

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 Seeds¹
(Question No EFSA-Q-2004-012)

Opinion adopted on 20 April 2005

SUMMARY

This document provides an opinion of the Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) on Bt11 maize, genetically modified to provide protection against specific lepidopteran pests. The maize also contains a gene providing tolerance to the herbicide glufosinate.

The opinion is based on a question raised by the Commission relating to an application for the placing on the market of Bt11 maize under Directive 2001/18/EC. The GMO Panel was asked to consider whether there is any scientific reason to believe that placing Bt11 maize on the market, for cultivation, import, processing and use as any other maize (excluding food uses), is likely to cause any adverse effects on human health and the environment (Notification C/F/96/05.10). The question followed a scientific assessment which was made initially by the Competent Authority of France and evaluated subsequently by all other Member States. An assessment of the Bt11 maize was requested by the Commission because of questions raised by several Member States following the evaluations at the national level. When this is the case, EU legislation requires that EFSA carries out a further assessment and provides an opinion.

Bt11 maize has been previously evaluated (SCP, 1998a) and approved (EC, 1998) for import, processing and feed use under Directive 90/220/EEC. Bt11 maize has also been evaluated for cultivation under the same Directive (SCP, 2000). Food and food ingredients derived from Bt11 maize have been authorised (EC, 1999) pursuant to Article 5 of Regulation (EC) 258/97. Bt11 sweet maize has also been evaluated (SCF, 2002) and approved (EC, 2004c) for food consumption in the framework of Regulation (EC) 258/97 (EC, 1997).

In delivering its opinion the Panel considered the application, additional information provided by the applicant and comments submitted by the Member States. Bt11 maize was assessed with reference to its intended use employing the appropriate principles as described in the 'Guidance Document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed' (EFSA, 2004a). The scientific assessment included examination of the DNA inserted into Bt11 maize and the nature and safety of the newly expressed proteins produced by the transgenic plants with respect to toxicology and allergenicity. Furthermore, a comparative analysis of agronomic traits and composition was undertaken and

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the safety of the whole product was evaluated. A nutritional and an environmental assessment, including monitoring plan, were both undertaken.

Bt11 maize has been developed for protection against specific lepidopteran pests such as the European corn borer (*Ostrinia nubilalis*) and *Sesamia* spp. Though the applicant considered that the *pat* gene for glufosinate ammonium tolerance was a marker gene and would only be used for that purpose, the Panel considered it likely that farmers would grow Bt11 maize with glufosinate herbicide applications. The Panel therefore decided that the environmental risk assessment and the post marketing environmental monitoring should also consider the direct and indirect impacts of the herbicide tolerance trait.

Insect resistance is achieved by production of a variant Cry1Ab protein from *Bacillus thuringiensis* and tolerance to the herbicide is conferred by a phosphinothricin-N-acetyltransferase (PAT) from *Streptomyces viridochromogenes*. Maize protoplasts were transformed with a DNA fragment containing two expression cassettes. As a result of the genetic modification, the Bt11 event contains an insert bearing both *cry1Ab* and *pat* genes, under the control of the 35S promoter.

Molecular analysis showed that Bt11 maize contains one copy of the DNA fragment used for transformation and that this is present at a single locus in the nuclear genome of the GM plant. The complete DNA sequence of the insert was provided. There is no evidence for the presence of partial insertions of *amp* gene sequences or non-coding vector backbone sequences. Analysis of DNA sequences flanking both ends of the insert shows that they correspond to maize genomic DNA. The insertion of the DNA fragment bearing both *cry1Ab* and *pat* genes does not disrupt any endogenous maize open reading frame. The genetic stability of the inserted DNA in event Bt11 was demonstrated and segregation data for the PAT and Cry1Ab traits were shown to follow Mendelian genetics.

Based on the results of compositional analysis, it is concluded that forage (silage) and kernels of Bt11 maize are compositionally equivalent to those of conventional maize, except for the presence of Cry1Ab and PAT proteins in Bt11 maize. No indications were found that unintended effects have occurred in Bt11 maize.

Notification C/F/96/05.10 concerns cultivation, import, processing and use as any other maize, excluding food uses. Bt11 maize is comparable with maize bred traditionally, except for the expression of tolerance to glufosinate herbicide and resistance to certain lepidopterans. Maize does not colonise and rarely survives outside the cultivated environment. It is winter-hardy only in parts of Southern Europe, and it has no cross-compatible wild relatives in Europe. Therefore, no unintended environmental effects due to the establishment and spread are anticipated. The likelihood of adverse effects on non-target organisms or on soil functions due to the expression of the *cry1Ab* gene or the *pat* gene is considered to be very low. The presence of the *pat* gene and the use of glufosinate ammonium are not likely to give an additional botanical diversity effect compared to other herbicides. The possible development of resistance of target organisms to Bt toxin has been identified as a potential risk due to large scale cultivation and/or long term exposure. Thus, an appropriate case-specific monitoring plan to record the development of resistance has been provided. In addition, the GMO Panel agrees in principle with the approach proposed by the applicant in the general surveillance plan.

From the data provided by the applicant, there was no evidence to indicate that Bt10 material was present in the Bt11 maize used for the biosafety studies. Therefore, the GMO Panel considers that the risk assessment of Bt11 maize has not been compromised by the presence of Bt10 maize.

In conclusion, the Panel considers that the information available for Bt11 maize addresses the outstanding questions raised by the Member States and considers that Bt11 maize will not have



an adverse effect on human and animal health or the environment in the context of its proposed use.

Key words: GMO, maize, *Zea mays*, Bt11, insect resistance, Cry1Ab, PAT, feed safety, human health, cultivation, environment, import, Regulation (EC) 258/97, Directive 90/220/EEC, Directive 2001/18/EC.

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BACKGROUND

The Commission received the C/F/96/05.10 notification from Syngenta, on 16 June 2003, together with a positive assessment report, from the lead Member State (France).

In accordance with Directive 2001/18/EC (EC, 2001), the notification was then transmitted to the Competent Authorities of the other Member States, a number of which have raised objections during the statutory 60-day period. The applicant provided the Member States with additional information in response to the objections raised during the 60-day period. The Member States had until 26 February 2004 to confirm or lift their objections. Where these objections are maintained, the Commission is required to consult the relevant Scientific Committees for opinion, now represented by EFSA.

Article 18(1) of Directive 2001/18/EC states that the period of time during which the Commission is awaiting the opinion of the Scientific Committee shall not exceed 90 days. The evaluation by EFSA started on 18 March 2004, after receipt of the complete background information (request from the Commission, dossier of the applicant and final objections maintained by the Member States). During the 90-day period, EFSA requested further clarification from the applicant. This procedure extended the final deadline set for the delivery of this opinion.

In delivering its opinion the Panel considered the notification, additional information provided by the applicant and the specific questions and concerns raised by the Member States.

Notification C/F/96/05.10, submitted under Directive 2001/18/EC, is for cultivation, feed use and industrial processing of Bt11 maize. Bt11 maize has been previously evaluated (SCP, 1998a) and approved (EC, 1998) for import, processing and feed use under Directive 90/220/EEC. Bt11 maize has also been evaluated for cultivation under the same Directive (SCP, 2000). Food and food ingredients derived from Bt11 maize have been authorised (EC, 1999) pursuant to Article 5 of Regulation (EC) 258/97. Bt11 sweet maize has also been evaluated (SCF, 2002) and approved (EC, 2004c) for food consumption in the framework of Regulation (EC) 258/97 (EC, 1997).

Following the information that came to EFSA on 23 March 2005 on the inadvertent release in the United States of a non-authorised GM maize line, called Bt10, and its unintended export as Bt11 for research purposes to Spain and France, the GMO Panel immediately sought information from the applicant to confirm that the risk assessment of Bt11 maize would not be compromised by the unintended presence of Bt10 maize. In response, the applicant confirmed that the material used in the safety studies is Bt11 maize. Based on internal Syngenta codes attributed to Bt maize lines that are Bt10, the applicant declared that it was able to identify what crosses/hybrids had been developed from the Bt10 breeding lines and that it was able to trace back the origin of the material used in the studies conducted to assess the safety of Bt11 maize. All the plant material

used in the safety studies listed in the application has been confirmed by the applicant to be Bt11 maize. With respect to Bt10 maize EFSA issued a statement on 12 April 2005 (EFSA, 2005c).

