OBJECTIONS TO THE APPLICATION MADE BY PIONEER HI-BRED RSA AND DOW AGROSCIENCE SOUTHERN AFRICA FOR COMMODITY CLEARANCE OF GRAIN AND DERIVED PRODUCTS FROM 1507X59122 MAIZE (HERCULEX XTRA) TO THE NATIONAL DEPARTMENT OF AGRICULTURE, SOUTH AFRICA

PREPARED BY

AFRICAN CENTRE FOR BIOSAFETY

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SCIENTIFIC ASSESSMENT

REFERENCES

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The scientific assessment is based on the information provided as part of the notifier application. The information provided for comment within a period of five days is in excess of 1000 pages comprising largely technical scientific data. The information provided is only that deemed unclassified. Notwithstanding this volume of information, there appear to be several omissions or references to previous applications. The section on toxicity of foreign gene products to humans and animals (page 17), for example, states that a 'very detailed evaluation of the potential toxicity to humans and animals of the Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins expressed in 1507 maize and 59122' was included in the respective applications. As a result, this information is not included in this application. A great deal of the information relating to the digestion assays was obtained from other researchers who have had sight of this data[27,40].

1. BACKGROUND

Maize

Maize or corn (Zea mays L.) is grown commercially in over 100 countries primarily for the kernel, which is processed into a wide range of food and industrial goods[1]. The greater proportion of maize produced is used for animal feed with under 10% of the maize used as human food products. Starch produced from maize is converted into sweeteners, syrups and fermentation products[1].

Bacillus thuringiensis: Mode of Insecticidal Action

Bacillus thuringiensis (Bt), a common soil bacterium produces insecticidal proteins during sporulation. Each of the several thousand strains of Bt that exist produces its own unique insecticidal crystal protein (delta endotoxin)[2], each of which displays differing insecticidal activity, but with a similar mode of action. Typically, ingested delta endotoxins are dissolved in the insect midgut liberating the protoxins of which they are comprised. These undergo proteolysis and one of the fragments binds to the cells of the insect midgut epithelium, disrupting the osmotic balance and forming pores in the cell membrane causing cell lysis, gut paralysis and death within a few hours of ingestion[2,3].

The Herculex Maize Varieties

The Herculex Insect Protection Family has been developed by Dow AgroSciences LLC and Pioneer Hi-Bred International, Inc.[4] The insect protection family, containing the original Herculex I, has now been expanded to include Herculex RW and Herculex XTRA[4]. The assessments of the Cry proteins are based on surrogate proteins rather than the transgenic protein produced by the genetically engineered crop. Especially, toxicity assessments, which require larger quantities of the protein for meaningful analyses are conducted using these

surrogate proteins. Cry34Ab1 and Cry35Ab1 were produced in P*seudomonas flourescens* for the purposes of the assessment of Herculex RW (page 22 of the notifier application for commodity clearance of Herculex RW). This practice has come under criticism because of the peculiarities of each transformation event, which by definition implies a unique gene arrangement[5]. Further, assuming the unlikely chance of precise incorporation into *P. flourescens*, the organism is kingdoms apart from maize with different protein generation and regulatory pathways.

Glufosinate Ammonium and the *pat* Gene

Glufosinate-ammonium salt (or phosphinothricin), often referred to as just glufosinate, is a broad-spectrum contact herbicide that behaves sufficiently like the amino acid glutamate to enable it to disrupt the conversion of glutamate to glutamine. It disrupts the enzyme mediated reaction by inhibiting glutamine synthetase activity in susceptible plants, resulting in reduced glutamine production. Glutamine synthetase also regulates ammonia levels by detoxification and disruption of the enzyme activity results in elevated ammonia levels[1,6]. The *pat* gene codes for phosphinothricin-N-acetyltransferase, an enzyme which catalyses phosphinothricin acetylation effectively rendering it inactive and thereby enabling transformed plants to withstand phosphinothricin based herbicide applications.

