Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the application (Reference C/BE/96/01) for the placing on the market of glufosinate-tolerant hybrid oilseed rape Ms8 x Rf3, derived from genetically modified parental lines (Ms8, Rf3), for import and processing for feed and industrial uses, under Part C of Directive 2001/18/EC from Bayer CropScience[1]

(Question No EFSA-Q-2005-003)

Opinion adopted on 14 September 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 oilseed rape Ms8, Rf3 and Ms8 x Rf3, genetically modified to introduce a pollination control system (hybrid system), linked with a tolerance to glufosinate-ammonium.

The opinion is based on a question raised by the Commission relating to an application (Ref. C/BE/96/01) from Bayer CropScience under Directive 2001/18/EC to place on the market oilseed rape Ms8, Rf3 and Ms8 x Rf3. The GMO Panel was asked to consider whether there is any scientific reason to believe that placing oilseed rape Ms8, Rf3 and Ms8 x Rf3 on the market for import, processing and uses as any other oilseed rape (excluding food uses), is likely to cause any adverse effects on human health and the environment. The question followed a scientific assessment which was made initially by the Competent Authority of Belgium and evaluated subsequently by all other Member States. An assessment of oilseed rape Ms8, Rf3 and Ms8 x Rf3 was requested by the Commission because of questions raised by several Member States following the evaluations at national level. When this is the case, EU legislation requires that EFSA carries out a further assessment and provides an opinion. In delivering its opinion the GMO Panel considered the application, additional information provided by the applicant and the specific questions and concerns raised by the Member States.

Oilseed rape lines Ms8, Rf3 and Ms8 x Rf3 were assessed with reference to their intended uses 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 oilseed rape Ms8, Rf3 and Ms8 x Rf3 and the nature and safety of the target proteins produced by the transgenic plants with respect to

[1] For citation purposes: Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the application (Reference C/BE/96/01) for the placing on the market of glufosinate-tolerant hybrid oilseed rape Ms8 x Rf3, derived from genetically modified parental lines (Ms8, Rf3), for import and processing for feed and industrial uses, under Part C of Directive 2001/18/EC from Bayer CropScience, *The EFSA Journal* (2005) 281, 1-23.

toxicology and allergenicity. Furthermore, a comparative analysis of agronomic traits and composition of Ms8 x Rf3 oilseed rape was undertaken and the safety of the whole feed was evaluated. A nutritional and an environmental assessment, including the import monitoring plan, were both undertaken.

The oilseed rape parental lines Ms8 and Rf3 have been developed for the production of hybrid seeds Ms8 x Rf3, combined with tolerance to the Liberty® herbicide (the active ingredient of which is glufosinate-ammonium/phosphinothricin). As a result of hybrid vigour cross-pollinated plants produce higher yield as compared to self-pollinated oilseed rape. The hybrid system is achieved using a pollination control system by insertion and expression of *barnase* and *barstar* genes from *Bacillus amyloliquefaciens* into two separate oilseed rape lines. Oilseed rape embryos were transformed by *Agrobacterium tumefaciens* to transfer DNA fragments containing these genes. The *barnase* and *barstar* genes are each linked with the *bar* gene from *Streptomyces hygroscopicus* which encodes the enzyme phosphinothricin acetyltransferase (PAT) and which confers tolerance to the herbicide glufosinate ammonium. Conventional crossing of the two GM lines is used to produce the Ms8 x Rf3 seeds.

The female line Ms8 (Male sterile) contains an insert bearing both *barnase* and *bar* genes, under the control of tapetum cell-specific PTA29 and PssuAra promoters, respectively. The *barnase* gene encodes a ribonuclease peptide (RNase) expressed only in the tapetum cells during anther development. The RNase affects RNA levels, disrupting normal cell functioning and arresting early anther development, thus leading to the lack of viable pollen and male sterility.

The male line Rf3 (Restorer of fertility) contains an insert bearing both *barstar* and *bar* genes, under the control of, respectively, tapetum cell-specific PTA29 and PssuAra promoters. The *barstar* gene codes for a ribonuclease inhibitor (Barstar peptide) expressed only in the tapetum cells of the pollen during anther development. The ribonuclease inhibitor (Barstar peptide) specifically inhibits the Barnase RNase expressed by the Ms8 line.

Together, the RNase and the ribonuclease inhibitor form a very stable one-to-one complex, in which the RNase is inactivated. As a result, when pollen from the restorer line Rf3 is crossed to the male sterile line Ms8, the resultant Ms8 x Rf3 progeny expresses the RNase inhibitor in the tapetum cells of the anthers allowing hybrid plants to develop normal anthers and restore fertility.

Appropriate molecular techniques were used to characterise the transformation events leading to the production of *Brassica napus* lines Ms8 and Rf3. Southern hybridisation was used to detect and characterise the transformation events, to establish the absence of unwanted vector sequences and to identify the transgenic lines. PCR analysis was used to characterise further the transgenic events and to determine the nucleotide sequences of the plant DNA flanking the inserts. Northern analysis was used to quantify transgene transcript levels in leaves, seeds and pollen. Western analysis was used to detect the protein products. ELISA and enzymatic method were used to detect and quantify the PAT protein and its activity. The DNA sequences of the insert in the hybrid Ms8 x Rf3 were investigated using PCR and DNA sequencing confirming that gross insert structures and insertion loci were retained.

The extensive comparative compositional analysis of Ms8 x Rf3 seeds from field trials in Europe (Belgium) showed that there was no indication of unintended effects of the genetic modification. Additional animal safety or nutritional studies are not necessary. Ms8 x Rf3 oilseed rape was considered comparable with conventional oilseed rape, except for the expression of the new proteins.

The application C/BE/96/01 for oilseed rape lines Ms8, Rf3 and Ms8 x Rf3 was only assessed for the import and processing of Ms8 x Rf3 seeds for feed and industrial uses. Therefore the GMO Panel did not assess the scientific information on possible environmental effects associated with the cultivation of oilseed rape Ms8, Rf3 and Ms8 x Rf3. The GMO Panel agrees with the conclusions of the environmental risk assessment by the applicant that the likelihood of unintended environmental effects due to the adventitious release and spread of Ms8, Rf3 and Ms8 x Rf3 oilseed rape will not be different from that of oilseed rape bred traditionally. The import monitoring plan provided by the applicant is in line with the intended uses of the GMO.