TERMS OF REFERENCE

EFSA was requested, under Article 29(1) and in accordance with Article 22(5)(c) of Regulation (EC) No 178/2002 (EC, 2002a), to provide a scientific opinion as to whether there is any scientific reason to believe that placing on the market of Bt11 maize for cultivation, feed and industrial processing is likely to cause any adverse effects on human health and the environment within the scope of Directive 2001/18/EC.

In particular, EFSA was requested to take account of the scientific objections raised by the Competent Authorities of Member States in this context.

EFSA was not requested to give an opinion on the non-scientific objections raised by Competent Authorities in their replies.

ASSESSMENT

1. Introduction

Bt11 maize was assessed with reference to its intended use and the appropriate principles described in the 'Guidance Document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed' (EFSA, 2004a). In its evaluation the Panel also considered the issues that were raised by Member States during the initial assessment of the application introduced under Directive 2001/18/EC, including additional information from the applicant in reply to questions from the Member States.

2. Molecular characterisation

2.1. Issues raised by Member States

(1) Questions related to the experimental approach used to search for genome wide insertion of DNA fragments and the molecular organization of the detected fragments in the genome were raised; (2) Concern was raised about possible differences between sequences in the plasmid used for transformation and those integrated into the transgenic plant.

The GMO Panel considered these issues and also requested additional information from the applicant on possible unintended secondary insertions of DNA fragments before an evaluation was finalised.

2.2. Evaluation of relevant scientific data

2.2.1. Transformation process and vector constructs

Bt11 maize was generated by transformation of *Zea mays* protoplasts according to the protocol described by Negrutiu *et al.* (1987). The protoplasts were transformed with the larger DNA fragment obtained by a restriction digest of the plasmid pZ01502 with the enzyme *Not* I. The *Not* I fragment was not expected to contain plasmid backbone DNA sequences. Regenerated plants were backcrossed to a selected line resulting in a plant which is called Bt11.

The DNA fragment used for transformation carried two expression cassettes: a selectable marker gene *pat*, encoding the PAT protein (phosphinothricin-N-acetyl transferase) and a variant *Bacillus thuringiensis cry1Ab* gene encoding a Cry1Ab endotoxin. Expression of both, the *cry1Ab* gene and the *pat* gene is controlled by the 35S promoter from the cauliflower mosaic virus (CaMV) (Gardner *et al.*, 1981), supplemented with the intron sequence II or VI from the alcohol dehydrogenase 1S (*adh*) gene from maize (Freeling and Bennet, 1985) to enhance gene expression in maize (Mascarenhas *et al.*, 1990). The polyadenylation signals are derived from the nopaline synthase (*nos*) gene from *Agrobacterium* (Bevan *et al.*, 1983).

2.2.2. Transgenic constructs in the genetically modified plant

Bt11 maize was subjected to molecular analysis in order to determine the number of integration sites within the maize genome and the copy number (the number of copies of the DNA fragment used for transformation that were inserted in the GM plant), the integrity of the inserted cassettes and the absence of backbone sequences.

Southern blot analyses were undertaken using a variety of DNA probes including the *pat* and *cry1Ab* genes, *amp* sequence and the entire plasmid to search for unintended insertions in the maize genome. The data demonstrated that the Bt11 event contains a single DNA insertion with one copy of both the *cry1Ab* and the *pat* cassettes. The insertion of small non-coding sequences from the vector backbone sequence in the locus of insertion can be excluded on the basis of the sequence data and a bioinformatics study confirming the homology of the flanking sequences with maize genome. Southern blots provided no evidence for the presence of partial insertions of *amp* gene sequences or non-coding vector backbone sequences.

PCR analysis and DNA sequencing were used to establish a detailed transgene locus structure and to verify the 5' and 3' junction sequences of the insert with the plant genome. This demonstrated the intactness of the 5' and 3' ends of the inserted cassettes. The sequence of the inserted fragment was obtained by sequencing PCR fragments amplified directly from the Bt11 genomic DNA. The resulting sequence was identical to the sequence of the corresponding fragment in the plasmid. Therefore it can be concluded that no rearrangements occurred within the *Not I* fragment during gene transfer and integration into the plant genome. Sequence information demonstrates that no vector backbone fragments, including *amp* sequences, are inserted and fused to the inserted *Not I* fragment.

These molecular analyses of the transgenic DNA present in maize event Bt11 indicate that only the two expected full-length proteins, Cry1Ab and PAT, will be expressed by the insert.

DNA sequences at the junctions between the insert and the parent genome were determined. At the 5' flank, approximately 350 bp of the plant DNA adjacent to the insert was sequenced. At the 3' flank, approximately 540 bp of the plant DNA adjacent to the insert was sequenced. The 5' and 3' flanking sequences were screened for homologies with sequences found in public databases. BLASTN analysis of both the 5' and 3' regions of the Bt11 insert revealed homology primarily to the *Zea mays* 180 bp knob-associated tandem repeat. Knobs are components of the maize heterochromatin, a class of chromatin known not to be expressed (Alberts *et al.*, 1994). The 180 bp tandemly repeated sequences have been characterized by Peacock *et al.* (1981) and Dennis and Peacock (1984). Therefore it can be concluded that the insertion of the *Not I* fragment in the maize genome does not disrupt any endogenous maize open reading frame.

2.2.3. Information on the expression of the insert

Expression analysis of the Cry1Ab and the PAT proteins were carried out by ELISA using leaf, pollen, silk, stalk, root, whole plant and grain. The Cry1Ab protein was found in all tissues examined, with a decrease in concentration at the time of plant maturation and senescence. The concentration of Cry1Ab protein is similar in leaf, husk and stalk (20 to 168 ng Cry1Ab

protein/mg total protein). The concentration is significantly lower in kernels (0.4 to 8.2 ng Cry1Ab protein/mg total protein).

With regard to PAT protein measurable levels were only found in leaves, silk and tassel. For kernels, pollen, root and stalk concentrations were below the limits of detection.

2.2.4. Inheritance and stability of inherited DNA

The genetic stability of the inserted DNA in event Bt11 was demonstrated by both a classical approach and a molecular approach using Southern blot analysis on genomic DNA. The Bt11 event was subjected to a backcrossing program with the elite line H8540. The lines BC3 and BC6, developed as part of this program, were used to evaluate genetic stability. BC3 was developed from 3 backcrosses with H8540 and BC6 from 6 backcrosses. No differences in banding pattern were observed between the DNA from these generations demonstrating the stability of the inserted DNA.

Segregation data for glufosinate ammonium tolerance and European corn borer resistance were collected at different points in the backcrossing experiment. BC3 and BC6 plants, identified as containing the *cry1Ab* and *pat* genes, were subjected to selfing experiments. This demonstrated the heritability and the stability of the two genes in the event Bt11. Data support the presence of a single insertion that segregates according to Mendelian laws of genetics.

The data provided by the applicant did not raise any concerns about instabilities of the insert in Bt11 maize. Minor differences between sequences both in the plasmid used for transformation and in the plant insert fall within the experimental errors of the sequencing experiments.

2.3. Conclusion

Bt11 maize was generated by transformation of *Zea mays* protoplasts, which were transformed with the larger DNA fragment obtained by a restriction digest of the plasmid pZO1502 with the enzyme *Not* I. Regenerated plants were backcrossed to a selected line resulting in a plant which is called Bt11. Detailed molecular analysis demonstrated that only the two expected full length proteins, Cry1Ab and PAT, would be encoded by the insert. DNA sequences at the junctions between the insert and parent genomes were determined and bioinformatic analysis was carried out. The insert was shown to occur in a section of the maize genome known not to be expressed. The genetic stability of the inserted DNA in event Bt11 was demonstrated by Southern blot analysis and segregation data for the PAT and Cry1Ab traits were shown to follow Mendelian genetics.

3. Comparative analysis

3.1. Issues raised by Member States

Possible higher contents of lignin in Bt11 maize were addressed.

3.2. Evaluation of relevant scientific data

3.2.1. Choice of the comparator and production of material for the compositional assessment

Studies compared the composition of maize kernels obtained from experimental plants of Bt11 maize with isogenic non-transgenic comparators. Field trials were conducted in the EU and the US. The analysis of kernels from maize plants grown in European greenhouses included moisture, nitrogen, ash, starch, cellulose, xanthophyll, fatty acids and amino acids. Kernels from

maize plants grown during parallel US field studies in 1995, each involving 3-6 sites, were analysed for protein, oil, starch, fibre, fatty acid- and amino acid- profiles, as well as copper, magnesium, manganese, zinc, folic acid, niacin, and vitamins B₁ and B₂. Data for the kernels harvested from field trials at two locations in France in 1998 included carbohydrate, protein, fat, fibre, fatty acid- and amino acid- composition, as well as trypsin inhibitor and phytic acid. Occasionally statistically significant differences between Bt11 and non-transgenic maize kernels were observed. These differences were not consistent between studies, seasons, and transgenic lines.