This Application

This application is commodity clearance of grain and derived products from 1507X59122 maize for use in foods, animal feeds and industrial products. This event, designated Herculex Xtra, was produced by conventional breeding of the progeny of the two genetically modified maize lines, TC1507 and Hercules RW (59122 maize). The following discussion details the main features of the individual events and those features or aspects of the application that are cause for concern for the respective events, which will ultimately manifest in Herculex Xtra.

2. TC1507: DESCRIPTION AND CHARACTERISTICS

Gene Modifications

TC1507 is a transgenic maize line that has been engineered to produce an insect control protein Cry1F as well as withstand the use of glufosinate-ammonium herbicides. This has been achieved by the introduction of two genes, *cry*1F and *pat* into the maize hybrid line Hi-II by biolistic (particle acceleration) transformation[1]. Cry1F protein confers resistance against lepidopteran insect pests, in particular the European corn borer (*Ostrinia nubilalis),* the pink borer *Sesamia* spp.), fall armyworm (*Spodoptera frugiperda*), black cutworm (*Agrotis ipsilon*) and southwestern corn borer (*Diatraea grandiosella*)[1,8] .

Molecular Characterisation and Gene Stability of TC1507

The application made by Dow AgroSciences in respect of event TC1507 to the National Department of Agriculture, South Africa was assessed by the African Centre for Biosafety (25th June 2004)[7]. Notwithstanding the summary responses in the application, coupled with the lack of provision of the associated documentation, the Summary Notification Information Format (SNIF), notification number C/ES/01/01, submitted jointly by Pioneer Hi-Bred and Mycogen Seeds (c/o Dow AgroSciences LLC) developers of TC1507 maize, to the Competent Authority of Spain, was used as a source for information relating to the genetic modifications[8].

Particle acceleration was used to introduce a linear fragment of DNA containing the *cry*1F and *pat* genes and their regulatory coding sequences into maize cells[8]. The cry1F gene isolated from *Bacillus thuringiensis* subsp.*aizawai* is under the control of a ubiquitin promoter, *ubi*ZM1 from *Zea mays* and an ORF25PolyA terminator from *Agrobacterium tumefaciens.* The *pat* gene derived from the soil actinomycete *Streptomyces viridochromogenes* is under the control of the Cauliflower Mosaic Virus CaMV35S promoter and terminator[8].

Detailed characterisation by Southern blot and DNA sequence analysis confirmed the presence of 6186bp of the 6235 insert containing the target *cry*1F and *pat* genes and associated regulatory sequences. Additionally, non-functional DNA fragments were inserted into the host plant. These include:

- A 335bp sequence of the *cry*1F gene with no *ubi*ZM1 promoter sequence and a 15bp sequence of the *cry*1F gene, both located at the 5' end of the insert;
- Two *pat* gene fragments lacking regulatory elements located at the 5' border and a fragment of the *pat* gene located at the 3' end;
- A fragment of the polylinker region and *ubi*ZM1 promoter at the 5' end, and
- An inverted sequence of a part of the ORF25PolyA terminator sequence located at the immediate 3' end[8].

Possible Unintended Effects of the non-functional DNA Fragments in TC1507

Despite the expression of the introduced gene sequences having been confirmed by molecular characterisation and protein expression analysis[8], unintended effects that are not detected in the lab and that may only become apparent in the long term, cannot be ruled out. Transformation by particle acceleration is associated with multiple fragments and gene rearrangements[9,10].

That this has occurred in the development of TC1507 is not in question. The inserted gene sequences may interrupt native gene sequences and/or their promoters[10]. What is of concern is the possible production of novel proteins from the transcription of the unintended

TC1507 fragments which have two open reading frames (ORF). The claim that these "non-functional" fragments are not transcribed[8] needs to be subjected to greater scrutiny and more investigation. Extra gene fragments in Monsanto's Roundup Ready Soya were also claimed to be non-functional and not-transcribed[11], but were later found to be transcribed to produce RNA[9,12,13].