In conclusion, the GMO Panel considers that the information available for oilseed rape Ms8, Rf3 and Ms8 x Rf3 addresses the outstanding questions raised by the Member States and therefore the placing on the market of Ms8, Rf3 and Ms8 x Rf3 oilseed rape for import and processing for feed and industrial purposes is unlikely to have an adverse effect on human or animal health or, in the context of its proposed uses, on the environment. This is in addition to the present uses of oil for food purposes and processed meal for feed purposes, both derived from Ms8 x Rf3 oilseed rape, which are already lawfully placed on the market.

The Panel advises that appropriate management systems are in place to minimize accidental loss and spillage of transgenic oilseed rape during transportation, storage, handling in the environment and processing into derived products.

Key words: GMO, *Brassica napus,* oilseed rape, Ms8, Rf3, Ms8 x Rf3, hybrid, glufosinate-tolerant, *bar*, PAT, *barnase, barstar*, human health, environment**,** import, Directive 90/220/EEC, Directive 2001/18/EC.

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BACKGROUND

The application (reference C/BE/96/01) was initially submitted by Bayer CropScience[2] to the lead Member State (Belgium) in 1996. At that time the scope of the dossier included import and processing for food, feed and industrial uses as well as cultivation in a stepwise approach. The application met the requirements laid down under Directive 90/220/EEC (EC, 1990) and the Commission therefore forwarded the application to the Scientific Committee on Plants for an opinion. The Scientific Committee on Plants was asked whether there is any reason to believe that the potential transfer of the herbicide resistant gene to wild *Brassica* relatives is likely to cause any adverse effects on the environment or whether the impact of such a transfer will be mainly of agricultural nature. On 19 May 1998 the Committee concluded that there is no evidence to indicate that the placing on the market of hybrid seed of oilseed rape (consisting of crossing of parental lines Ms8 and Rf3) with the purpose to be used as any other oilseed rape is likely to cause adverse effects to human health and the environment (SCP, 1998).

The applicant updated the application in order to meet the requirements of Directive 2001/18/EC (EC, 2001). The Commission received the Summary Notification Information Format (SNIF[3]) from the applicant on 5 February 2003. The full application, together with the assessment report[4], was received by the Commission from the lead Member State on 3 February 2004. The lead Member State was positive for import and processing for feed and industrial uses of Ms8 (Unique Identifier ACS-BN005-8), Rf3 (Unique Identifier ACS-BN003-6) and Ms8 x Rf3 but excluded the cultivation in the EU of varieties derived from these GMOs.

In accordance with Directive 2001/18/EC, the application was then transmitted to the Competent Authorities of the other Member States, a number of which raised objections during the statutory 60-day period or supported the lead Member State to exclude cultivation. The applicant provided the Member States with additional information in response to the

[2] Previously called '*Plant Genetic System*, *PGS*'

[3] http://gmoinfo.jrc.it/csnifs/C-BE-96-01.pdf

[4] http://gmoinfo.jrc.it/csnifs/C-BE-96-01_AssessmentReport.pdf

objections raised during the 60-day period. The applicant did not provide further data to support cultivation. The Member States had until 10 December 2004 to confirm or withdraw their objections. Where these objections are maintained, the Commission is required to consult the relevant Scientific Committees for opinion, now represented by EFSA. Some Member States maintained specific objections, in particular with respect to the cultivation of oilseed rape Ms8, Rf3 and Ms8 x Rf3: either these Member States did not assess the cultivation part of the application or they did not support the marketing of the oilseed rape for cultivation purposes.

In parallel, following the initial environmental risk assessment made by the lead Member State, the applicant confirmed its interest in the approval of oilseed rape Ms8, Rf3 and Ms8 x Rf3, excluding the cultivation of these lines in the EU.

EFSA was then asked by the Commission: ' *Whether there is any scientific reason to believe that the placing on the market of oilseed rape Ms8, Rf3 and Ms8 x Rf3 for import, processing and cultivation 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 objections raised by competent authorities of Member States in this context*'.

However, this request, including cultivation, was in contradiction with the supporting documents provided by the Commission (*e.g.* support by Competent Authorities to exclude cultivation). As the risk assessment carried out by the lead Competent Authority was not clear with respect to cultivation, the assessment of the data related to cultivation by the other Member States was uncomplete and not further supported by the applicant.

Article 29(4) of Regulation (EC) 178/2002 (EC, 2002a) states that, where the request to EFSA is not clear, the Authority may either refuse or propose amendments to a request for an opinion in consultation with the Institution or Member States that made the request. Therefore, pending further clarifications about the limitation of scope of the application, EFSA suggested an amendment of the terms of reference (see below) and the GMO Panel did not to assess the data provided by the applicant related to the cultivation of oilseed rape Ms8, Rf3 and Ms8 x Rf3.

Article 18(1) of Directive 2001/18/EC states that the period during which the Commission is awaiting the opinion of the Scientific Committee shall not exceed 90 days. The evaluation by EFSA started on 8 April 2005, 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 clarifications from the applicant.

In delivering its opinion the GMO Panel considered the application, additional information provided by the applicant and the specific questions and concerns raised by the Member States, excluding those data related to cultivation.

Purified oil produced from oilseed rape Ms8, Rf3 and Ms8 x Rf3 was notified under the novel foods Regulation (EC) 258/97 (EC, 1997). Both purified oil and processed meal for feed uses have been inserted onto the Community Register of genetically modified food and feed according to Article 28 of Regulation (EC) 1829/2003 (EC, 2003). These products, that were lawfully placed on the market before the entry into force of Regulation (EC) 1829/2003, may continue to be placed on the market[5].

[5] http://europa.eu.int/comm/food/dyna/gm_register/index_en.cfm

TERMS OF REFERENCE

EFSA was requested, under Article 29(1) and in accordance with Article 22(5)(c) of Regulation (EC) No 178/2002, to provide a scientific opinion as to whether there is any scientific reason to believe that the placing on the market of oilseed rape Ms8, Rf3 and Ms8 x Rf3 for import and 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, to highlight diverging scientific views, if any, and how these are resolved in the opinion.

EFSA was not requested to give an opinion on the non-scientific objections raised by Competent Authorities in their replies, in the context of the entry into force of forthcoming legislation or requests for further legislative/implementing measures.