In addition, data on silage composition were available from feeding study reports. Experimentally produced silage from Bt11 maize and non-transgenic controls, which were grown in the US in 1998 and used for a feeding study with beef- and dairy- cattle, was analysed for composition, including dry matter, nitrogen (protein and non-protein), ash, fibre, starch, and lignin (Folmer *et al.*, 2002).

3.2.2. Compositional analysis

Bt11 forage samples and non-GM control cultivated at several locations in the USA (1995) were analysed for proximates (dry matter, crude protein, available crude protein, acid detergent fibre, neutral detergent fibre, total digestible nutrients), and four minerals (calcium, phosphorous, potassium, magnesium).

Silage from maize grown at one location in the USA (1996) and prepared for feeding studies with Bt11, Bt 176, and non-transgenic control was analysed for proximates, *i.e.* moisture, protein, acid detergent fibre, neutral detergent fibre (NDF), total digestible nutrients, 12 minerals (calcium, phosphorous, potassium, magnesium, sulphur, sodium, zinc, manganese, copper, iron, cobalt, aluminium), and energy (metabolisable energy, net energy lactation, net energy growth, net energy maintenance).

Several small increases were observed for lignin content and *in vitro* NDF digestibility, the magnitude of which is not considered to be relevant. It has been suggested that lignin levels might be increased in transgenic maize lines expressing *B. thuringiensis* insecticidal proteins (Saxena and Stotzky, 2001a; Flores *et al.*, 2005). However, a broader and more extensive study on lignin content in Bt maize does not support this conclusion (Jung and Sheaffer, 2004).

Aside from minor modifications, the selection of compounds analysed was similar to those now recommended by OECD (OECD, 2002).

3.2.3. Agronomic traits and GM phenotype

During a field trial in 1995 in France, agronomic data (anthocyanin coloration at the level of the ear, tassel, leaf, internodes and glomes; plant and tassel length, grain type, resistance to pests and diseases, number of primary lateral branches, height of insertion of ears, length of pedunculi, shape/length of ears, number of rows of grain) were collected and confirmed the equivalence of Bt11 maize phenotype to its non-transgenic counterpart.

Furthermore, no differences in the agronomic and phenotypic characteristics were found between the Bt11 maize and the non-transgenic counterpart during field trials at different locations (Spain, France, Italy and Portugal) conducted between 1994 and 2003 that would indicate unexpected pleiotropic effects of the genetic modification.

3.3. Conclusion

Based on the results of compositional analysis, it is concluded that forage (silage) and kernels of Bt11 maize are compositionally equivalent to those of conventional maize, except for the presence of Cry1Ab and PAT proteins in Bt11 maize.

In addition, experimental field trials in Europe did not show indications for unexpected changes of agronomic characteristics and performance.

4. Feed safety assessment

4.1. Issues raised by Member States

(1) Further data on toxicity and allergenicity obtained with the whole GMO and not only with the isolated newly expressed proteins were requested; (2) it was noted that the feeding/toxicology studies on poultry and cows were short term studies and essentially referred to detection of possible residues of the newly expressed proteins in animal products; (3) the whole design and presentation of toxicity and allergenicity studies was criticized by one Member State.

4.2. Evaluation of relevant scientific data

4.2.1. Product description and intended use

This evaluation of Bt11 maize, in the frame of Directive 2001/18/EC, is for cultivation, feed use and industrial processing. In this application, Bt11 maize is not intended for food use.

Maize kernels are a rich source of carbohydrate, while starch production produces by-products, such as maize gluten and maize gluten feed, which are used as animal feed.

Maize kernel products are used in various animal feeds, including cattle, swine, poultry, and in fish feed.

As the modification in Bt11 maize is only intended to improve the agronomic performance but not to influence nutritional aspects, production processes and overall use of maize as a crop are not expected to be influenced as a result of the introduction of the GM plants to the market.

4.2.2. Stability during processing

Based on the data of the compositional analysis of the raw agricultural commodities of Bt11 maize and the non-GM maize, the Panel is of the opinion that there are no reasons to assume that the stability of the processed products derived from Bt11 maize would be different from the non-GM processed products.

4.2.3. Toxicology

4.2.3.1. Cry1Ab and PAT proteins used for safety assessment

(a) Cry1Ab protein

Cry1Ab protein is detectable in all plant tissues. The highest Cry1Ab concentration is found in the leaf tissue, especially at the younger stages of tissue development. The measured concentration of Cry1Ab protein in grain and leaves is respectively 1.4 and 3.26 µg Cry1Ab protein/g fresh weight.

Cry1Ab test protein was produced in recombinant *E. coli* strains and was shown to be equivalent to that expressed in the plant using SDS-PAGE analysis, immunoblot analysis, trypsin digestion, N-terminal sequencing, amino acid composition analysis, glycosylation analysis and insect bioassays.

(b) PAT protein

In addition to Cry1Ab, Bt11 maize also expresses the phosphinothricin-N-acetyl transferase enzyme (PAT) which is responsible for the degradation of phosphinothricin in plants tolerant to the action of the herbicide BASTA®. The PAT protein is present at less than 0.000008% fresh weight and 0.00016% of the total maize grain protein.

PAT expression is low in Bt11 maize plants and it was not possible to extract it in sufficient quantities to be used in model digestion system or in safety testing. The PAT protein was therefore derived from expression of the recombinant protein in *E. coli*. The dossier does not provide experimental evidences for the equivalence of recombinant PAT from Bt11 maize and bacteria although it is stated by the applicant that they are identical. The applicant refers to previous studies conducted on the first Bt maize developed by Novartis although it expressed a PAT encoded by the bar gene and not by the *pat* gene as in Bt11. However the two proteins were considered structurally and functionally equivalent based on their molecular weight, their rapid degradation and loss of enzymatic activity during *in vitro* digestion with digestive stomach fluids from various species, their immuno cross reactivity and their characteristics in terms of enzymatic activity. It also provides similar studies conducted by Hoechst/Agrevo in 1993 on PAT protein of transgenic oilseed rape plants.

4.2.3.2. Toxicological assessment of expressed novel proteins in Bt11 maize

(a) Cry1Ab protein

Safety assessment of Cry1Ab is dealt with in several reports. The applicant refers to acute oral toxicity on mice, to *in vitro* digestibility studies and search for homology with known toxins. It essentially refers to the published literature, to the outcome of an EU funded project (*i.e.* Safety assessment of the Bt insecticidal crystal protein Cry1Ab expressed in transgenic tomatoes, Noteborn, 1994) and to studies on the safety of Cry1Ab which were performed previously for the development of another Bt maize (Bt176). Cry1Ab expressed in Bt11 maize is 33 amino acid residues shorter than the one expressed in the Bt176 maize. Based on structural data obtained for similar Cry proteins (Grochulski *et al.*, 1995; Li *et al.*, 1991), the Panel considers that this truncation does not raise particular issues regarding the applicability of those studies to the assessment of Cry1Ab expressed in Bt11 maize.

The Panel also refers to the safety assessment of Cry1Ab made in previous applications already approved such as MON 810 (SCP, 1998b).

Short term feeding/toxicity studies on poultry, pigs, calves and cattle also provided additional information on the behavior of Cry1Ab protein in the gastro intestinal tract (Jennings *et al.*, 2003; Chowdhury *et al.*, 2003; Einspanier *et al.*, 2004; Lutz *et al.*, 2005). Cry1Ab was not completely degraded in the gastro-intestinal tract and fragments of the gene and/or immunoreactive protein fragments were still present in the intestinal content and in the faeces, but no residual DNA/protein could be found in animal tissues nor in the peripheral blood. No recent scientific information has become available which could trigger any new concern on Cry1Ab toxicity and require additional testing.

An evaluation of exposure has been attempted from the information on the Cry1Ab levels in Bt11 hybrids used in the feeding studies although they were very variable depending on the plant material used to feed the animals, *i.e.* freshly chopped plant, silage or stalks.

A 14-day lactating dairy cow study performed in 1996 reported a concentration of ca. 0.6 ppm (i.e. 0.6 mg Cry1Ab / kg fresh weight) in freshly chopped plants at one sampling time point. Assuming a daily intake of 30 kg fresh plant (corresponding to 23 kg dry matter), the total daily intake would then be ca. 15 mg Cry1Ab for a cow fed with Bt11 maize.