Further, it is not clear if the insert or fragments thereof lie on any maize transposons and what the impact of the DNA insert is on flanking sequences. The lack of sophisticated methods for targeted insertion, especially in higher organisms[10] necessitates more rigorous research into possible position effects prior to the granting of any release of transgenic organisms into the environment.

The assertion by Dow AgroSciences (question 4.4 of the application to the Department of Agriculture in South Africa for a trial release of TC1507) that the inserted gene is no different from naturally occurring plant genes and that any instability will only affect the transformed plant is not so clear cut. Firstly, the basis on which Dow AgroSciences made these claims could not be properly assessed as they cited no sources or data to substantiate their claims. Secondly, if transgenes behave just like naturally occurring genes, then they have the potential to be inherited in the same way and persist indefinitely in cultivated or free-living populations. Any mixing of native and transgenic plants whether by dispersal, improper handling etc., can result in the spread of transgenes. The consequences, both ecological and evolutionary of crop-to-crop gene flow are only now beginning to be investigated in any meaningful way and the possible exposure of non-target organisms, including humans to novel proteins cannot be discounted[10].

As a final point regarding the molecular characterisation of TC1507, it is important to note that the UK competent authority, ACRE (Advisory Comment on Releases to the Environment) in response to notification ES/01/01, dated 29 April 2004, did not give its consent for cultivation and requested further clarification of the PCR-based event-specific detection protocol because of an apparent contradiction in the information provided on the characterisation of the insert.

CaMV Promoter

The cauliflower mosaic virus (CaMV) is a DNA-containing para-retrovirus replicating by means of reverse transcription. It contains within its genome a viral promoter called 35S, a general strong plant promoter which has been used to secure expression of transgenes in a large proportion of commercialised GMOs. There are several studies indicating the potential for transcriptional activation of the 35S CaMV promoter in mammalian systems[14,15].

The CaMV 35S promoter has been found to have a recombination hotspot where it tends to fragment and join with other double stranded DNA in a very non-specific manner[16]. These

hotspots are flanked by multiple motifs involved in recombination and functions efficiently in all plants, green algae, yeast and *Escherichia coli*. The potential exists for the viral genes to recombine with other viruses to generate new infectious viruses[17], carcinogens and mutagens as well as to reactivate dormant viruses.

Detractors claim that virus infected cabbages and cauliflowers have been consumed for years with no ill effects and that similar pararetroviral sequences occur widely in plants, causing no apparent harm[18]. That the intact virus causes no obvious harm in the natural host is related to the fact that its integrity is maintained and that it is adaptive to the host biology. This is unlike the fragments of naked DNA as in the transformed plant where the natural regulatory mechanisms are not present[17]. A call has been made that the use of the CaMV promoter in transgenic plants be phased out due to the structural instability arising out of its use[19].

3. HERCULEX™ RW: DESCRIPTION AND CHARACTERISTICS

Gene Modifications

Maize line 59122 otherwise known as Herculex™ RW is a transgenic maize line that has been engineered to produce two insect control proteins Cry34Ab1 and Cry35Ab1 as well as the PAT protein to withstand the use of glufosinate-ammonium herbicides. The Cry proteins Cry34Ab1 and Cry35Ab1 act synergistically to confer resistance against coleopteran insect pests, in particular corn rootworm larvae (*Diabrotica* spp.).

4. THE STARLINK CORN CONTROVERSY

StarLink corn hybrids produced by Aventis Crop contain a plant pesticide protein (Cry9C) derived from *Bacillus thuringiensis* which kills certain destructive pests of corn such as the European corn borer. In 1998 the Environmental Protection Agency (EPA) approved Starlink corn for use only as animal feed and set a zero-tolerance level for its use in human food based on the fact that this particular Bt protein does not break down easily in the human digestive system, is heat resistant, and could prove allergenic. In 2002 however, StarLink corn was detected in taco shells[20]. The potential for allergenicity of Starlink corn was not completely ruled out because some tests showed that the Cry9C protein could survive cooking or processing and was hard to digest. The contamination of the human food chain led to a public outcry and massive recall of all products thought to contain the Starlink variety.