ASSESSMENT

1. Introduction

Oilseed rape Ms8, Rf3 and Ms8 x Rf3 were assessed with reference to the intended uses 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*'. In its evaluation the GMO Panel also considered the issues, except the ones related to cultivation (see the '*BACKGROUND*' section), that were raised by Member States during the initial assessment of the application introduced under Directive 2001/18/EC. The assessment presented here is based on the information provided in the application, as initially submitted under Directive 2001/18/EC, as well as on all additional information from the applicant in reply to Member States and EFSA questions.

The oilseed rape parental lines Ms8 and Rf3 have been developed for the production of hybrid seeds Ms8 x Rf3, combined with tolerance to the Liberty® herbicide (the active ingredient of which is glufosinate-ammonium/phosphinothricin). As a result of hybrid vigour cross-pollinated plants produce higher yield as compared to self-pollinated oilseed rape. Oilseed rape is capable of both self-pollination and cross-pollination. When grown conventionally, only ~30% of progeny result from cross pollination; the remaining ~70% resulting from self-pollination. To produce F_1 hybrids, control of self-pollination is required.

The hybrid system was achieved using a pollination control system by insertion and expression of *barnase* and *barstar* genes from *Bacillus amyloliquefaciens* into two separate oilseed rape lines Ms8 and Rf3 (Agbios, 2003 for detailed description). Conventional crossing of the two GM lines is used to produce the Ms8 x Rf3 seeds for food, feed and industrial purposes as any other oilseed rape. Oil and meal derived from Ms8 x Rf3 oilseed rape for food and feed purposes respectively can be lawfully placed on the market (EC, 2003).

2. Molecular characterisation

2.1. Issues raised by Member States

(1) Clarification was sought on the putative open reading frames (ORFs) at the junction regions in transformation events Ms8 and Rf3 and possible homology to known toxic proteins. (2) One Member State was not satisfied with the characterisation of the hybrid.

The GMO Panel considered these issues and requested additional information from the applicant with respect to the junction ORFs.

Comments raised by the Member States on specific molecular detection methodologies as well as on delivery of appropriate reference material are not within the scope of the GMO Panel remit.

2.2. Evaluation of relevant scientific data

2.2.1. Transformation process and vector constructs

Male sterility in *Brassica napus* line Ms8 is achieved through production of a ribonuclease, Barnase, expression of which is regulated with respect both to tissue and to stage of development. The fertility-restorer Rf3 produces a ribonuclease inhibitor, Barstar. This inhibitor is highly specific for the *barnase* gene product. When crossed with Ms8, Barstar will overcome the effects of Barnase, restoring fertility to the resultant Ms8 x Rf3 hybrids. Selection for both Barnase and Barstar in their respective plant lines was achieved by using the *bar* selectable marker gene from *Streptomyces hygroscopicus* which encodes the enzyme phosphinothricin acetyltransferase (PAT) and which confers tolerance to the herbicide phosphinothricin (glufosinate ammonium). The traits were introduced by transformation of each parental line mediated by modified *Agrobacterium tumefaciens* T-DNA.

Male-sterile transformant Ms8

The female line, Ms8 (Male-sterile), was the product of transformation with plasmid pTHW107. Between the left and right T-DNA borders of this plasmid lies a gene from *Bacillus amyloliquefaciens* encoding Barnase. Expression of this gene is regulated by a promoter, PTA29, from *Nicotiana tabacum* that is only expressed in tapetum cells during pollen development and by the 3' terminator of the nopaline synthase gene of *Agrobacterium tumefaciens*. The RNase affects RNA levels, disrupting normal cell functioning and arresting early anther development, thus leading to the lack of viable pollen and male sterility. Linked with the *barnase* is the *bar* gene, which encodes resistance to phosphinothricin. Expression of the *bar* gene in these plants is driven by the PssuAra promoter from the atS1A ribulose 1,5-biphosphate carboxylase small subunit from *Arabidopsis thaliana* and the terminator sequence is the 3' untranslated DNA from the TL-DNA gene 7 (3' g7) of pTiB6S3. The regulatory sequences controlling expression of *bar* are active in all green plant tissues.

Fertility-restorer transformant Rf3

Transformation of *Brassica napus* with plasmid pTHW118 resulted in the production of the male line Rf3 (Restorer of fertility). The gene on plasmid pTHW118 that permits restoration of

fertility to male-sterile plants is the *barstar* gene, isolated from *Bacillus amyloliquefaciens*. This gene is regulated by the same promoter and terminator sequences as is the *barnase* gene located on plasmid pTHW107. Expression of the *barstar* gene in the tapetum cells of the pollen during anther development results in the production of a specific RNase inhibitor that overcomes the activity of the RNase encoded by the *barnase* gene when both are expressed in the same cell. Resistance to phosphinothricin on pTHW118 is encoded by the same sequences as for plasmid pTHW107.

To aid the manipulation of plasmids in bacterial hosts, a gene encoding resistance to streptomycin and spectinomycin was present in the transforming plasmid. The inclusion of either of these antibiotics in the growth medium will provide a positive selection for cells carrying the plasmids.

2.2.2. Transgenic constructs in the genetically modified plant

2.2.2.1 Male-sterile Ms8 transformant

Segregation analysis demonstrated that the transgenic event in the male-sterile line Ms8 occurs at a single genetic locus. The site of insertion of the transgenic cassette in Ms8 was characterised using PCR amplification and nucleotide sequence determination. A single copy of the cassette has been incorporated in line Ms8. There are rearrangements of the DNA sequence at the site of insertion but these do not lead to the expression of a new trait and are not considered to pose a safety risk. The nucleotide sequences flanking the insert in Ms8 were determined and subjected to BLAST analysis using the GenBank, EMBL, DDBJ and PDB databases. No meaningful matches were found from the sequence flanking the 3' end of the insert. At the 5' end, the flanking sequences showed similarity to sequences found in *Arabidopsis thaliana* located on Chromosomes 3 and 5. This similarity is not surprising, given the close relationship between the genomes of *Arabidopsis thaliana* and *Brassica* spp.

2.2.2.2 Fertility-restorer Rf3 transformant

Segregation analysis demonstrated that the transgenic event in the fertility-restorer line Rf3 occurs at a single genetic locus. Molecular analysis shows that one T-DNA copy is arranged as an inverted repeat with a second, incomplete copy of the T-DNA. The second copy includes a functional part of the promoter and the coding region of *barstar* together with its terminator sequence and a non-functional part of the promoter associated with *bar*. The inverted repeat structure of the insert is thought to result from strand switching during repair at the T-DNA ends. There are rearrangements of the DNA sequence at the site of insertion but these do not lead to the expression of a new trait and are not considered to pose a safety risk. The nucleotide sequences flanking the insert in Ms8 were determined and subjected to BLAST analysis using the GenBank, EMBL, DDBJ and PDB databases. No meaningful matches were found from the sequence flanking the 5' end of the insert. At the 3' end, the flanking sequences showed similarity to genomic sequences found in *Arabidopsis thaliana* located on Chromosomes 1 and 5. This similarity is not surprising, given the close relationship between the genomes of *Arabidopsis thaliana* and *Brassica* spp.