(b) PAT protein

A repeated dose 14-day oral toxicity study has been conducted in rats (5 male and 5 female per group) which received a diet containing 0, 5,000 or 50,000 ppm PAT protein (i. e. average intake of 0, 712 and 7,619 mg/kg/day in males and of 0, 703 and 7,965 mg/kg/day in females). The study was made according to the OECD guidelines. No mortalities occurred and no clinical signs were observed in any of the groups. Food consumption and body weight were not influenced by the treatment. Organ weights, gross pathology and histopathology findings did not indicate differences between control and treated groups. No changes were found in haematology or urine analysis. Only in clinical biochemistry the following effects were observed: slightly higher cholesterol and phospholipid level in treated groups (particularly in males). These findings were considered to reflect an increased load for metabolic function but were not considered as being toxicologically significant.

Search for sequence homology showed no homologies with known toxins.

4.2.3.3. Toxicological assessment of new constituents other than proteins

Since no new constituents other than the above mentioned proteins were expressed in Bt11 maize, nor were levels of endogenous compounds altered, a toxicological assessment of such compounds is not applicable.

4.2.4. Toxicological assessment of the whole GM feed

The results of the compositional analysis, the molecular characterisation, and of the phenotypic analysis did not reveal unintended differences between Bt11 maize and non-transgenic maize. Further, animal testing data from short term feeding studies (see 4.2.6.), that were available for the assessment, did not show any adverse effects and confirmed the equivalence of Bt11 maize when compared to non-transgenic maize. Therefore the GMO Panel is of the opinion that no additional subchronic toxicity studies are necessary.

4.2.5. Allergenicity

The strategies in assessing the allergenic risk concentrate on characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons and whether the transformation may have altered the allergenic properties of the modified food. A weight of evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (EFSA, 2004a; CAC, 2003).

4.2.5.1. Assessment of allergenicity of the newly expressed proteins

(a) Cry1Ab protein

An allergy risk evaluation of Cry1Ab protein has been completed using different approaches, which led to indirect evidence for an allergenicity risk being very low. This included the absence of known allergenicity of the source, absence of sequence homology with known allergens and rapid and extensive degradation by pepsin (Metcalf *et al.*, 1996; EC, 2003; CAC, 2003). Previous applications of Cry1Ab using the same strategy were evaluated and approved by the national

competent authorities and the EC Scientific Committees (SCF, 2002; SCP, 1998a, 1998b; SCP, 2000). The Panel is not aware of any new information on allergenicity, which requires a change of this opinion. Also the Panel is not aware of any new validated tests which produce more relevant or accurate information on possible allergenicity of the protein and which provide a higher guarantee of safety.

One Member State referred to an article by Stickler *et al.* (2003) which would indicate that Cry1Ab could be an allergen. Actually this paper describes a methodology to identify immunodominant T cell epitopes on proteins that could be potential allergens. Bt proteins were used as models and the paper did not conclude that Cry1Ab is allergenic.

(b) PAT protein

The PAT protein has previously been evaluated for its safety in the frame of other applications for the placing of PAT expressing GM crops on the market (EFSA, 2004b; 2005a; 2005b).

Search for sequence homology showed no homology with known allergens; *in vitro* digestibility studies showed rapid degradation with loss of biological activity, essentially by action of pepsin.

In addition PAT protein was also shown not to be stable under processing conditions and not to be glycosylated.

Based on all information made available, the Panel considers that the newly expressed proteins are not likely to be allergenic.

4.2.5.2. Assessment of allergenicity of the whole GM crop

Allergenicity of the whole crop could be increased as an unintended effect of the random insertion of the transgene in the genome of the recipient, for example through qualitative or quantitative modifications of the expression pattern of endogenous proteins. This issue does not appear relevant to the Panel since maize is not considered a major allergenic food and possible over-expression of any endogenous protein that is not known to be allergenic would be unlikely to alter the overall allergenicity of the whole plant. The same considerations also apply for exposure by inhalation.

In addition it must be stressed that the present application is for feed only and allergy is not a relevant issue for animal health.

4.2.6. Nutritional assessment of GM feed

The data considered by the Panel on nutritional studies in domestic animals concerned two studies with laying hens and dairy cattle that had been provided with the dossier, as well as two further articles on broilers and dairy- and beef-cattle published in scientific literature.

(a) 14-day feeding study on laying hens

Laying hens with high egg production were fed diets containing Bt11 maize or non-transgenic counterpart during 14 days. No effect of the diet was observed upon survivability, health, egg production or egg weights. In addition, no residue of Cry1Ab and PAT protein was detected in eggs nor in animal tissues.

(b) Evaluation of Bt11 maize in broiler chickens (42-day feeding study)

Rapidly growing broilers have been used for nutritional testing of Bt11 maize (Brake *et al.*, 2003). The animals received one of four diets containing kernels derived from Bt11 maize (treated and

non-treated with glufosinate ammonium), a non-transgenic control line, and a commercial reference line. The amino acid balance for the four lots of maize was similar relative to their crude protein content; however, the conventional maize had higher protein content. Diets were amended so that the metabolisable energy and crude protein content were similar.

Growth, mortality, feed conversion ratio and carcass yield at 48 days were similar in the chickens fed with the different diets containing Bt11 maize and were sometimes better than in the chicken fed with the control diet with conventional maize. In conclusion, no consistent effects of the intake of Bt11 maize on the performance of chicken broilers have been observed

(c) 14-day feeding study in high producing dairy cows

Three groups of 4 dairy cows were fed fresh chopped whole plant maize (ca. 22.7 kg of dry matter per animal and per day) of either Bt11 maize, another insect tolerant transgenic maize (*i.e.* event 176) and the non-transgenic, near isogenic counterpart of event 176. Both Bt11 and event 176 have been modified with the Cry1Ab and PAT proteins. Event 176 derived from plants contained intermediate levels, and plants from the Bt11 variety contained relatively high levels of Cry1Ab protein.

While the aim of this study was to determine whether transfer of Cry1Ab and PAT to milk from cows fed transgenic maize would occur, it also provides information on animal performance.

Milk production, feed intake, milk composition, and udder health were similar for all study groups. Cry1Ab and PAT proteins could not be detected in milk of cows fed the genetically modified maize lines.

Utilization of Bt maize residues by grazing beef steers and Bt maize silage and grain by growing beef cattle and lactating dairy cows has been reported by Folmer *et al.* (2002).

Sixteen lactating dairy cows received diets containing silage of an early- and late-maturing variety of Bt11 maize or a control with the corresponding non-transgenic near isogenic maize line during 21-day feeding periods. No differences were observed between Bt11 and control maize for feed intake, body weight, milk production, and milk composition (lactose, protein, fat), as well as ruminal pH and volatile fatty acids. In addition no effects either were observed of the transgenic trait on *in situ* ruminal digestion of neutral detergent fibre of maize.

The same silages as those used for the dairy cow study were used in a beef cattle study which lasted for 101 days. Measurements included feed intake and body weight. Dry matter intake was significantly higher in steers fed early- and late-maturing Bt11 maize when compared with those fed diets containing non-GM silage. In addition, average daily weight gain in early maturing Bt11 maize-fed steers was higher than in control-fed steers, while final body weight and feed efficiency was decreased in steers fed late maturing Bt11 maize compared with steers fed control maize. In conclusion, the slightly higher dry matter intake was not associated with other effects on performance of beef cattle fed Bt11 maize that would be consistent for diets of both Bt11 maize lines.

In conclusion, results showed no significant differences between dietary treatments and indicate nutritional equivalence between the transgenic Bt11 maize and the non-GM control.

4.2.7. Post-market monitoring of GM feed

The composition of Bt11 maize and its nutritional value have not been altered by the genetic modification. Therefore the GM plants will be used as any other maize and only replace a part of the overall maize products within the European market. The risk assessment concluded that no data have emerged to indicate that Bt11 maize is any less safe than its non-GM comparators. The

opinion of the applicant that a post-market monitoring of the GM feed is not necessary is in line with the Guidance Document of the GMO Panel for the risk assessment of genetically modified plants and derived food and feed and is shared by the GMO Panel.