5. ALLERGENICITY

The nature of genetic modification of higher plants results in the production of novel proteins which might cause allergic reactions. Allergies to food are potentially life threatening

for an estimated 2% of adults and 8% of children. One reason for the failure of identification of GM crops as allergenic is related to the fact that the testing and assessment thereof is left up to the developer of the transgenic organism and that no standardised agreed-upon protocols exist for such testing[21]. No test exists that is fully predictive of potential allergenicity[22]. The need for the assessment of allergenicity was first recognised when Pioneer transferred Brazil nut genes for a high methionine 2S albumin into soybeans and detected its allergenic potential and voluntarily stopped development of the product[23,22]. This highlighted the need for a sound assessment strategy for allergenicity and over the past ten years, several bodies have applied themselves to this including the International Life Sciences Institute, the International Food Biotechnology Council, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO)[22,24.]

Assessment of Allergenicity

Several elements were considered for testing including the source of the gene, sequence homology to known allergens, specific serum screening, comparative resistance to pepsin, target serum screening (the immunoreactivity of the novel protein with serum IgE from individuals with known allergies to species that are broadly related to the source of the transferred DNA) and the use of animal models. The latter two methods were not considered sufficiently well understood or developed methodologies for regulatory purposes and to date, the allergenicity assessment of genetically modified food crops relies on the four former-mentioned methods[22].

The gastric stability assay has been widely accepted as an important part of allergenicity assessments of genetically modified products and support in the literature continuing through the FAO/WHO consultation in 2001 resulted in acceptance by the Codex Alimentarius[25,26,27.]. This experiment is based on the hypothesis that food allergens must exhibit sufficient gastric stability to have a chance of reaching the intestinal mucosa where absorption and sensitising will occur[22,28]. Typically the test is a measure of comparative resistance to pepsin proteolysis[22]. In the face of the lack of definitive tests for determining potential allergenicity, it is the most reliable test[27,22,29].

For the assessment of allergenicity of Herculex RW, Dow conducted tests at an extremely acidic pH (1.2), the acidic end of fasting pH, which is more likely the reason why there was such rapid degradation rather than an inherent instability. The FAO/WHO 2001 protocol conditions for assessment of gastric stability is a ph of 2.0[25,26,27], which is more representative of the range of the human gut with gastric pH typically being between 1–2 under fasting conditions, rising to a value of over 5 during a meal[30]. Had the tests been carried out at this higher pH, as outlined in the accepted protocol, judging from the behaviour of other Cry proteins, the chances of Cry34Ab1[31] being judged a potential allergen would have been greater.

Reliability of Gastric Assays

It is important to note that whilst gastric assays remain the most reliable form of currently used allergenicity tests, there have been several instances where food allergens have been found to be unstable in the gastric assay, as well as some instances where supposed non-allergenic proteins have been stable. This makes interpretation more difficult, but does not invalidate the assay[27]. Also the assays may not always be appropriately applied. If, for example, if stability is correlated with allergenicity because the protein must reach immune tissue in the intestines for sensitization to occur, then oral allergy syndrome allergens may not fit the model because sensitization may occur through the respiratory homologue of the food allergen[27]. Similarly, assays of the uncooked form of the protein may not be relevant if the food is always eaten in a cooked form, which degrades the GE protein or makes it more susceptible to digestion. The application of the gastric digestion should therefore be correctly applied and interpreted.

Allergenicity to *Bacillus thuringiensis*

There have been reports of allergenicity to *B. thuringiensis*. In instances where there has been exposure, e.g. on farms where farm workers were exposed to conventional Bt sprays, 2 out of 123 workers exhibited sensitivity to Bt formulations[32]. Allergic symptoms include allergic rhinitis, angioedema, dermatitis, pruritus, swelling, erythema with conjunctival injection, exacerbations of asthma, angioedema and rash. Aerial spraying of Bt pesticides precipitated increased respiratory health effects in local residents[33].

