allergenicity, not just in the use of animal models. Indeed, a recent call for research proposals from the Food Standards Agency in the UK specified the need for state of the art scientific techniques in bioinformatics and proteomics for identification of potential allergens in novel foods[48]. Until there are validated and accepted methods for detection of potential allergenicity, there should be no further approvals of GM crops and foods, and existing approvals should be suspended.

Rather than simply increasing the use of animal testing, which will not necessarily reflect human allergic reactions, there is a need to question the actual need for a GMO before the testing phase is reached. The need for the product must justify both the expense and the ethical issues involved in its testing. Where the need can be justified, the full range of available and applicable tests, such as targeted human serum screens, should be carried out prior to the use of animal models.

Recommendations

- EFSA's guidance for allergenicity testing must be tightened up to at least the level of the preceding Joint Working Group guidelines and include the full recommendations of the

FAO/WHO guidelines

- There should be much greater scrutiny of the methods used in applications to justify the conclusion of lack of significant allergenic risk, for example pepsin resistance tests must use realistic simulations, and should not be used as a rationale for no further testing
- The use of surrogate proteins is not acceptable – protein produced by the GM plant that will actually be eaten must be used in allergenicity assessments.
- Assumptions of safe historic use/low level of protein expression are not acceptable in allergenicity assessments.
- Proteins must be considered on a case by case basis, not using prior evaluations for different crops
- The entire GM plant must be considered in allergenicity assessments, not just the protein it is genetically modified to produce
- Lack of consideration of animal allergenicity must be properly justified.
- Currently approved GMOs must be reconsidered in the light of the findings of the transgenic pea research
- The need for a GMO must justify the issues involved in its testing, including ethical issues around the use of animal models as well as whether this is the best use of resources
- Where the need can be justified, the full range of available and applicable tests must be used, including targeted human serum screens, prior to animal testing
- Until there are validated and accepted methods for full detection of potential allergenicity, there should be no further approvals of GM crops and foods, and existing approvals should be suspended

References

[1] Bernstein JA, Bernstein IL, Bucchini L, Goldman LR, Hamilton RG, Lehrer S, Rubin C & Sampson HA (2003). *Clinical and laboratory investigation of allergy to genetically modified foods.* Environmental Health Perspectives 111(8):1114-21

[2] Australia's national research organisation, the Commonwealth Science and Industrial Research Organisation

[3] Prescott VE, Campbell PM, Moore A, Mattes J, Rothenberg ME, Foster PS, Higgins TJV and Hogan SP (2005). *Transgenic expression of bean alpha-amylase inhibitor in peas results in altered structure and immunogenicity*. Journal of Agricultural and Food Chemistry 53:9023-30.
A fact sheet can be found at: http://www.pi.csiro.au/GMpeas/GMpeas.htm

[4] Schubert D (2002). *A different perspective on GM food*. Nature Biotechnology 20:969

[5] EFSA (2005). *Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed*.
http://www.efsa.eu.int/science/gmo/gmo_guidance/660_en.html

[6] Codex ad hoc Intergovernmental Task Force on Foods Derived from Biotechnology (2003). *Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants.*
http://www.codexalimentarius.net/download/standards/10021/CXG_045e.pdf

[7] Food and Agriculture Organization of the United Nations/World Health Organization (2001). *Evaluation of allergenicity of genetically modified foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology 22-25 January 2001*. FAO, Rome.
http://www.fao.org/es/ESN/food/pdf/allergygm.pdf

[8] The Joint Working Group on Novel Foods and GMOs (2003). *Guidance document for the risk assessment of genetically modified plants and derived food and feed. 6-7 March 2003.*
http://europa.eu.int/comm/food/fs/sc/ssc/out327_en.pdf

