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Opinion of the Scientific Panel on Genetically Modified Organisms on applications (references EFSA-GMO-UK-2005-25 and EFSA-GMO-RX-T45) for the placing on the market of the glufosinate-tolerant genetically modified oilseed rape T45, for food and feed uses, import and processing and for renewal of the authorisation of oilseed rape T45 as existing product, both under Regulation (EC) No 1829/2003 from Bayer CropScience[1]

(Questions No EFSA-Q-2005-278 and EFSA-Q-2007-154)

Adopted on 30 January 2008

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SUMMARY

This document provides an opinion of the Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) on genetically modified oilseed rape T45 (Unique Identifier ACS-BNØØ8-2) developed to provide tolerance to glufosinate-ammonium herbicides.

In delivering its scientific opinion, the GMO Panel considered the new application EFSA-GMO-UK-2005-25, additional information provided by the applicant (Bayer CropScience) and the scientific comments submitted by the Member States. The scope of application EFSA-GMO-UK-2005-25 is for food and feed uses, import and processing of oilseed rape T45 and all derived products, but excluding cultivation of the crop in the EU. Information provided in the context of the application for renewal of the authorisation of oilseed rape T45 as existing product, submitted under Regulation (EC) No 1829/2003 (Reference EFSA-GMO-RX-T45), was also taken into account. The scope of application EFSA-GMO-RX-T45 covers the continued marketing of existing food additives and feed materials produced from oilseed rape T45.

A single risk assessment for all intended uses of genetically modified oilseed rape T45 has been performed by the GMO Panel and one single scientific opinion for both applications submitted under Regulation (EC) No

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1829/2003 is issued. The GMO Panel assessed oilseed rape T45 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 (EFSA, 2006). The scientific assessment included molecular characterization of the inserted DNA and expression of the new protein. A comparative analysis of agronomic traits and composition was undertaken and the safety of the newly expressed protein and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional quality. An assessment of environmental impacts and the post-market environmental monitoring plan were undertaken.

Oilseed rape T45 was transformed by *Agrobacterium tumefaciens*-mediated gene transfer technology. Oilseed rape T45 expresses the *pat* gene leading to the production of the enzyme phosphinothricin acetyl-transferase (PAT) that acetylates L-glufosinate-ammonium. The PAT enzyme confers tolerance to glufosinate-ammonium herbicides (trade names: Liberty®, Ignite®, Finale®, Basta®).

The molecular characterisation data established that only one copy of the gene cassette (corresponding to the T-DNA region of plasmid pHOE4/Ac(II)) is integrated in the oilseed rape genomic DNA. Appropriate analyses of the integration site including sequence determination of the inserted DNA and flanking regions and bioinformatic analysis have been performed. Bioinformatic analysis of junction regions demonstrated the absence of any potential new ORFs coding for known toxins or allergens. The expression of the genes introduced by genetic modification has been sufficiently analysed and the stability of the genetic modification has been demonstrated over several generations.

The GMO Panel is of the opinion that the molecular characterisation of the DNA insert and flanking regions of oilseed rape T45 does not raise any safety concern, and that sufficient evidence for the stability of the genetic modification was provided.

Analyses carried out on materials from T45 oilseed rape and its non-GM comparators in a representative range of environments and seasons do not provide indication of biologically relevant compositional and agronomical changes. The GMO Panel is of the opinion that the composition of oilseed rape T45 does not deviate from that of conventional oilseed rape varieties, except for the introduced trait.

The PAT protein induced no adverse effects in acute dose oral toxicity studies in rodents. In addition, the PAT protein is rapidly degraded in simulated gastric fluid and inactivated during heat treatments.

A 42-day feeding study with broilers did not indicate differences in the nutritional value of T45 oilseed rape versus the non-GM comparator and confirms the nutritional equivalence of T45 oilseed rape containing diet in comparison with a conventional diet in broiler chickens.