Allergenicity of Novel Proteins

Cry34 proteins impacts on human exposure are little reported and understood. The value of sequence homology is not immediately apparent as questions regarding homology and allergenicity still have to be answered. For Herculex RW, The EPA reported that Dow submitted a study showing no overall sequence similarities or homology at the level of 8 contiguous amino acid residues to known allergens. This choice of 8-AA sequences was recommended by Metcalfe in 1996[34]. There have since been several refinements and alterations suggested including the allowance of substitution of chemically similar amino acids in the 8-AA sequence[35] and comparisons based on identity of 6 rather than 8 contiguous amino acids[26]. this suggest that it would be prudent to conduct further testing, applying the more rigorous homology criteria, particularly in view of the suggestive evidence of allergenicity of Bt spore preparations described above[36].

Matched sequences in this instance will require more study as the Cry34 protein is still an unknown quantity given that they have not been food constituents, and are not similar to food proteins or known allergens. The currently known allergens and their related gene sequences do not therefore represent the full range of possible protein sequences capable of producing an allergic reaction[27] and negative results in sequence homology searches are not

necessarily proof of lack of allergenicity. Allergenic responses to new proteins that have not previously formed part of the food supply cannot therefore be ruled out.

Heat Stability and Significance

Loss of function from heating is not necessarily an indicator of non-allergenicity. Some milk allergens for example, can have either conformational or linear epitopes, where the latter may reflect sensitization to the denatured form of the protein[37,38]. More generally, loss of function may merely indicate denaturation rather than degradation into short peptides, and could therefore still be allergenic.

Protein Abundance

Abundance of a particular protein in food has also been used in predicting the likelihood of allergenicity, since the bulk of known food allergens are typically plentiful proteins[27]. Levels as low as 20 PPB on Starlink were considered unacceptable because a lower limit for sensitization could not be determined[39]. The FAO/WHO assessment was not made by reference to heat stability or protein abundance, but noted that '…allergens can sensitize susceptible individuals at less than milligram levels, possibly at less than microgram levels," and "Thus, level of expression cannot yet be incorporated into the assessment of the allergenicity of genetically modified foods."[26,27].

Proteolysis of Cry34 and Cry35 protein

Dow submitted two sets of digestion data for the Cry34 proteins. In the first study (MRID 452422-12), Cry34Ab1 was digested within 30 minutes[40]. The Cry34 results prompted Dow to submit a second set of digestion data with the tests carried out under the same conditions with the addition of shaking during incubation[40] (MRID 455845-02). The results that Dow report as being the final results are that the Cry34Ab1 protein was digested under simulated gastric conditions in 6.5 minutes and the Cry35Ab1 protein in under 5 minutes. Gurian-Sherman (2003)[27] in an assessment of the methodology used by Dow to assess allergenicity found that the protocol was flawed. The notifiers in support of their application have developed a kinetic assessment of the degradation of the novel Cry protein Cry34Ab1[40]. Dow measured rate of digestion to determine 90% digestion as opposed to using the longest time-point where SGD test protein can be detected. The kinetic approach to assessing digestion is not widely accepted and the value and significance of this approach is currently the subject of discussion by an open meeting of the FIFRA Scientific Advisory Panel[41].

The regression analysis provided by Dow to determine the 90% digestion time was not accompanied by any statistical analysis of variance, such as a confidence interval. This coupled with apparent variability in the detection gels studied by Gurian-Sherman suggested that the time point 6.2min (DT_{90} – time taken for 90% of the sample to decay) might not be statistically significant. Also, Dow used more than three-fold higher proportion of pepsin-to-

test-protein (Cry34Ab1) in its SGD assay which may make Cry34Ab1 appear to be less stable than it would if carried out according to the literature[27].