[9] Spök A, Gaugitsch H, Laffer S, Pauli G, Saito H, Sampson H, Sibanda E, Thomas W, van Hage M, Valenta R (2005). *Suggestions for the assessment of the allergenic potential of genetically modified organisms.* International Archives of Allergy and Immunology 137:167-80.
[10] Ibid
[11] Freese F & Schubert D (2004). *Safety testing and regulation of genetically engineered foods.* In Harding SE (Ed) Biotechnology & Genetic Engineering Reviews 21.
[12] Chowdhury EH, Kuribara H, Hino A, Sultana P, Mikami O, Shimada N, Guruge KS, Saito M, Nakajima Y (2003) *Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11.* J. Anim. Sci. 81(10): 2546-2551.
[13]EFSA (2005). *Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/F/96/05.10) for the placing on the market of insect resistant genetically modified maize Bt11, for cultivation, feed and industrial processing under Part C of Directive 2001/18/EC from Syngenta Seeds.* The EFSA Journal 213:1-33.
http://www.efsa.eu.int/science/gmo/gmo_opinions/922/gmo_opinion_ej213_bt11maize_cultivation_en1.pdf
[14] Op cit 11
[15] Brooks SA (2004). *Appropriate Glycosylation of Recombinant Proteins for Human Use: Implications of Choice of Expression System*. Molecular Biotechnology 28(3):241-256
[16] Berstein IL, Bernstein JA, Miller M, Tierzieva S, Bernstein DI, Lummus Z, Selgrade JK, Doerfler DL & Seligy VL (1999). *Immune responses in farm workers after exposure to Bacillus thuringiensis pesticides. Environmental Health Perspectives* 107(7):575-82.
[17] EFSA (2004). *Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Notification (Reference C/NL/98/11) for the placing on the market of herbicide-tolerant oilseed rape GT73, for import and processing, under Part C of Directive 2001/18/EC from Monsanto.* The EFSA Journal 29:1-19.
http://www.efsa.eu.int/science/gmo/gmo_opinions/174/opinion_gmo05_ej29_gt73_en1.pdf
[18] EFSA (2003). *Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safety of foods and food ingredients derived from herbicide tolerant genetically modified maize NK603, for which a request for placing on the market was submitted under Article 4 of the Novel Food Regulation (EC) No 258/97 by Monsanto*. The EFSA Journal 9:1-14.
http://www.efsa.eu.int/science/gmo/gmo_opinions/177/opinion_gmo_02_final_en1.pdf
[19] Op cit 13
[20] EFSA (2005). *Opinion of the Scientific Panel on Genetically Modified Organisms on an application (reference EFSA-GMO-NL-2004-02) for the placing on the market of insect-tolerant genetically modified maize 1507, for food use, under Regulation (EC) No 1829/2003 from Pioneer Hi-Bred International/Mycogen Seeds.* The EFSA Journal 182:1-22.
http://www.efsa.eu.int/science/gmo/gmo_opinions/826/gmopanelriskassessment1.pdf
[21] Op cit 13
[22] Scientific Committee on Plants (1998) *Opinion of the Scientific Committee on Plants regarding the genetically modified, insect resistant maize lines notified by the Monsanto Company.* 10 February 1998.
http://www.europa.eu.int/comm/food/fs/sc/scp/out02_en.html
[23] Scientific Committee on Plants (2000). *Opinion of the Scientific Committee on Plants on the submission for placing on the market of genetically modified insect resistant and glufosinate ammonium tolerant (Bt-11) maize for cultivation. Notified by Novartis Seeds SA Company (notification C/F/96/05-10) (opinion adopted by the Scientific Committee on Plants on 30 November 2000).*
http://www.europa.eu.int/comm/food/fs/sc/scp/out86_gmo_en.html
[24] Scientific Committee on Plants (1998). *Opinion of the Scientific Committee on Plants on the genetically modified maize lines notified by the Novartis Company (Notification C/GB/96/M4/1) (Submitted by the Scientific Committee on Plants, 10 February 1998).* http://www.europa.eu.int/comm/food/fs/sc/scp/out05_en.html
[25] Scientific Committee on Food (2002). *Opinion of the Scientific Committee on Food on a request to place genetically modified sweet maize line Bt11 on the market.*
http://www.europa.eu.int/comm/food/fs/sc/scf/out129_en.pdf
[26] EFSA (2004). *Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safety of foods and food ingredients derived from insect-protected genetically modified maize MON 863 and MON 863 x MON 810, for which a request for placing on the market was submitted under Article 4 of the Novel Food Regulation (EC) No 258/97 by Monsanto.* The EFSA Journal 50:1-25. http://www.efsa.eu.int/science/gmo/gmo_opinions/383/opinion_gmo_07_en1.pdf
[27] Op cit 22