The applications for oilseed rape T45 concern food and feed uses, import and processing of oilseed rape T45 and all derived products, but excluding cultivation of the crop in the EU. There is therefore no requirement for scientific assessment of possible environmental effects associated with the cultivation of oilseed rape T45. There are no indications of increased likelihood of establishment or survival of feral oilseed rape plants in case of accidental release into the environment of oilseed rape T45 seeds during transportation and processing. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of oilseed rape T45 since cultivation is excluded. The monitoring plan provided by the applicant is in line with the EFSA Guidance document (EFSA, 2006) and the Opinion of the GMO Panel on post-market environmental monitoring (EFSA, 2006). However, the GMO Panel advises that appropriate management systems should be in place to minimise accidental loss and spillage of transgenic oilseed rape during transportation, storage, handling in the environment and processing into derived products.

In conclusion, the GMO Panel considers that the information available for oilseed rape T45 addresses the scientific comments raised by the Member States and that the GM oilseed rape T45 is as safe as its non genetically modified counterpart with respect to potential effects on human and animal health or the environment. Therefore the GMO Panel concludes that oilseed rape T45 is unlikely to have any adverse effect on human or animal health or on the environment in the context of its intended uses.

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

Key words: GMO, *Brassica napus,* oilseed rape, T45, glufosinate-ammonium herbicide, glufosinate-tolerant, *pat* gene, PAT protein, ACS-BNØØ8-2, human and animal health, environment, import, processing, Regulation (EC) No 1829/2003, renewal, existing product.

TABLE OF CONTENTS

BACKGROUND

APPLICATION EFSA-GMO-UK-2005-25

On 4th November 2005, EFSA received from the Competent Authority of United Kingdom an application (Reference EFSA-GMO-UK-2005-25), for authorisation of the glufosinate-tolerant genetically modified oilseed rape T45 (Unique Identifier ACS-BNØØ8-2), submitted by Bayer CropScience within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed (EC, 2003) for food and feed uses, import and processing.

After receiving the application EFSA-GMO-UK-2005-25 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the Member States as well as the European Commission and made the summary of the dossier publicly available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 2 March 2007 EFSA received additional information requested under completeness check (requested on 5 December 2006) and on 13 April 2007 EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the European Commission and consulted nominated risk assessment bodies of the Member States, including the national Competent Authorities within the meaning of Directive 2001/18/EC (EC, 2001) following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. The Member State bodies had three months after the date of receipt of the valid application (until 13 July 2007) within which to make their opinion known.

The GMO Panel carried out a scientific assessment of genetically modified (GM) oilseed rape T45 taking into account 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, 2006).

On 29 August 2007 and 06 December 2007, the GMO Panel asked for additional data on oilseed rape T45. The applicant provided the requested information on 10 October 2007 and on 18 December 2007. After receipt and assessment of the full data package, the GMO Panel finalized its risk assessment of oilseed rape T45.

The GMO Panel carried out a scientific assessment of the GM oilseed rape T45 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003, taking into consideration the scientific comments of the Member States and the additional information provided by the applicant.

In giving its opinion on GM oilseed rape T45 to the European Commission, the Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of six months from the receipt of the valid application. As additional information was requested by the EFSA GMO Panel, the time-limit of 6 months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

According to Regulation (EC) No 1829/2003, the EFSA opinion shall include a report describing the assessment of the food and feed and stating the reasons for its opinion and the information on which its opinion is based. This document is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation and thus will be part of the overall opinion in accordance with Articles 6(5) and 18(5).

APPLICATION EFSA-GMO-RX-T45

On 29th June 2007, EFSA received from the European Commission an application for renewal of the authorisation of GM oilseed rape T45 (EFSA-GMO-RX-T45) (Unique Identifier ACS-BNØØ8-2), submitted by Bayer CropScience within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed (EC, 2003).

After receiving the application EFSA-GMO-RX-T45 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the Member States and the European Commission and made the summary of the dossier available to the public on the EFSA website.

EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3), 5(5), 17(3) and 17(5) as well as 8(1) and 20(1) of Regulation (EC) No 1829/2003. On 7 September 2007 EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

All data required for the risk assessment of the application EFSA-GMO-RX-T45 have also been provided in application EFSA-GMO-UK-2005-25.