6. GENE TRANSFER

Horizontal Gene Transfer (HGT)

Horizontal gene transfer (HGT) is the transfer of genetic material between organisms, outside the context of parent to offspring reproduction[42,43]. It is most commonly recognized as infectious transfer[44]. HGT frequencies are now known to be much higher than originally thought. The evolution of antibiotic resistance, for example, is an indicator of the frequency of gene transfer, given that antibiotics have been used in medicine only for about 50 years[44]. The intentional modification of plants could through horizontal gene transfer result in the unintentional modification of other organisms. What the possible impacts of such gene transfer might be is not known.

Resistance of DNA to Digestion

There are several reported cases in the literature of both the persistence and transfer of gene sequences after ingestion of GM products. Polymerase chain reaction (PCR) has been used to demonstrate the presence of large fragments of M13 phage DNA, which had been fed to mice, in the faeces and bloodstream and in white blood cells[45]. Research published by the UK government in 2002 has shown that bacteria in human intestines had in fact taken up a novel gene from processed food containing GM Soya[46]. It has been reported that people with ileostomies (i.e. who make use of a colostomy bag) are capable of acquiring and harbouring DNA sequences from GM plants in the small intestine[47]. Recombinant DNA fragments and Cry1Ab protein was also found in the gastrointestinal contents of pigs fed genetically modified corn[48].

REFERENCES

[1] DAS-01507-1 (TC1507). *AGBIOS Database Product Description*. http://www.agbios.com/static/cropdb/SHORT_TC1507_printer.html

[2] *Neppl, C. C. (2000) Management of resistance to Bacillus thuringiensis toxins. The Environmental Studies Program, University of Chicago. May 26, 2000. http://camillapede.tripod.com/bapaper.html*

[3] Tappeser, B. The differences between conventional *Bacillus thuringiensis* strains and transgenic insect resistant plants. Possible reasons for rapid resistance development and susceptibility of non-targeted organisms. Third World Network. Briefing Paper No. 1. http://www.twnside.org.sg/title/diffe-cn.htm

[4] Dow AgroSciences, Pioneer announce next Herculex™ traits. 19 July 2004. http://www.pioneer.com/pioneer_news/press_releases/products/herculex_rw_xtra.htm

[5] Freese, W. (2003) Genetically Engineered Crop Health Impacts Evaluation - GAPS Analysis. Friends of the Earth

[6] Greenpeace (1997) Glufosinate and genetic engineering. economic and environmental implications of herbicide resistance. *Greenpeace, International Genetic Engineering Campaign, Background Information*. 04/97.

[7] Objections to the application made by Dow AgroSciences in respect of event TC1507 to the National Department of Agriculture, South Africa. http://www.biosafetyafrica.net

[8] Summary Notification Information Format for products containing genetically modified higher plants (GMHPs) in accordance with directive 2001/18/EC. C/ES/01/01. http://gmoinfo.jrc.it/csnifs/C-ES-01-01.pdf

[9] Greenpeace comments on: SNIF for the deliberate release and placing on the EU market of the 1507 maize, C/ES/01/01. http://www.greenpeace.se/files/200-2399/file_2308.pdf

[10] Snow, G. A., Andow, D. A., Gepts, P., Hallerman, E. M., Power, A., Tiedje, J. M., and Wolfenberger, L. L. (2004) Genetically engineered organisms and the environment: Current status and recommendations. *Ecological Society of America Position Paper*. ESA Public Affairs Office. February 26, 2004. http://www.esa.org/pao/esaPositions/Papers/geo_position.htm

[11] Monsanto (2000) *Dossier containing molecular analysis of Roundup Ready Soya.* http://www.foodstandards.gov.uk/pdf_files/acnfp/dossier.pdf available at http://www.foodstandards.gov.uk/committees/acnfp/acnfpassessments.htm

[12] Monsanto (2002a) Transcript analysis of the sequence flanking the 3' end of the functional insert in Roundup Ready Soybean event 40-3-2. http://www.food.gov.uk/science/ouradvisors/novelfood/assess/assess-uk/60500