4.3. Conclusion

The Panel considers that, given the background of experience and knowledge already accumulated on the newly expressed proteins in Bt11 maize, there is no evidence for particular concerns for their safety when Bt11 maize is cultivated and/or used for feed purposes. Based on the results of the compositional analysis and of the molecular characterisation, the GMO Panel is of the opinion that no additional subchronic toxicity studies are necessary. Furthermore, results showed no significant differences between dietary treatments and indicate nutritional equivalence between the transgenic Bt11 maize and the non-GM control. From the studies conducted on Bt11 maize there is no indication of the occurrence of unintended effects.

The safety of residues of glufosinate applied to Bt11 maize and of any metabolites has to be evaluated for market approval under Directive 91/414/EEC (EC, 1991), and is therefore not within the remit of this opinion. The Panel is aware that glufosinate containing herbicides have been evaluated (EFSA, 2005d) within the framework of Directive 91/414/EEC.

5. Environmental risk assessment

5.1. Issues raised by the Member States

(1) The scope of the application has not to be limited to insect resistance but has to be considered as both insect resistance and herbicide tolerance; (2) direct and indirect effects of the Cry1Ab toxin on non-target organisms, specifically soil biota, arthropods, butterflies, and other invertebrates, should be addressed; (3) further data on the effects and persistence of Bt toxin in soil is requested; (4) more information on the general surveillance and monitoring of non-target effects is needed; (5) a considerable modification of the case-specific monitoring plan to take into account the additional requirements for the environmental risk assessment is requested; (6) concerns about potential harm to endangered Lepidopteran species are expressed and the possible need to protect endangered butterfly species is emphasised; (7) it is recommended that potentially altered lignin contents and the biodegradability of plant litter as well as possible long-term persistence of the Cry1Ab protein should be considered.

The GMO Panel considered these issues and also requested the applicant to provide a full environmental risk assessment of the *pat* gene in connection with the possible use of the complementary herbicide consistent with the requirements of Directive 2001/18/EC before an evaluation was finalised.

5.2. Evaluation of relevant scientific data

5.2.1. Potential unintended effects on plant fitness due to the genetic modification

Maize is highly domesticated and not generally able to survive in the environment without cultivation. Maize plants are not winter hardy in many parts of Europe. They have lost their ability to release seeds from the cob and they do not occur outside cultivated or disturbed land in Europe, despite cultivation for many years. In addition, there are no cross-compatible wild relatives in Europe, and gene flow via pollen is largely restricted to neighbouring crops.

Bt11 maize has no altered survival, multiplication or dissemination characteristics except in the presence of glufosinate ammonium. The Panel is of the opinion that the likelihood of unintended

environmental effects due to the establishment and spread of Bt11 maize will be no different to that of traditionally bred maize.

5.2.2. Potential for gene transfer

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, DNA in case of horizontal gene transfer and pollen in case of vertical gene flow through cross-pollination.

Exposure of microorganisms to transgenic DNA derived from GM maize plants takes place in the environment during natural decay of transgenic plant material, such as GM plant parts, in agricultural areas and/or pollen in nearby natural ecosystems as well as in cropped fields.

Transgenic DNA is a component of some or most of the food and feed products derived from the GM maize. Therefore microorganisms in the digestive tract of humans and animals (domesticated animals and other animals feeding on fresh and decaying GM plant material) may be exposed to transgenic DNA.

Transgenic pollen is shed and distributed from cultivated GM hybrids or from plants resulting from the adventitious presence of GM kernels in conventionally bred maize seeds. A further but less likely pathway of dispersal of transgenic maize pollen is the flowering of volunteer GM maize plants originating from accidental seed spillage during transport and/or processing. For *Zea mays* any vertical gene transfer is limited to other maize plants as populations of sexually compatible wild relatives of maize are not known in Europe.

(a) Plant to bacteria gene transfer

Based on present scientific knowledge and elaborated recently in more detail (EFSA, 2004c), gene transfer from GM plants to bacteria under natural conditions is extremely unlikely, and would occur primarily through homologous recombination in microbes.

The *cry1Ab* gene and the *pat* gene expressed in the Bt11 maize are under the control of eukaryotic promoters with limited if any activity in prokaryotic organisms. Genes under control of prokaryotic regulatory elements conferring the same traits as expressed in the GM plants are widespread in microorganisms in natural environments.

Taking into account the origin and nature of these genes and the lack of selective pressure in the intestinal tract and/or the environment, the likelihood that horizontal gene transfer would confer selective advantages or increased fitness on microorganisms is very limited. For this reason it is very unlikely that genes from Bt11 maize would become established in the genome of microorganisms in the environment or human and animal digestive tract. In the very unlikely event that such a horizontal gene transfer would take place, no adverse effects on human and animal health and the environment are expected as no principally new traits would be introduced into microbial communities.

(b) Plant to plant gene transfer

The extent of cross-pollination to conventionally bred hybrids will mainly depend on the scale of accidental release and/or adventitious presence in conventional seeds.

As shown in several field trials there are no indications for an altered ecological fitness of the GM maize in comparison to conventionally bred hybrids with similar genetic background.

The herbicide tolerance trait can only be regarded as providing a selective advantage where and when glufosinate-ammonium containing herbicides are applied, *i.e.* mainly on arable land. Insect protection against lepidopteran pests is also not regarded as providing a selective advantage for maize in Europe, as the survivability is mainly limited by the absence of a dormancy phase, susceptibility to fungi and susceptibility to cold climate conditions. Therefore, as for any other maize cultivars, it is considered very unlikely that volunteers could survive until subsequent seasons or would establish undesirable populations under European environmental conditions.

5.2.3. Interactions between the GM plant and target organisms

According to the statement made by the Scientific Committee on Plants (SCP, 1999) and in line with Annex II of Directive 2001/18/EC the Panel considers that the evolution of resistance in target pests is an environmental and agronomic concern. Up to now, resistant *Ostrinia nubilalis* or *Sesamia nonagrioides* have not been found in fields in the US or in Europe (Evans, 2002; Tabashnik *et al.*, 2003; Bourguet *et al.*, 2003; Farinós *et al.*, 2004). Although laboratory tests showed that corn borer populations are capable of developing some degree of tolerance to the Cry1Ab protein (Huang *et al.*, 2002), laboratory selection and F2 screening to generate highly resistant *O. nubilalis* strains have failed so far (Bourguet, 2004). However, another lepidopteran pest (*Plutella xylostella*) has developed resistance to Bt toxins (Tabashnik *et al.*, 2003). The Panel concludes that large scale cultivation of Bt11 maize over several years will increase the selection pressure on corn borers, which might result in the development of resistance. This could have several consequences including the use of alternative phytosanitary measures to control the pest including involving the use of insecticides other than Bt toxins. The Panel agrees that the likelihood of occurrence is low since, under field conditions and several years of cultivation, no resistance has been reported. However, cultivation of Bt maize in Europe is currently on a small scale and limited to a few geographic regions. Thus it is difficult to predict future responses of corn borer populations in Europe. Therefore, the Panel advises that potential target pest resistance development should be monitored under case-specific monitoring.

5.2.4. Interactions of the GM plant with non-target organisms

(a) Effects on predators and parasitoids of the target organisms

The abundance of non-target predators preying upon the target organisms *Ostrinia* or *Sesamia* will vary with the abundance of their prey. Thus, a reduction in prey either by cultivation of Bt maize or by insecticides may negatively effect the food source of predators like *Chrysoperla carnea* (Hilbeck *et al.* 1998a,b). However, current knowledge on toxicity and exposure give sufficient scientific evidence that Bt maize poses no risk to this predator (Dutton *et al.* 2003a, b; Romeis *et al.* 2004). Most field studies confirm that predator and parasitoid abundances and biocontrol functions are very similar in Bt and non-Bt fields (Candolfi *et al.*, 2004; Pons and Stary, 2003; Musser and Shelton, 2003). Reductions of population densities of specialist *Ostrinia* predators and parasitoids are expected as this pest is the target to be controlled in Bt maize fields. Bourguet *et al.* (2002) and Siegfried *et al.* (2001) have found that populations of specific natural enemies of *Ostrinia* are less abundant in Bt maize fields than in non-Bt maize fields. This is not thought to be due to the direct effects of the Cry toxin consumed while predating or parasitizing *Ostrinia* but is due to decreased availability of specific prey. Results of field studies comparing the effects of Bt maize with insecticide treatments against the target pest show that broad-spectrum insecticides, like pyrethroids, reduce abundances of a range of predator and parasitoid species not specific to *Ostrinia*. Such effects have not been reported in Bt maize.