The GMO Panel performed one single comprehensive risk assessment for all intended uses of genetically modified oilseed rape T45 and issued a single comprehensive scientific opinion for both applications submitted under Regulation (EC) No 1829/2003.

TERMS OF REFERENCE

The GMO Panel was requested to carry out a scientific assessment of the genetically modified oilseed rape T45 for food and feed uses and import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of GMOs or food/feed containing or consisting of GMOs, conditions for the protection of particular

ecosystems/environments and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)e of Regulation (EC) No 1829/2003.

The EFSA GMO Panel was not requested to give an opinion on information required under Annex II to the Cartagena Protocol, nor on the proposals for labelling and methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to GMO risk management.

ASSESSMENT

1. Introduction

The genetically modified (GM) oilseed rape T45 (Unique Identifier ACS-BNØØ8-2) was assessed with reference to its intended uses, taking account of 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, 2006). In its evaluation the GMO Panel also considered the scientific comments that were raised by Member States on application EFSA-GMO-UK-2005-25. The risk assessment presented here is based on the information provided in the applications relating to oilseed rape T45 submitted in the EU including additional information from the applicant.

The applicant stated in the applications, that the sale of oilseed rape T45 derived lines by its retailers was discontinued and all oilseed rape T45 lines have been deregistered as of 2003 with the exception of line LL2393 that was still for sale in 2005 until exhaustion of inventory. According to the applicant, stocks of all other oilseed rape T45 lines has been recalled from distribution and destroyed. The applicant commits not to commercialize the event in the future and the import will therefore be restricted to adventitious levels in oilseed rape commodity. Thus, the incidence of oilseed rape T45 in the EU is expected to be limited.

2. Molecular characterisation

2.1. Issues raised by the Member States

Member States raised questions concerning the suitability of the control variety used for the molecular characterisation and the need for sequence data of the regions flanking the insert.

2.2. Evaluation of relevant scientific data

2.2.1 Transformation process and vector constructs

The glufosinate-tolerant oilseed rape transformation event T45 contains a synthetic version of the native *pat* gene isolated from *Streptomyces viridochromogenes* (ATCC14920), a common soil microbe, not known to be a human, animal or plant pathogen. In the synthetic gene the nucleotide content was adapted to the codon usage of the plant. The *pat* gene codes for the phosphinothricin acetyl-transferase (PAT) that acetylates glufosinate and thereby inactivates the herbicide.

The recipient organism is oilseed rape, *Brassica napus* subspecies *oleifera* cultivar AC Excel. *Brassica napus* protoplasts were transformed using *Agrobacterium tumefaciens* containing plasmid pHOE4/Ac(II). Selection of transformed plants was based on increased tolerance to glufosinate. The plasmid pHOE4/Ac(II) comprises the origin of replication from *E. coli* vector PiAN7 and the *ori*V and *ori*T regions from the vector RK2, allowing replication in *A. tumefaciens*; the *aadA* gene conferring resistance to streptomycin and spectinomycin; an artificial T-DNA region consisting of the left border sequence from pTiAch5 and the right border from pTiT37 and a multilinker cloning site in which the chimeric *pat* gene construct (P*35S*::*pat*::T*35S*) has been inserted. P*35S*::*pat*::T*35S* contains the *35S* promoter of the Cauliflower Mosaic Virus and the *pat* coding sequence followed by the *35S* terminator of the Cauliflower Mosaic Virus.

2.2.2. Transgenic constructs in the genetically modified plant

The inserted DNA within event T45 is fully characterised by PCR, Southern analysis and sequencing. Southern analysis demonstrated that only one copy of the gene cassette (corresponding to the T-DNA region of plasmid pHOE4/Ac(II)) is integrated in the oilseed rape genomic DNA. The fact that the transgene is inherited as a single dominant trait supports this observation. The absence of vector sequences from pHOE4/Ac(II) was demonstrated by Southern analysis and PCR.