[13] Monsanto (2002b) Additional characterisation and safety assessment of the DNA sequence flanking the 3' end of the functional insert of Roundup Ready Soybean event 40-3-2. http://www.food.gov.uk/science/ouradvisors/novelfood/assess/assess-uk/60500

[14] Ryabova, L. A. & Hohn, T. (2000) Ribosome shunting in the cauliflower mosaic virus 35S RNa leader is a special case of reinitiation of translation functioning in plant and animal systems. *Genes & Development*. **14**:817

[15] Ho, M-W. et al. (2000) Hazardous CaMV promoter? *Nature Biotechnology*. **18(4)**, 363

[16] Kohli, A., Griffiths, S., Plcios, N., Twyman, R. M., Vain, P., Laurie, D. A., Christou, P. (1999) Molecular characterization of transforming plasmid rearrangements in transgenic rice reveals a recombination hotspot in the CaMV 35S promoter and confirms the predominance of microhomology mediated recombination. *The Plant Journal*. **17**, 591.

[17] Ho, M-W. A brief history of the CaMV 35S promoter controversy. http://www.i-sis.org.uk

[18] Hull, R., Covey, S. N. & Dale, P. (2000) Genetically modified plants and the 35S promoter: assessing the risks and enhancing the debate. *Microbial Ecology in Health and Disease*. **12**, 1.

[19] Christou, P., Kohli, A., Syoger, E., Twyman, R. M. Agrawal, P., Gu, X., Xiong, J., Wegel, E., Keen, D., Tuck, H., Wright, M., Abranches, R. & Shaw, P. (2000) Transgenic plants: a tool for fundamental genomics research. *John Innes Centre & Sainsbury Laboratory Annual Report 1999/2000*. p29.

[20] Segarra, A. E. & Rawson, J. M. (2001) StarLink™ Corn Controversy: Background. 10 January. http://www.ncseonline.org/nle/crsreports/agriculture/ag-101.cfm

[21] European Communities – Measures affecting the approval and marketing of biotech products (WT/DS0291, 292 and 293). *Amicus Curiae Brief*. June 1, 2004. http://www.ciel.org/Publications/ECBiotech.AmicusBrief_2June04.pdf

[22] Taylor. S. L. Review of the development of methodology for evaluating the human allergenic potential of novel proteins. http://www.ilsi.org/file/Chapter2Taylor.pdf

[23] Hansen, M. (2002) Science-based Approaches to Assessing Allergenicity of New Proteins in Genetically Engineered Foods. 14 August. Presentation to FDA Food Biotechnology Subcommittee, Food Advisory Committee. http://www.organicconsumers.org/gefood/hansen081402.cfm

[24] Metcalfe, D. D. (2003) Introduction: What Are the Issues in Addressing the Allergenic Potential of Genetically Modified Foods? Environ Health Perspect **111**,1110–1113 http://dx.doi.org/

[25] Metcalfe, DD et al. (1996) Assessment of the allergenic potential of foods from genetically engineered crop plants. Crit. Rev. Food Sci. Nutr. **36(S)**, 165-186

[26] FAO/WHO (2001) Evaluation of allergenicity of genetically modified foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology. January 22-25, Rome, Italy

[27] Gurian-Sherman, D. (2003) Comments to the Scientific Advisory Panel Evaluating Cry34Ab1/35Ab1, March 1-2, 2005, Docket No. OPP-2004-0395. The Centre for Food Safety.

[28] EuropaBio. The European Association for Bioindustries. (2003) Safety Assessment of GM Crops. Document 4.3. Protein safety evaluation. February. http://www.europabio.org/relatedinfo/CP2.pdf

[29] Codex Alimentarius. Foods derived from Biotechnology. Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants. CAC/GL 45-2003

[30] Thomas, K. et al (2004). "A multi-laboratory evaluation of a common in vitro pepsin digestion assay protocol used in assessing the safety of novel proteins," *Regulatory Toxicology and Pharmacology* 39: 87-98.