(b) Effects on other non-target organisms

It is well documented that a range of lepidopteran species may be affected by Bt toxins and some may be present in maize fields (Schmitz *et al.*, 2003; for a review see Evans, 2002). However,

exposure of any populations of lepidoptera to the toxin is restricted to those consuming the Bt plant or its products. In the vicinity of the Bt maize field larvae may be most exposed to the toxin when Bt maize pollen is deposited on plants on which they are feeding. Maize, a recently introduced species into Europe, is not a significant food source for endemic lepidoptera and impacts due to pollen dispersal are likely to be transient and minor as demonstrated by studies on monarch butterflies in the USA (Dively *et al.*, 2004). Published studies investigating potential effects of GM plants due to the expression of Bt toxins have been mainly performed with maize Bt11 and Bt176, both producing Cry1Ab.

Three year experimental studies of Bt maize (Bt176 expressing Cry1Ab) in Spain did not show effects on mortality, developmental and pre-reproductive times, fecundity, and intrinsic rate of population increase comparing the offspring of apterous aphids maintained on Bt or non-Bt maize for several generations (Lumbierres *et al.*, 2004), which is in line with the absence of Bt toxin in the phloem (Raps *et al.*, 2001).

Direct and indirect effects of GM plants in general on animals higher in the food chain including both invertebrates and vertebrates (birds, mammals) have been discussed in some publications (Kjellson and Strandberg, 2001; Firbank *et al.*, 2003). No indications of intoxication have been reported or are indicated from first and second tier exposure studies or from feeding studies with diets containing Bt toxin. It should also be considered that under field conditions most animals higher in the food chain would be eating diets consisting of a range of food sources.

No evidence of accumulation of Bt toxins in the food chain has been reported and is not expected as the toxin is an easily degradable protein. In most situations the toxin appears to be degraded through passage of the gut, although detectable amounts of the Bt toxin can still be found in faeces and therefore pass into the environment. In cattle, the influence of Cry1Ab transgenic maize on rumen bacterial microflora was investigated compared with isogenic material through analysis of 497 individual bacterial 16S rDNA sequences. In principle, specific bacterial species could be identified in all bovine rumen extracts, but no significant influence of Bt maize feed (Bt176) was found on the composition of the microbial population (Einspanier *et al.*, 2004). It therefore appears that the environmental impact of Bt toxin through manure is negligible, as only very small amounts of the toxin are expected to be excreted to the environment through manure and significant long-lasting changes in the composition of microbial communities of the manure seem unlikely.

Reduction of prey/feed abundance can be a consequence of many types of crop management practices. The Panel has no reason to consider that Bt11 maize will cause changes to non-target species that differ significantly from those caused by conventional farming.

5.2.5. Potential interaction with the abiotic environment and potential effects on biogeochemical processes

As a consequence of the cultivation of Bt maize the respective Bt toxins will be incorporated into the soil (root exudates, Bt toxin containing plant material like plant litter and pollen). Some scientific publications indicate that this might affect soil organisms. Assumptions were raised that the Bt toxin may persist and accumulate in soil during cultivation of Bt maize in subsequent years. Therefore, both direct and indirect impacts of the toxin or the Bt maize (e.g. potential increase of lignin content in combination with a possible delay in decomposition) on non-target organisms and soil function should be considered (Saxena *et al.*, 2002; Zwahlen *et al.*, 2003a). There was a concern that Bt maize might negatively affect species other than lepidoptera and consequently biodiversity. The suggested species range comprises soil and plant associated insects in food chains including those involved in plant decomposition.

Cry proteins are rapidly decomposed in soil (Glare and O'Callaghan, 2000). Saxena and Stotzky (2001b) reported Cry1Ab had no apparent effect on earthworms and nematodes in a 45-

days study. Zwahlen *et al.* (2003b) reported a 200-day study investigating the impact of Bt11 maize on immature and adult *Lumbricus terrestris* in a single worst-case laboratory study and in a single small scale field test. At the end of the laboratory test the earthworms showed a significant weight loss of 18% (compared with their initial weight) when fed (Bt+) maize litter whereas a weight gain of 4% occurred with non-GM control maize. No difference was found in the higher tier small scale field test. Due to the experimental design, the authors stated that they were unable to exclude the possibility that the weight loss of earthworms fed with Bt maize in the laboratory test was due to other factors.

The effects of Bt11 maize on soil microbial community structure were assessed in growth chamber experiments using three soil types with different textures (Blackwood and Buyer, 2004). Very few significant effects on soil microbial communities due to the presence of the Bt toxin were found, whereas the soil type significantly influenced the composition of the soil microflora. Similarly, other studies on transgenic plants expressing Bt toxins did not reveal any negative, long-lasting impact on the soil or plant-associated microorganisms (Flores *et al.*, 2005; Devare *et al.*, 2004; Donegan *et al.*, 1995). Brusetti *et al.* (2004) presented data obtained from short-term and greenhouse studies, which showed that root exudates from Bt176 maize could determine the selection of different bacterial communities from those of non-Bt maize. Turrini *et al.* (2004) reported that root exudates of Bt176 maize significantly reduced hyphal growth of arbuscular mycorrhizal fungi, a group of organisms that is fundamental for soil fertility and plant nutrition. In the same study, Bt11 maize did not affect the plant-mycorrhiza symbiosis (Turrini *et al.*, 2004). Koskella and Stotzky (2002) reported that Bt proteins showed no toxicity to bacteria, fungi and algae.

For Bt11 maize, it has been suggested that biodegradation and mineralisation of plant litter was delayed by higher lignin content (Zwahlen *et al.*, 2003a; Saxena and Stotzky, 2001a). Zwahlen *et al.* (2003a) published the results of two field studies in the temperate maize-growing region of Switzerland investigating the degradation of Cry1Ab toxin in Bt maize leaves during autumn, winter and spring periods. Each of the two field trials (in 1999/2000 and 2000/2001) covered a period of 200 days. The results suggest that the Cry1Ab protein is not completely degraded within the period tested. The authors discuss their findings in the light of potential differences in lignification (Saxena and Stotzky, 2001a), although lignin content was not determined. A more comprehensive study suggests that the extent of lignification of Bt transgenic maize (several lines derived from MON 810 and Bt11) does not differ from the non-transgenic controls (Jung and Sheaffer, 2004). Compositional analysis performed on Bt11 maize silage by Folmer *et al.* (2002) revealed small increases for lignin content which are within the ranges of conventional maize varieties.

A four-year study on the decay of transgenic maize Bt toxin (event Bt176) was published (Hopkins and Gregorich, 2003). The authors followed the rate at which the toxin in Bt maize leaves decomposed in soil from a field in which Bt maize had been cultivated for four years. The results suggested that much of the Bt toxin in crop residues is highly labile and quickly decomposes in soil, but that a small fraction may be protected from decay in relatively recalcitrant residues. It is known from experience with conventional Bt sprays, that Bt toxins as crystals can persist in soils, e.g. for at least 28 months (Vettori *et al.*, 2003). Recently, the decomposition of different plant species expressing Bt toxins was analysed in laboratory experiments and results were discussed in relation to lignin contents and potential environmental consequences (Flores *et al.*, 2005). Generally, Bt plants showed less decomposition than non-Bt plants. However, this effect was not clearly related to lignification or reduced microbial activity in soil. The authors concluded that lower decomposition rates may be beneficial as organic matter derived from plants would persist for a longer period improving soil structure and reducing erosion. In addition, Flores *et al.* (2005) discussed potential effects on target and non-target insects due to the longer persistence of Bt toxins in soil. In relation to soil organic content, it has been shown that even distinct increases in decomposition resistant compounds such as lignin result in only modest increases in organic carbon in the topsoil. Changes in soil management have a much more pronounced effect

(Sessitsch *et al.*, 2004). Considering the available information on potential effects of Bt plants on the soil environment and in particular on soil non-target organisms, adverse effects due to slightly altered decomposition rates are unlikely.

The published results from laboratory and field trials showed that on short to medium time scales (up to 3 years) and under field conditions, the effects on soil functions and biodiversity (Blackwood and Buyer 2004; Motavelli *et al.*, 2004; Evans, 2002) does not exceed the range of the “natural” variability. No conclusive evidence has yet been presented that currently released Bt toxin transgenic crops are causing significant direct effects on the soil environment. The effects of transgenic Bt maize in these experiments were small, if they existed at all. In addition, the available data do not indicate a chain of events that might result in long-term effects. Therefore, it seems likely that in commercial cropping conditions, where crop rotations are used, the consequences of effects on soil functions and soil organisms are negligible. However, long-term effects may become detectable in cultivation systems without crop rotation where repeated cropping of Bt11 maize might result in accumulation of effects.