2.2.2.3 Hybrid Ms8 x Rf3

A conventional cross between the two primary transgenic lines was used to construct the hybrid Ms8 x Rf3. The molecular structures of the DNA inserts present in the hybrid were investigated using PCR. In PCR amplifications using an endogenous control, the hybrid carried target sequences for primers designed to amplify DNA from both Ms8 and Rf3 events whereas the respective parental lines carried only the target appropriate to the specific transformation event as expected, demonstrating that gross insert structures and loci of insertion were retained.

2.2.2.4 Absence of plasmid backbone sequences

PCR analysis established the absence in the transgenic lines Ms8 and Rf3 of the marker gene encoding resistance to streptomycin and spectinomycin that was present in the original transforming plasmid. To demonstrate the absence of DNA encoding streptomycin and spectinomycin resistance, three amplification protocols were employed: one targeting the 5' sequence of the marker gene, the second targeted at the 3' end and the third targeted at almost the complete coding sequence. Positive control samples yielded amplimers of the expected size whereas no amplification was apparent in negative controls and in test material. Using the same control material, PCR amplification was used to demonstrate that plasmid backbone sequences flanking the left and right T-DNA borders were absent from transgenic plants of lines Ms8 and Rf3.

The results of the PCR analysis were supported by Southern transfer and hybridisation experiments. Three probes were used to assess the sequences present in line Ms8: one derived from the origin of replication derived from plasmid pBR322, one derived from the plasmid pVS1 and related sequences on plasmid pTHW107 and one derived from the DNA sequence encoding resistance to streptomycin and spectinomycin. Probe DNA was generated by PCR amplification of plasmid pTHW107, linearised by digestion with restriction endonuclease *Eco*RV. The probe used to demonstrate the absence of DNA sequences encoding resistance to streptomycin and spectinomycin was isolated from the intermediate vector as a *Hin*dIII and *Xba*I fragment.

Similar experiments demonstrated the absence of backbone vector in line Rf3, using pTHW100 as the positive control DNA added to digested genomic plant DNA and using plasmid pTHW118 for PCR amplification of probe DNA targeted at the origins of replication and using the *Hin*dIII-*Xba*I fragment of plasmid pTHW100 to demonstrate the absence of DNA sequences encoding resistance to streptomycin and spectinomycin.

2.2.3. Information on the expression of the insert

2.2.3.1 Expression of the introduced genes

Northern blotting was used to determine the level of transcription of transgenes (mRNA of *bar*, *barnase* and *barstar* genes) in different tissues from Ms8, Rf3 and Ms8 x Rf3 oilseed rape. Leaves, flower buds, pollen and seeds were examined. The *bar* mRNA, encoding glufosinate-ammonium/phosphinothricin tolerance, was detected in leaves and flower buds but was not detected in dry seeds where the limit of detection was 0.1 pg/µg total RNA. Since the promoter driving expression of *bar* is associated with gene expression in green tissues, this result is to be expected. Expression of *barnase,* the gene responsible for male-sterility, in tissues from plants belonging to the male-sterile line Ms8 and of *barstar,* the gene responsible for fertility-restoration, in tissues from plants of the fertility-restorer line Rf3 was

not detectable in any of the tissues tested, except *barstar* in flowering buds, where the expression was found to vary between 1.2 and 2.4 pg/µg total RNA. The inability to detect *barnase* transcripts in flower buds of *Brassica napus* line Ms8 has been attributed to the activity of the Barnase protein in this tissue. The limit of detection was 0.1 pg/µg total RNA in these experiments.

Western blotting was used to detect proteins in different plant tissues from Ms8, Rf3 and Ms8 x Rf3 oilseed rape, specifically leaves (both young and mature), roots, flower buds, pollen and dry seed. Although PAT was found in all tissues tested, it was only found in trace levels except in green tissues, where its levels were elevated above the baseline. Barnase was not detected in any tissue from *Brassica napus* line Ms8; Barstar was found only in the flower buds and then only during pollen development in *Brassica napus* line Rf3. In flower buds from Ms8 x Rf3 plants, both Barnase and Barstar could be detected during pollen development. The limits of detection were 1 ng PAT in all tissues tested, 5 ng Barstar in all tissues tested and 5 ng Barnase in pollen and dry seed and 1 ng Barnase in other tissues. Using ELISA, PAT was detected in seeds of *Brassica napus* lines Ms8 and Rf3. The level of PAT protein was, however, very low, representing less than 0.001% of the total extractable protein.

2.2.3.2 Measurement of PAT activity

Activity of the PAT enzyme in Ms8, Rf3 and Ms8 x Rf3 oilseed rape was demonstrated phenotypically by the glufosinate-ammonium dot and spray assays. A spectrophotometric assay was used to determine activity of this enzyme in leaf tissue and in seeds. When seeds were assayed, PAT activity was not observed in transgenic plants above the background acetyl transferase activity in control plants lacking *bar*.

2.2.3.3 Putative cryptic open reading frames (ORF) in *Brassica napus* transformation events Ms8 and Rf3

Examination of the DNA sequences of the transgenes and the plant DNA that flanks them in Ms8 and Rf3 oilseed rape has revealed eleven of the fifty putative open reading frames to be located in the junction regions in which expression may lead to a new protein. Three are found in Ms8 and eight are in Rf3. These were subjected to an *in silico* search, the purpose of which was to identify sequences that might code for an allergenic epitope or that code for a known toxin. No identity with known allergen epitopes was found. Likewise, no meaningful similarity with known toxins was discovered. The applicant reported that ORF-20 in Rf3 had some matches with a venom precursor from the monocled cobra (*Naja naja*), described in the PIR database but the identity between the ORF-20 sequence and the venom factor precursor was limited to a small number of amino acids representing only 12/1642 (0.7%) of the venom factor precursor. The matching amino acids in the venom precursor and in ORF20 were not contiguous, and the identities were scattered over a stretch of 44 amino acids. The GMO Panel accepts that it is extremely unlikely that this small ORF fragment, if it were to be expressed, encodes a venom toxin. No relevant similarities between the other translated ORF sequences and other known toxins were found.