[28] Op cit 23
[29] Op cit 5
[30] See, for example, Wilson A, Latham J, Steinbrecher R (2004). *Genome Scrambling – Myth or Reality?* Econexus Technical Report October 2004. http://www.econexus.info/pdf/ENx-Genome-Scrambling-Report.pdf
[31] Op cit 18
[32] Op cit 11
[33] Op cit 17
[34] Op cit 5
[35] http://europa.eu.int/comm/food/dyna/gm_register/index_en.cfm
[36] AgrEvo (undated). *Submission for placing on the market (in accordance with the Part C of the Directive 90/220/CEE) of glufosinate tolerant corns (Zea mays) transformation event T25*. AgrEvo France.
Monsanto (1994). *Application to the United Kingdom Advisory Committee on Novel Foods and Processes for Review of the safety of glyphosate tolerant soybeans by the Agricultural Group of Monsanto Company*. July 27 1994.
Monsanto (2000). *Application to place on the market genetically modified higher plants pursuant to Council Directive 2001/18/EC. Application for consent to place on the market NK603 Roundup Ready maize for use as any other maize, excluding the marketing of varieties, in the European Union.* Monsanto Company, December 2000
Monsanto (2002). *Additional information to complement oilseed rape notification C/NL/98/11, as requested by the entering into force of Directive 2001/18/EC*. 12th December 2002.
Monsanto (2002). *Notification concerning the placing on the market of genetically modified higher plant pursuant to Part C of Council Directive 2001/18/EC and anticipating Directive 2001/18: Placing on the market of maize grains derived from insect-protected maize line MON863 and maize hybrid MON863xMON810.* Monsanto Company, July 2002 (amended Feb 2003).
Monsanto (undated). *Submission to the French Commission du Génie Biomoléculaire: Application to place on the market genetically modified higher plants: Insect protected maize (MON810).* Monsanto Company.
Monsanto (undated). Application for consent to market grain and derived products from Roundup Ready maize line GA21 and its progeny for the purposes of handling in the environment and use for animal feed.
Novartis Seeds AG (1994). *Genetically modified maize plant: maize protecting itself against corn borers – Bt-176 maize: Food and feed safety assessment*. November 1994.
Pioneer/Mycogen (2000). *Notification to market products containing genetically modified organisms in accordance with Directive 90/220/EEC. Import of 1507 maize*. 23 November 2000.
Syngenta (undated). *Notification for Part C Consent, Ref C/F/96/05/10 for the marketing (including cultivation) of maize genetically modified for insect resistance and herbicide tolerance.*
Op cit 17, 18, 20, 22, 26, 37, 38, 42, 43, 44
[37] Scientific Committee on Food (2002). Opinion of the Scientific Committee on Food on the safety assessment of the genetically modified maize line GA21, with tolerance to the herbicide glyphosate (expressed on 27 February 2002). http://www.europa.eu.int/comm/food/fs/sc/scf/out121_en.pdf
[38] EFSA (2005). *Opinion of the Scientific Panel of Genetically Modified Organisms on a request from the Commission related to the Notification (Reference C/DE/02/9) for the placing on the market of insect-protected genetically modified maize MON863 x MON810, for import and processing, under Part C of Directive 2001/18/EC from Monsanto*. The EFSA Journal 251:1-22.
[39] Op cit 17
[40] Op cit 18
[41] Op cit 22
[42] Scientific Committee on Food (1996). *Opinion on the potential for adverse health effects from the consumption of genetically modified maize (Zea mays L) (Expressed on 13 December 1996, updated to include references 21 February 1997*. http://www.europa.eu.int/comm/food/fs/sc/oldcomm7/out02_en.html
[43] Scientific Committee on Plants (1998). *Opinion of the Scientific Committee on Plants regarding "Submission for placing on the market of glufosinate tolerant maize (Zea mays) transformation event T25" by the AgrEvo company (now Aventis CropScience). (Amended opinion adopted by the Scientific Committee on Plants on 20 July 2001)*. http://www.europa.eu.int/comm/food/fs/sc/scp/out108_gmo_en.pdf
[44] Op cit 38
[45] GM Science Review Panel (2004). *GM Science Review Second Report: An open review of the science relevant to GM crops and food based on interests and concerns of the public*. January 2004. http://www.gmsciencedebate.org.uk/report/pdf/gmsci-report2-full.pdf

[46] Penninks AH (2001). *Joint FAO/WHO Expert Consultation of Foods Derived from Biotechnology: Topic 8: Animal Model for Allergenicity Assessment*.
[47] Op cit 7
[48] Food Standards Agency (2005). *Research Requirements Document, Issue 20: Requirements for Research and Surveys, December 2005*. http://food.gov.uk/multimedia/pdfs/rrd20.pdf