Effects on biogeochemical processes resulting from *pat* expression and glufosinate ammonium treatment are likely to be the same as effects resulting from cultivation of non-GM maize. Glufosinate ammonium is a non-persistent herbicide. Therefore, after treatment the duration of any effect due to the herbicide is limited compared with currently applied herbicides on maize.

5.2.6. Potential impacts of the specific cultivation, management and harvesting techniques

The applicant considered that the *pat* gene for glufosinate tolerance was a marker gene and would only be used for that purpose. According to the application, Bt11 maize will not be commercialized as an herbicide tolerant crop in the EU. A proposal for labelling has been submitted by the applicant to inform potential users of Bt11 maize seeds that they should not use glufosinate ammonium for weed control in Bt11 maize. However, the Panel considered it likely that farmers would grow Bt11 maize with glufosinate herbicide applications. The Panel therefore considered that the environmental risk assessment and the post marketing environmental monitoring should also consider the direct and indirect impacts of the herbicide tolerance trait both in isolation and combined with the *cry1Ab* gene.

The environmental risk assessment made no comparisons of the environmental profile of the use of glufosinate ammonium on maize in comparison with other herbicides. Indeed, this would be difficult to do because of the range of other herbicides used and the range of agricultural systems and environments in which maize is grown and the wide diversity of weed species and associated flora and fauna that will be found in maize fields. Glufosinate ammonium is a contact, non-persistent and non-systemic broad-spectrum herbicide with activity against a wide range of plants though some tolerance occurs in some *Viola* species and some species of grasses. In the UK Farm Scale Evaluation study the glufosinate herbicide programmes studied on farms resulted in reduced botanical diversity in spring oilseed rape crops but had less impact on botanical diversity than the standard herbicide programmes used on maize crops (Heard *et al.*, 2003). No adverse effects have been found on higher trophic levels in herbicide tolerant maize (Brooks *et al.*, 2003). The most commonly used comparator in maize was atrazine for which authorisations had to be withdrawn in most EU countries by 10 September 2004 (EC, 2004a). However, other herbicides were used and a recent report (Perry *et al.*, 2004) indicated that regimes applying glufosinate ammonium either had a better or similar biodiversity impact compared with these herbicides.

The Panel considers that the presence of the *pat* gene and the use of glufosinate ammonium are not likely to give an increased impact on biodiversity in most situations. The Panel therefore comes to the conclusion that case-specific monitoring regarding any consequences due to the application of glufosinate ammonium in combination with the cultivation of Bt11 maize is not

required. The Panel, however, recommends that observation of general weed abundance and diversity should be included in the general surveillance plan.

5.3. Conclusions

The notification C/F/96/05.10 for Bt11 maize is for cultivation, and thus the environmental risk assessment and the monitoring plan have to consider the environmental impact of full scale commercialisation. The Panel is of the opinion that no significant risk has been identified in the environmental risk assessment with the exception of resistance development of the target insects, which affects the case-specific monitoring plan.

Bt11 maize has no altered survival, multiplication or dissemination characteristics except in the presence of glufosinate ammonium. The Panel agrees with the assessment that the likelihood of unintended environmental effects due to the establishment and spread of Bt11 maize will be no different from that of traditionally bred maize.

On the basis of the available data delivered either by the applicant or by literature survey, the likelihood of adverse effects on non-target organisms or on soil function is foreseen to be very low.

The Panel considers that the presence of the *pat* gene and the use of glufosinate ammonium are not likely to give an additional botanical diversity effect compared to other herbicides.

The safety of residues of glufosinate ammonium applied to Bt11 maize and of any metabolites has to be evaluated under Directive 91/414/EEC (EC, 1991), and is therefore not within the remits of this opinion. The Panel is aware that glufosinate containing herbicides have been evaluated (EFSA, 2005d) within the framework of Directive 91/414/EEC (EC, 1991).

6. Post-market environmental monitoring plan

6.1. Issues raised by the Member States

(1) It was stated that a detailed monitoring plan is required comprising general surveillance as well as case-specific monitoring. In addition, a more detailed insect resistance management plan was demanded; (2) it was argued that the implications of the presence and use of *pat* gene, in addition to the *cry1Ab* gene, should be considered in the post-market environmental monitoring plan; (3) more information on the general surveillance and monitoring of non-target effects is needed; (4) a considerable modification of the case-specific monitoring plan to take into account the additional requirements for the environmental risk assessment is requested.

The GMO Panel considered these issues and also critically examined the monitoring plan initially submitted by the applicant. The GMO Panel requested improvements and clarification from the applicant before an evaluation was finalised.

6.2. Evaluation of relevant scientific data

Notification C/F/96/05.10 for Bt11 maize is for cultivation, and thus a monitoring plan is required that considers the environmental impact of full commercial scale, cultivation and production.

6.2.1. General aspects of monitoring

The objectives of a post market environmental monitoring (PMEM) plan according to Annex VII of Directive 2001/18/EC (EC, 2001) are to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment are correct and to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the environmental risk assessment (EFSA, 2004a).

The Panel is of the opinion that the structure of the environmental monitoring plan provided by the applicant complies with the requirements defined in the Directive 2001/18/EC, the Guidance Notes to Annex VII (EC, 2002b) and the Guidance document provided by EFSA (EFSA, 2004a). The monitoring plan (referring to both case-specific monitoring as well as general surveillance) describes objectives, responsibilities and tasks, flow of information and monitoring methods (including statistical approaches).

6.2.2. Interplay between environmental risk assessment and monitoring

From the environmental risk assessment it can be concluded that the development of resistant corn borer populations could be induced by the cultivation of Bt11 maize. Therefore, a case-specific monitoring of resistance development in corn borers is required, and an appropriate monitoring plan was provided by the applicant.

The GMO Panel considered whether the abundance of non-target lepidoptera in or close to maize fields should also be monitored. The environmental risk assessment has not identified any risks specifically linked to Bt maize fields. The influence of Bt11 maize on the variability of abundance of lepidoptera is expected to be minimal compared with other impact factors (general agricultural management; insecticide usage on neighbouring fields, weed abundance; climate). In addition it will be difficult to compare populations of lepidoptera in conventional maize fields (sometimes treated with insecticides) with populations in Bt maize fields. Consequently, a significant and unequivocal correlation of detected differences with the cultivation of Bt11 maize is highly unlikely. Furthermore, the recording of statistically sufficient data on the abundance of lepidoptera would demand a high input of personnel and costs (Lang, 2004), especially if larvae, as the most susceptible and immobile development stage, are to be monitored. In addition maize, a species recently introduced into Europe, is not a significant food source for endemic lepidoptera and impacts due to pollen dispersal are likely to be transient and minor as demonstrated by studies on monarch butterflies in the USA (Dively *et al.*, 2004). The case-specific monitoring of the abundance of non-target lepidoptera in Bt11 maize does not comply with the required cost-effectiveness according to Council Decision 811/2002/EG (EC, 2002b). However, management recommendations for the cultivation of Bt11 maize, as given by the applicant to users of Bt11 maize, considers measures to reduce exposure of non-target lepidoptera (as well as the target pests), such as the use of non-transgenic border rows as refugia for the targets that would also reduce exposure of field margin weeds (and hence non-target lepidoptera) to pollen from Bt maize.

The Panel agrees with the risk assessment that no adverse effects on other non-target organisms are anticipated and thus this should not be included in the case-specific monitoring plan.

The Panel considers the spread of transgenes not relevant for an environmental monitoring regime since natural relatives of maize are not present in the EU. Furthermore horizontal gene transfer to microorganisms is extremely unlikely, and would occur primarily through homologous recombination in microbes.

The environmental risk assessment provided by the applicant did not identify risks specific to the GMO associated with the *pat* gene or the management of herbicide tolerance. The GMO Panel

agrees with this assessment. Thus, considering all the conclusions described above, the Panel considers that monitoring of target insect resistance is the only case-specific monitoring requirement for Bt11 maize.