2.2.4. Inheritance and stability of inherited DNA

Stability of integration of the inserts in lines Ms8 and Rf3 was established by segregation analysis, with the phenotype showing a simple Mendelian inheritance pattern. Multiple crosses and back-crosses, up to BC_3, have been studied and there was no loss of phenotype nor were new traits observed.

The stability was further established using Southern transfer and hybridisation analysis of plant DNA taken from different generations of Ms8 x Rf3 oilseed rape. The hybridisation patterns of DNA digested with *Eco*RV and probed with inserts DNA were indistinguishable in plants from generations 1 and 3 and in a back-cross, establishing that the inserts are inherited stably across generations and in different genetic backgrounds.

2.3. Conclusion

Appropriate molecular analyses were used to characterise the transformation events leading to the production of *Brassica napus* lines Ms8 and Rf3. Southern transfer and hybridisation was used to detect and characterise the transformation events, to quantify the transgenes in the transformed plant lines, to establish the absence of unwanted vector sequences and to identify transgenic lines. PCR analysis has been used to characterise further the transgenic events and to determine the nucleotide sequences of the plant DNA flanking the inserts. Northern blotting was used to analyse the expression of transgenes in leaves, seeds and pollen. Western blotting was used to detect the products of transgene expression and this was supplemented with ELISA to detect PAT. Enzyme assays were used to quantify the activity of the PAT enzyme. The molecular structures of the DNA inserts in the Ms8 x Rf3 oilseed rape were investigated using PCR and this confirmed that gross insert structures and loci of insertion were retained.

In conclusion, the GMO Panel is of the opinion that the transgenic inserts in *Brassica napus* lines Ms8 and Rf3 have been analysed and described sufficiently. Neither insertion event provides grounds for specific concern. The stability of inheritance of the introduced traits has been demonstrated, as has the expression of the transgenes.

3. Comparative analysis

3.1. Issues raised by Member States

(1) Comments were made on the compositional equivalence of the hybrid oilseed rape Ms8 x Rf3 with its non-transgenic counterpart; (2) the validity of the statistical analysis of the compositional data was questioned.

The GMO Panel considered these issues and requested additional information from the applicant on the statistical analysis of the compositional data.

3.2. Evaluation of relevant scientific data

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

The comparator used in the comparative analysis of Ms8 x Rf3 oilseed rape was the open pollinated winter oilseed rape line PP0005B. Both the Ms8 and Rf3 events were originally constructed in a spring oilseed rape (*Brassica napus,* variety Drakkar), but backcrossed into the winter oilseed rape line PP0005B, using conventional backcrossing techniques to produce a comparable genetic background for the hybrid and the comparator. The Ms8 x Rf3 oilseed rape used in the field trials resulted from crossing plants from the Ms8 event that were backcrossed to the PP0005B line seven times and plants from the Rf3 event that were backcrossed five times and then subjected to three selfings to produce a homozygous Rf3/Rf3 PP0005B parental line. Seeds from glufosinate-treated and untreated Ms8 x Rf3 hybrids and the non-transgenic comparator line were harvested for compositional and nutritional analysis.

3.2.2. Compositional analysis

Field trials were performed in twelve different locations in Belgium during the seasons 2000-2001 and 2001-2002 to assess the agronomic performance of oilseed rape Ms8 x Rf3 and using the raw agricultural commodity seed for compositional analysis. The field trial design was a randomised split-block design to compensate for environmental effects within each site. At each location the agronomic performance of oilseed rape Ms8 x Rf3, half of them treated according to conventional herbicides regimes and the other half treated with glufosinate, was compared with the non-transgenic comparator and a local oilseed rape, both treated according to conventional herbicides regimes.

For compositional analysis at each location seeds were harvested from twelve plots, four Ms8 x Rf3 conventionally treated, four glufosinate treated and four non-transgenic counterpart conventionally treated samples, delivering a total of 144 samples. These samples were analysed on key nutrients and key toxicants (OECD, 2001), including proximates, micro-nutrients, such as minerals and tocopherols, anti-nutriens as phytic acid and glucosinolates, total spectrum of amino acids and fatty acids. The statistical analysis of the data performed with a 20% bioequivalence range was provided by the applicant. In the absence of an international agreement on the use of this methodology, the GMO Panel does not agree with this approach to be applied as a general principle. However the raw data on the above mentioned compounds, with the exception of glucosinolates content, do not indicate the occurrence of unintended effects as result of the genetic modification. An ANOVA statistical analysis of glucosinolate data as provided by the applicant shows some statistically significant differences between the contents of alkenyl glucosinolates and total glucosinolates in transgenic and non transgenic plants. However these differences were small and not considered relevant given the reported natural variations in these compounds in oilseed rape (OECD, 2001). The absolute differences in glucosinolate levels between the transgenic and non-transgenic oilseed rape seeds samples amounted up to 4 µmol/g on a mean total glucosinolate level in transgenic seed not exceeding 16 µmol/g, a level clearly below the threshold glucosinolate content of 25 µmol/g, set by the European Commission for certified seed of “double zero” varieties listed in the Common Catalogue of Varieties of Agricultural Plant Species (EC, 1999).

The difference in glucosinolate levels between the Ms8 x Rf3 oilseed rape and the non-transgenic comparator is explained by the applicant as a difference in genetic background. The GM line and comparator are not fully isogenic. In the development of the founder Ms8 and Rf3/Rf3 lines selection might have occurred, despite intensive backcrossing into the comparator line. For parameters that are relatively stable throughout the population it is without consequences, but for parameters that show great inter-individual variation, such as glucosinolate content, a minor difference in genetic background may cause consistent differences. The GMO Panel accepts this explanation since the applicant in the mean time

produced, by an extensive classical selection activity, Ms8 x Rf3 varieties with identical genetic modification, but with reduced glucosinolate levels.

3.2.3. Agronomic traits and GM phenotype

The agronomic performance was monitored from germination until harvest for a number of key agronomic parameters, such as establishment, vigour, flowering, height, maturity, lodging and yield. Agronomic performance was not affected except for higher yield due to hybrid vigour.