[31] Freese, W. (2005) Friends of the Earth. *Personal communication.*

[32] Bernstein, I. L., Bernstein, J. A., Miller, M., Tierzieva, S., Bernstein, D. I., Lummus, Z., Selgrade, M. K., Doerfler, D. L., Seligy, V. L. (1999) Immune response in farm workers after exposure to Bacillus thuringiensis pesticides. Environ. Health Perspect. **107(7)**, 575

[33] Petrie, K., Thomas, M. & Broadbent, E. (2003) symptom complaints following aerial spraying with biological insecticide Foray 48B. New Zealand Medical Journal. **116(1170),** U354

[34] Metcalfe, D.D., Astwood, J.D., Townsend, R., Sampson, H.A., Taylor, S.L. And Fuchs, R.L. (1996). Assessment of the Allergenic Potential of Foods Derived from Genetically Engineered Crop Plants. *Critical Reviews in Food Science and Nutrition* **36**(S), S165-186.

[35] Gendel (1998). "The use of amino acid sequence alignments to assess potential allergenicity of proteins used in genetically modified foods," Advances in Food and Nutrition Research 42, pp. 45-62; "Mammalian Toxicity Assessment Guidelines for Protein Plant Pesticides," FIFRA Scientific Advisory Panel to the EPA, SAP Report No. 2000-03B, September 28, 2000.

36 Freese, W. (2005) Comments on the Application by Pioneer Hi-Bred RSA And Dow Agroscience Southern Africa for Commodity Clearance of Genetically Modified 59122 Maize to the National Department Of Agriculture, South Africa. 9th March.

[37] Kohno, Y et al. (1994) Preferential recognition of primary protein structures of alpha-casein by IgG and IgE antibodies of patients with milk allergies. *Ann. Allergy* **73**, 419-422

[38] Vila, L et al. (2001) Role of conformational and linear epitopes in the achievement of tolerance in cow's milk allergy. Clin. Exper. Allergy **31(10**), 1599-1606

[39] SAP Report No. 2001-09, Assessment of Additional Scientific Information Concerning StarLink Corn, July 2002, Arlington, VA http://www.epa.gov/scipoly/sap/2001/july/julyfinal.pdf

[40] Position Paper (2005) Scientific Issues Associated with the Human Health Assessment of the Cry34Ab1 Protein. 9 February. http://www.epa.gov/scipoly/sap/2005/march/positionpaper.pdf

[41] FIFRA Scientific Advisory Panel Open Meeting (2005) Scientific issues associated with the human health assessment of the Cry 34Ab1 protein. 1-2 March. Docket Number: OPP-2004-0395. http://www.epa.gov/scipoly/sap

[42] Heinemann, J. A. (2003) *Bioscience*. 12, 51 cited in [43].

[43] European Communities: Measures Affecting the Approval and Marketing of Biotech Products (DS291, DS292, DS293). (2004) Third Party Submission by Norway.

[44] Heinemann, J. A. Gene Ecology Guide to: Measuring Horizontal Gene Transfer. Condensed version of paper published in Nature Biotechnology in September 2004. *Personal Communication*.

[45] Schubbert, R., Lettmann, C. Doerfle, W. (1994) Ingested foreign (phage M13) DNA survives transiently in the gastrointestinal tract and enters the bloodstream of mice. *Mol. Gen Genet*. **242**, 495.

[46] Netherwood, T., Matin-Orue, S. M., O'Donnell, A. G., Gockling, S., Gilbert, H. J. & Mathers, J. C. (2002) Transgenes in genetically modified Soya survive passage through the human small bowel but are completely degraded in the colon. UK Food Standards Agency. *Research Report G01008: Evaluating the risks associated with using GMO in human foods.*

[47] Heritage, J. (2004) The fate of transgenes in the human gut. *Nature Biotechnology.* **22(2)**, 170. http://www.nature.com/cgi-taf/DynaPage.taf?

[48] Chowdhury, E. H., Kuribara, H., Hino, A., Sultana, P. and Mikami, O. (2003) Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn. *Journal of Animal Science.* **81**, 2546.