6.2.3. Case-specific monitoring of Bt11 maize

The case-specific monitoring plan clearly describes the responsibilities and activities of the applicant. These include organising the establishment and activities of this case-specific monitoring, co-ordinating third parties' contributions to the studies and establishing a reporting system to the EU and the Competent Authorities of Member States. Since the development of resistance is more likely to occur with increased time and scale of cultivation, the monitoring period has been selected appropriately for the period of the market release. The applicant documented that previous commercial releases of Bt maize in Europe and North America have already been managed in order to reduce selection pressure. It is appropriate that Resistance Management Strategies are fully integrated into PMEM plans so that information provided by the monitoring concerning resistance development is used to refine strategic options for managing resistance. In addition, the level of susceptibility and the geographical information of occurrence of resistance will be linked to a stepwise pest management strategy so that methodological improvements can be reviewed and adopted - if appropriate. The direct assessment of susceptibility in the corn borer populations allows the detection of resistance at an early stage of development, so that detection can be rapidly linked to pest management measures. Such a strategy of insect resistance management and monitoring should provide an efficient stewardship of Bt11 maize and other similar maize cultivars, as well as an efficient pest control regime.

The Panel concludes that large scale cultivation of Bt11 maize is likely to increase the selection pressure on corn borers to develop tolerance to its Cry1Ab toxin and possibly to other Bt toxins. This could have several consequences including the use of alternative phytosanitary measures to control the pest including the use of insecticides other than Bt toxins. The Panel agrees that the likelihood of occurrence is low since under field conditions and several years of cultivation no resistance has been reported (Farinós *et al.*, 2004). However, cultivation of Bt maize in Europe is currently at small scale and limited to few geographic regions and thus it is difficult to predict future responses of corn borer populations in Europe.

6.2.4. General surveillance of the impact of Bt11 maize

The objective of general surveillance is to identify unforeseen adverse effects of the GM plant or its use on human health and the environment, which were not predicted in the risk assessment. The methods and approaches should be appropriate, proportional and cost-effective to allow for the detection of GMO effects. Potential data sources and related networks should be identified.

The GMO Panel gives its opinion on the scientific quality of the general surveillance as part of the environmental monitoring plan provided by the applicant. The Panel welcomes the approach of the applicant to establish new general surveillance networks by using farmer questionnaires as a reporting format. The questionnaires to farmers exposed to or using Bt11 maize provided by the applicant are regarded as an adequate starting point for addressing several aspects of general surveillance. The Panel considers the format of the questionnaires provided by the applicant as comprehensive.

However the GMO Panel proposes the following modifications to the farmer questionnaires:

- The questionnaire should be designed to allow for the input of general farm information (data on fertilizer usage, soil fertility, crop rotations, crop performance, crop yields, pests and diseases, pesticide use and weed abundance) as well as field-specific information for several

fields when more than one field of a specific farmer is included in the monitoring. The questionnaire should include an advisory note explaining that separate data sets are required for each Bt11 maize field to be monitored on a single farm.

- The farmers' questionnaire sent to the farmers for the year(s) after the Bt maize cultivation needs to be adapted for the monitoring of the specific crops (maize or different) that follow the Bt11 maize cultivation. It should be in a format that is statistically compatible with the questionnaires supplied for the Bt11 maize growing season.

Considering Bt11 maize, the GMO Panel is not aware of any existing surveillance networks that would substantially fulfil the scientific requirements for the detection of any unforeseen environmental effect in relation to Bt11 maize cultivation. Thus the Panel agrees with the proposal of the applicant to describe the generic approaches for using other existing surveillance networks. The applicant has also given consideration to the use of any future surveys of conservation goals as defined in the Directive 2004/35/EC on environmental liability (EC, 2004b) in farming regions where Bt maize is cultivated and intends to investigate their suitability for providing data on potential changes in biota.

6.2.5. Reporting the results of monitoring

The GMO Panel is content with the proposal made by the applicant on the reporting intervals. The GMO Panel recommends that effective reporting procedures are established with the Competent Authority and the Commission as required under the Directive 2001/18/EC.

6.3. Conclusion

An appropriate case-specific monitoring plan to record the development of Cry1Ab toxin resistance in target populations of lepidoptera has been provided by the applicant. The time period and design of this case-specific monitoring should consider the rate at which resistance is likely to evolve, resistance management strategies, the scale and the geographical dispersal of Bt11 maize cultivation.

The GMO Panel agrees in principle with the general methods and approaches of the general surveillance plan.

Management options for the cultivation of Bt11 maize should include measures to reduce exposure of non-target lepidoptera and to delay the development of resistance to the Cry1Ab protein in target insects.

CONCLUSIONS AND RECOMMENDATIONS

Maize line Bt11 has been developed for protection against lepidopteran pests by expressing the Cry1Ab protein. The introduction of a *pat* gene confers tolerance to glufosinate ammonium. The GMO Panel has assessed information provided on the molecular characteristics of the insertion, the safety of the proteins expressed and the potential for risks associated with any changes to the nutritional, toxicological and allergenic properties of Bt11 maize. Analysis of the chemical composition of the maize and field trial data were also used to assess the potential for changes to safety, nutritional as well as agronomic parameters. No data have emerged to indicate that maize line Bt11 is any less safe than its non-GM comparators.

The Panel considers that Bt11 maize will have impacts similar to those of comparable non-GM maize cultivars on the environment. The only adverse effect identified was the possibility that resistance to Cry1Ab protein might evolve in corn borers exposed to Bt11 maize following

cultivation for some years. The Panel accepts the monitoring plan developed by the applicant to monitor specifically for resistance in corn borers and recommends that cultivation should be accompanied by appropriate risk management strategies to minimise exposure of non-target insects and to delay the development of resistance to the Cry1Ab protein in target insects. In addition, the Panel accepts in principle the general surveillance plan submitted by the applicant.

From the data provided by the applicant, there was no evidence to indicate that Bt10 material was present in the Bt11 maize used for the biosafety studies. Therefore, the GMO Panel considers that the risk assessment of Bt11 maize has not been compromised by the presence of Bt10 maize.

The EFSA GMO Panel is therefore of the opinion that there is no evidence to indicate that placing of maize line Bt11 and derived products on the market is likely to cause adverse effects on human or animal health or the environment in the context of its proposed use.

The authorisation of the complementary herbicide is not within the remits of this opinion and is covered by other legal frameworks of the EU and Member States.

DOCUMENTATION PROVIDED TO EFSA

1. Note to Mr. Koëter, dated 28 January 2004 with ref. C4/HM/KT/sf D(2004)440073, from Mr. J. Delbeke – advance copy of a request to EFSA concerning notification C/F/96/05.10 (Bt11 maize).
2. Note to Mr. Koëter, dated 17 March 2004 with ref. C4/KT/ D(04) 440330, from Mr. J. Delbeke – transmission of Member State objections concerning notification C/F/96/05.10 (Bt11 maize).
3. Initial comments and final objections from Member States with regard to notification C/F/96/05.10 (Bt11 maize).
4. Submission from Syngenta Seeds SAS (16 March 2004) to EFSA regarding the notification to place on the market Bt11 maize, for cultivation, feed and industrial processing, under Part C of Directive 2001/18/EC (Ref C/F/96/05.10), containing:
 - a) a letter from Syngenta to the Competent Authority of France concerning submission of the notification,
 - b) the summary of the notification,
 - c) the assessment report of the notification carried out by the Competent Authority of France,
 - d) the complete notification submitted by Syngenta,
 - e) additional information submitted by Syngenta in response to comments and objections raised by the Competent Authorities of the Member States.
5. Letter from EFSA to applicant with request for clarification/additional information (ref. SR/ (2004) 346, 18 May 2004).
6. Letter from EFSA to applicant with request for clarification/additional information (ref. EVH/ (2004) 532, 22 July 2004).
7. Letter from EFSA to applicant with request for clarification/additional information (ref. SR/ (2004) 950, 3 November 2004).

8. Additional information submitted by Syngenta on 28 December 2004 in response to EFSA's request for further information.
9. Additional information submitted by Syngenta on 27 January 2005 in response to EFSA's request for further information.
10. Letter from EFSA to applicant with request for clarification/additional information (ref. SR/MR/jq (2005) 193, 14 February 2005).
11. Letter from Syngenta (15 February 2005) notifying change of contact person at Syngenta for notification C/F/96/05.10.
12. Additional information submitted by Syngenta on 21 February 2005 in response to EFSA's request for further information.
13. Letter from EFSA to applicant with request for clarification/additional information (ref. SR/MR (2005) 293, 9 March 2005).
14. Additional information submitted by Syngenta on 16 March 2005 in response to EFSA's request for further information.
15. Letter from EFSA to applicant with request for clarification/additional information (ref. SR/ (2005) 369, 24 March 2005).
16. Additional information submitted by Syngenta on 15 April 2005 in response to EFSA's request for further information.

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