3.2.4. Nutritional assessment

In the human diet rapeseeds are only used after processing into food-grade vegetable oil. Rapeseed meal is used exclusively as a high protein feed supplement for livestock and poultry. For a long time, the use of rapeseed meal was limited by the presence of glucosinolates in the seeds (OECD, 2001). Glucosinolates themselves are generally considered to be innocuous, but their hydrolysis products have negative effects on animal production and feed palatability. Nowadays the “double zero” varieties with low erucic acid and low glucosinolates content are acceptable for monogastric animals at inclusion rates of 15% and in ruminants up to a maximum of 30% of the total diet. The VLGR (very low glucosinolate residue) cultivars with less than 20 µmols/g glucosinolates can be utilised without consequences for performance (Etienne and Dourmad, 1994). The mean total glucosinolate content of Ms8 X Rf3 seeds remained below 16 µmols/g dry matter and therefore can still be considered a VLGR variety. An additional feeding study in rabbits with Ms8 x Rf3 seeds revealed a comparable protein and fat digestibility of more than 75 and 90% respectively for both the transgenic and the comparator rapeseed, fed at an inclusion rate of 30%.

3.3 Conclusion

Investigation of all key nutrients and key toxicants in glufosinate and conventionally treated Ms8 x Rf3 oilseed rape and the non-transgenic comparator crop, grown for two seasons at twelve different locations, did not reveal compositional changes other than an increase of glucosinolate content in seeds of the genetically modified crop. These altered glucosinolate levels are considered to be a consequence of genetic variation between the GM and comparator line, rather than a result of the genetic modification. The average glucosinolate levels remained well below the maximum glucosinolate content set by the EC (1999) and at normal dietary inclusion rates this glucosinolate content will not affect the performance of livestock and poultry. As the extensive comparative compositional analysis provides no indication for unintended effects of the genetic modification, additional animal safety or nutrition studies are not appropriate.

4. Feed safety assessment

4.1. Issues raised by Member States

(1) Additional animal feeding studies, *i.e.* a broiler chicken study and a 90-day rat toxicity study, were requested to verify the absence of unintended toxic effects; (2) one Member State requested, as a principle, a comprehensive toxicological risk assessment instead of

deciding case-by-case which toxicological tests might be appropriate to perform a safety assessment.

4.2. Evaluation of relevant scientific data

4.2.1. Product description and intended use

The application concerns the import and processing of Ms8 x Rf3 oilseed rape for feed and industrial uses. Only seeds are used in the human food and animal feed chain. In the human diet rapeseed is only used after processing in food grade vegetable oil. The only oilseed rape product for human use is the refined oil, which has already been notified within the European Union[6]. The main side product from oil processing, the mechanically and/or solvent extracted meal is used as a protein rich feed for all classes of livestock. In seeds and unprocessed meal the PAT protein is present in low amounts (see 2.2.3.1). Barnase and Barstar proteins are not detected in these products. These proteins or the Barnase-Barstar complex are not detected in plant tissue outside the flower buds.

4.2.2. Stability during processing

Since Ms8 x Rf3 oilseed rape has been found to be substantially equivalent to conventional oilseed rape, except for the introduced traits, considerations of the stability of any altered nutritional components do not pertain to this application.

4.2.3. Toxicology

4.2.3.1. PAT protein used for safety assessment

From the novel proteins expressed in Ms8 x Rf3 oilseed rape only presence of the PAT protein in the animal feed chain can be expected. As the expression level of the PAT protein in the transgenic rape is very low, the safety studies are conducted with the protein produced in *E. coli*. Extensive examination of the nature of the plant and bacterial PAT proteins have shown a high degree of similarity, based on their size and sequence homology, their enzymatic activity, immunoreactivity and their absence of glycosylation (Herouet *et al.*, 2005). Therefore the GMO Panel accepts the test material derived from *E. coli* for the safety assessment of PAT protein in oilseed rape.

4.2.3.2. Toxicological assessment of expressed novel proteins in Ms8 x Rf3 oilseed rape

(a) Acute toxicity testing

[6] http://europa.eu.int/comm/food/dyna/gm_register/index_en.cfm

Because of the extremely fast proteolytic degradation the intrinsic toxic effect of the PAT protein was studied by using intravenous injection in mice at dose levels of 1 and 10 mg/kg body weight. Even at this relative high exposure level no signs of systemic toxicity were observed. Oral toxicity studies with the *bar* encoded PAT protein are not available, but a 14-day repeated dose oral toxicity study conducted in rats fed with the PAT protein encoded by the *pat* gene up to dietary levels of 50.000 ppm did not induce toxic effects. The PAT/pat and PAT/bar proteins have been shown to be structurally and functionally equivalent (Wehrmann *et al.*, 1996; Herouet *et al.*, 2005). Therefore, the GMO Panel accepts the data from the study with PAT/*pat* protein in order to assess the safety of the PAT/*bar* protein.

(b) Degradation in simulated digestive fluids

In vitro digestibility studies have shown the fast degradability of PAT protein, both in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). In SGF the bar gene encoded PAT protein was digested within 30 seconds of incubation in the presence of pepsin at pH 2.0. In SIF the PAT protein was digested into smaller fragments within seconds, but the complete degradation of a 7 kDa fragment was achieved within 5 minutes of incubation in the presence of pancreatin at pH 7.5.

4.2.3.3. Toxicological assessment of new constituents other than proteins

As the comparative analysis does not give any indication for unintended effects no new constituents other than the novel proteins are to be expected.

4.2.4. Toxicological assessment of the whole GM feed

Although some of the Member States asked for additional safety studies of the whole seed, the GMO Panel considers the safety of the oil and seed assured by the extensive comparative assessment, showing the compositional and nutritional equivalence of Ms8 x Rf3 oilseed rape and its non GM counterpart.

4.2.5. Allergenicity

The strategies used when assessing the allergenic risk focus on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in persons who are already sensitised 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 (CAC, 2003).

4.2.5.1. Assessment of allergenicity of the newly expressed proteins

The PAT protein is the only newly-expressed protein present in Ms8 x Rf3 seed. Barnase and Barstar proteins are only expressed in the tapetum cells of the flower buds and therefore will not occur in food or feed derived from Ms8 x Rf3 seed. The PAT protein has been previously evaluated for its safety in the context of other applications for the placing on the market of GM crops expressing PAT. In a recent study, Kleter and Peijnenburg (2002)

investigated whether the identical stretches of six or more contiguous amino acids were shared by transgenic proteins expressed in genetically modified crops and allergenic proteins. In the case of PAT encoded by the *bar* gene of *Streptomyces hygroscopicus*, no identities of six or more amino acids were observed by these authors. The applicant also showed the absence of amino acid sequence homology of the three newly-expressed proteins with known allergens and toxins.

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 transgenes in the genome of the host, for example through qualitative or quantitative modification of the pattern of expression of endogenous proteins or the expression of new unintended proteins coded by newly created putative cryptic open reading frames (ORF). The endogenous proteins do not appear relevant to the GMO Panel since oilseed rape is unknown as an allergenic feed ingredient. In humans, rare cases of occupational allergy to inhaled dust/flour derived from oilseed rape has been reported (Monsalve *et al.*, 1997; Suh *et al.*, 1998), but there is no reason to expect that the genetic modification might alter the allergenicity of the Ms8 x Rf3 oilseed rape. This conclusion is also supported by an *in silico* analysis on epitope homology, which indicates that the putative ORF sequences do not code for proteins with potential allergenic properties. None of the eleven putative ORF sequences in Ms8 x Rf3 oilseed rape codes for a protein that shares more than 35% identity on a window of 80 amino acids with an allergenic protein.

4.2.6. Nutritional assessment of GM feed

Compositional analysis is the starting point and cornerstone for the nutritional assessment of feed resources. A number of publications in the scientific literature have reported that once compositional equivalence has been established, when comparing conventional and GM feeds modified for agronomic input traits, nutritional equivalence can be assumed as livestock feeding studies have added little to the nutritional assessment of the GM crop (Clark and Ipharraguerre, 2001; Flachowsky and Aulrich, 2001; OECD, 2003, Flachowsky *et al.*, 2005). This fact is recognized 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), as once substantial equivalence has been demonstrated; there is no absolute requirement to conduct animal feeding studies.

As the extensive comparative compositional analysis of Ms8 x Rf3 oilseed rape provides no indication for unintended effects of the genetic modification under consideration in this opinion, additional animal safety or nutrition studies are not required.

4.2.7. Post-market monitoring of GM feed

Ms8 x Rf3 oilseed rape is, from a nutritional point of view, equivalent to conventional oilseed rape and will be used as any other oilseed rape. The GMO Panel is of the opinion that a post-market monitoring of the GM feed is not necessary.

4.3. Conclusion

Evidence has been provided that there is no acute toxicity from the PAT protein, the only transgenic protein expressed in the seeds. The GMO Panel is satisfied that the structural and functional identity of this protein produced in *E. coli* and in Ms8, Rf3 and Ms8 x Rf3 oilseed rape was established.

As the molecular characterisation of Ms8 x Rf3 oilseed rape did not reveal unexpected changes, the putative ORF sequences did not code for potential toxic proteins and the compositional comparison with the non GM comparator only revealed a slightly increased glucosinolate content, the GMO Panel is of the opinion that additional animal safety or nutritional testing is not necessary.

An allergy risk evaluation of the newly expressed PAT, Barnase and Barstar proteins as well as the proteins encoded by the putative ORF sequences has been performed, providing indirect evidence for a low probability of allergenicity. During seed processing occupational exposure to the oilseed rape may occur. Therefore, an allergenic risk from inhalatory exposure to dust/flour may exist, but there is no indication that the allergenic potency of Ms8 x Rf3 has changed due to any unintended effect. Since this application is for import, processing for feed and industrial uses of Ms8 x Rf3 oilseed rape only, and not for cultivation, the presence of possible toxic or allergenic proteins in the pollen is not considered relevant. Therefore the GMO Panel considers that additional experimental data on possible toxicity and allergenicity is not required.

5. Environmental risk assessment and monitoring plan

5.1 Issues raised by the Member States

(1) Release of seeds and plants into the environment through accidental spillage during transport and/or handling of seeds was considered to represent a risk that was insufficiently addressed by the applicant; (2) two Member States requested a full environmental risk assessment of Ms8, Rf3 and Ms8 x Rf3 oilseed rape; (3) comments were made on the monitoring plan which was found was insufficiently detailed or not in line with regulatory requirements.

5.2. Evaluation of relevant scientific data

5.2.1. Environmental risk assessment

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

Since cultivation was excluded from the assessment by EFSA (see BACKGROUND and TERMS OF REFERENCES), the application C/BE/96/01 for Ms8, Rf3 and Ms8 x Rf3 oilseed rape was assessed for import and processing for feed and industrial uses only, and thus the Panel did not assess the scientific information on environmental effects associated with the cultivation of Ms8, Rf3 and Ms8 x Rf3 oilseed rape. Oilseed rape is an out-crossing, open pollinating crop with effective dispersal systems for pollen. It also produces large quantities of small seeds. These seeds are very robust and can remain viable in soil for many years. Oilseed rape also exists as a weed in other crops and can colonise semi-natural habitats in Europe. It can survive winter temperatures as low as -20°C. Unintended seed dispersal and gene flow via pollen thus pose the potential for exposure to the conventional varieties and several wild relatives in several European countries (Devos *et al.*, 2004; Pessel *et al.*, 2001). In February

2005, the Japanese Environmental Studies Institute indeed reported the presence of oilseed rape genetically modified for tolerance to an herbicide around Japanese port facilities (EC, 2005a and 2005b). However, if escape into the environment occurs the events would only show enhanced fitness in the presence of the glufosinate herbicide. This herbicide is not widely used in arable farming systems except as a pre-harvest desiccant, but it is used in uncultivated land in orchards and other perennial crops or, occasionally, as a herbicide used for general weed control on uncultivated land. The GMO Panel is thus of the opinion that the Ms8 x Rf3 oilseed rape would generally not show any enhanced fitness and would behave as conventional oilseed rape. However, volunteers or feral plants may establish in certain farming systems and thus the evolution of transgenic populations is feasible. These plants can be managed by other herbicides (Warwick *et al.*, 2004; Devos *et al.*, 2004) Thus gene escape through spillage during handling and transport should be minimised by adequate management measures and quality control. This should be in line with the recommendation previously published by the Commission on how to deal with accidental spillage if it should occur (EC, 2005b).

5.2.1.2. Potential for gene transfer

(a) Plant to bacteria gene transfer

No horizontal gene flow effects or consequences are indicated by this transformation event particularly as the *bar, barstar* and *barnase* genes originate from soil bacteria *Streptomyces hygroscopicus* and *Bacillus amyloliquefaciens* respectively and thus already occur in some soil microbial populations.

(b) Plant to plant gene transfer

Oilseed rape can also establish feral populations in Europe and cross with other *Brassica*-relatives especially *Brassica rapa* and disperse genes through this species (OECD, 1997; Chèvre *et al.*, 2004). Thus spilled seeds could result in escaped GM plants that survive and establish which could outcross and disperse genes to other plants or plant species. However, if gene flow or escape into the environment occurs the events would only show enhanced fitness in the presence of the complementary herbicide as demonstrated for herbicide tolerant GT73 oilseed rape (Crawley *et al.*, 2001). As stated under section 5.2.1.1, this herbicide is not widely used in arable farming systems. The GMO Panel is thus of the opinion that hybrids with other oilseed rape varieties or wild relatives would generally not show any enhanced fitness and would behave as conventional plants. However many of these species are successful weeds, volunteers or feral plants in certain farming systems. Thus, in line with section 5.2.1.1, gene escape should be minimised by adequate management measures and quality control during transportation, storage, handling in the environment and processing into derived products.

5.2.1.3. Potential interactions of the GM plant with non-target organisms

This point was not considered as an issue by the Member States and by the GMO Panel.

5.2.1.4. Monitoring

The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC are to (1) 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 (2) identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment which were not anticipated during the environmental risk assessment. The scope of the monitoring plan provided by the applicant is in line with the intended uses for the GMO since the application does not cover cultivation. No potential risks requiring the establishment of a case-specific monitoring plan were identified in the environmental risk assessment.

General surveillance is related to risk management, and thus a final adoption of the general surveillance plan falls outside the mandate of EFSA. However, the GMO Panel gives its opinion on the scientific quality of the general surveillance plan provided by the applicant (EFSA, 2004b). The only significant exposure of the environment to the transgenic oilseed rape would be related to accidental spillage. In this respect, the applicant describes adequately the stewardship of the handling of the imported seeds to minimise spillage.

In this instance the GMO Panel is of the opinion that the general approaches and measures proposed by the applicant are appropriate. The GMO Panel recommends that critical points for accidental release and spillage are identified on each route of importation and processing, and that appropriate management, surveillance and inspection be developed for these points.

The GMO Panel is content with the proposal made by the applicant on the reporting intervals and procedures.

5.3. Conclusion

Oilseed rape events Ms8, Rf3 and Ms8 x Rf3 are comparable with oilseed rape bred traditionally, except for the expression of tolerance to glufosinate herbicide and a hybrid pollination control system. According to the terms of reference, the GMO Panel only considered the transformation events Ms8, Rf3 and the Ms8 x Rf3 hybrids to be imported and processed in feeds and for industrial uses, but did not address the cultivation. The GMO Panel is of the opinion that sufficient information has been provided by the applicant to demonstrate that Ms8, Rf3 and Ms8 x Rf3 oilseed rape are unlikely to pose a risk to the environment given their intended uses. No potential risks requiring the establishment of a case-specific monitoring plan were identified in the environmental risk assessment.

Although no environmental risk has been identified, there is a small likelihood of environmental exposure through accidental or unintentional spillage. The likelihood of spillage is highest at the transport and seed handling stages where seed lots are exposed to the open environment. The applicant has provided a plan for monitoring and mitigation, in order to minimise dispersal. The scope of the monitoring plan for import provided by the applicant is in line with the intended uses for the GMO.

The GMO Panel recommends that critical points for accidental release and spillage are identified on each route of importation and processing, and that appropriate management, surveillance and inspection be developed for these points.

CONCLUSIONS AND RECOMMENDATIONS

The GMO Panel considered all the information made available by the applicant as sufficient to assess the safety of Ms8, Rf3, Ms8 x Rf3 oilseed rape with reference to the intended uses (import and processing of Ms8 x Rf3 oilseed rape for feed and industrial purposes) and to address all the specific questions raised by the Member States related to risk assessment, except the ones with respect to cultivation.

The GMO Panel has considered information provided on (1) the molecular inserts within the transgenic events Ms8 and Rf3 and the resulting hybrid, (2) the compositional analysis of Ms8 x Rf3 oilseed rape and its non transgenic comparator, (3) the safety of the proteins expressed and (4) the potential for risks associated with any changes to the toxicological, allergenic or nutritional properties of Ms8 x Rf3 oilseed rape.

From all evidences provided, the GMO Panel is of the opinion that Ms8, Rf3, Ms8 x Rf3 oilseed rape is as safe as conventional oilseed rape for humans and animals and, in the context of the proposed uses, for the environment.

The GMO Panel considers the environmental risk assessment and monitoring plan for import acceptable in the light of the intended uses. The GMO Panel advises that appropriate management systems should be in place to minimize accidental loss and spillage of transgenic oilseed rape during transportation, storage, handling in the environment and processing into derived products.

DOCUMENTATION PROVIDED TO EFSA

1. Note from Mrs. C. Day - DG Environment, European Commission – to Mr. Koëter, dated 10 January 2005, concerning a request for EFSA opinion on application C/BE/96/01 (Ms8, Rf3 and Ms8 x Rf3 oilseed rape) under Directive 2001/18/EC (ref. ENVB4/KT/bv/D(04)000043) and related enclosures.

2. Letter from EFSA to applicant, dated 4 April 2005, with request for clarification of the scope (ref. SR/EVH/jq (2005) 389).

3. Letter from the applicant to EFSA, dated 8 April 2005, providing clarification on the scope of the application C/BE/96/01 and submission to EFSA of the application for the placing on the market of genetically modified oilseed rape Ms8, Rf3 and Ms8 x Rf3 in accordance with Directive 2001/18/EC.

4. Note from EFSA to Mrs Day - DG Environment, European Commission –, dated 17 May 2005, as acknowledgment receipt of the request for an opinion on application C/BE/96/01 (ref HK/SR/SM/jq (2005) 563).

5. Letter from EFSA to applicant, dated 23 June 2005, concerning a request for additional information (ref. SR/SM/jq (2005) 773).

6. Additional information submitted by the applicant on 1st July 2005 in response to EFSA's request for further information.

7. Letter from EFSA to applicant, dated 26 July 2005, concerning a request for additional information (ref. SR/SM/sp (2005) 945).

8. Additional information submitted by the applicant on 31 August 2005 in response to EFSA's request for further information.

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ACKNOWLEDGEMENT

The GMO Panel wishes to thank Gijs Kleter and Richard Phipps for their contributions to the opinion.