The inserted DNA (1364 bp) as well as the 5' (994 bp) and 3' (911 bp) flanking regions were sequenced. The DNA sequence of the T45 insert is identical to the corresponding fragment of the plasmid used for transformation. The inserted T-DNA copy starts with the last eight bases of the left border sequence and ends at bp 3 of the right border sequence. A fragment of 48 bp present at the wild type locus is deleted in the transgenic locus. At the 5' junction, 9 bp are inserted as a putative duplication of a sequence that is present in the 3' flanking region. In addition, two bp are inserted at the 3' junction.

Bioinformatic analysis was performed on the 5' and 3' junction regions of oilseed rape event T45. A total of thirty six open reading frames (ORFs, minimal size of 3 amino acids) were identified of which four were newly created due to the insertion event. Analysis of the first ATG codon context sequence, the promoter and 3' untranslated sequences revealed that none of these ORFs can be considered as trancriptionally and/or translationally active. No significant sequence similarities were found with known toxins or allergens.

BLASTn analysis indicated that stretches of homology with oilseed rape DNA can be found in the 5' and 3' flanking regions. These sequences are likely to be repetitive elements. There is no indication that the T-DNA has been integrated in a known functional gene of the oilseed rape genome.

2.2.3. Information on the expression of the insert

Northern analysis shows that the *pat* gene is expressed in leaves, stems and roots but was not detected in seeds. However, the presence of PAT protein was demonstrated in seeds by ELISA, with PAT representing 0.0027% (w/w) of the total extractable protein or 930 ng/g dry weight.

2.2.4. Inheritance and stability of inserted DNA

The genetic stability of transformation event T45 was demonstrated at the genomic level over multiple generations by Southern analysis. Segregation analysis shows that the T45 event is inherited as a dominant, single locus trait. Phenotypic stability has been confirmed by stable tolerance to the herbicide for T45 lines and varieties derived from this event grown in Canada since 1993.

2.3. Conclusion

The molecular characterisation provided for the transformation event T45 is sufficient for the safety assessment. The bioinformatic analysis of the inserted DNA and flanking regions does not raise any safety concern. The expression of the genes introduced by genetic modification has been sufficiently analysed and the stability of the genetic modification has been demonstrated over several generations. The GMO panel considers that the molecular characterization does not indicate any safety concern.

3. Comparative analysis

3.1 Issues raised by Member States

Questions were raised regarding the statistically significant differences in the compositional analysis of T45 oilseed rape compared to the non-GM comparators.

3.2 Evaluation of relevant scientific data

Having considered the information provided in the applications and the Member States comments, the GMO Panel requested to the applicant further information on the number of replicates in the compositional analysis, on the pedigree and the choice of the comparator, and on the statistical evaluation of the agronomic characteristics of T45 oilseed rape.

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

For compositional studies T45 oilseed rape was compared to its non-transgenic parent variety AC Excel or to other comparators[2], derived from AC Excel. As all of these materials are varietal associations (i.e. mixtures of male sterile and male fertile plants) it is not possible to have isogenic counterparts according to the Guidance Document (EFSA, 2006), but only genetically related controls. Additional comparators used in the comparative assessment were Cyclone, Legend, Innovator, AC Elect and Variety A[3]. All field trials were carried out in Canada at various locations during the years 1995, 1996, 2000 and 2004. The studies were performed in a complete randomized block design with 3-8 replicates, but experimental conditions selected for each trial do not allow a full comparison across sites and seasons. The Panel noted that the dataset obtained over the years did not indicate consistent differences between the GM and the various comparators (see section 3.2.2).

3.2.2. Compositional analysis

For compositional analysis seeds were harvested from field trials performed at six different locations in Canada in 2000 and 2004. Within each site, T45 sprayed with the herbicide glufosinate-ammonium and conventionally treated T45 oilseed rape was cultivated together with a non-transgenic comparator (in 2000 the oilseed rape variety AC Elect, and in 2004 variety A) sprayed with conventional herbicide.

[2] Full comparators' name is confidential (see text from Company)
[3] Full comparator name is confidential (see text from Company)

The compositional analysis of T45 oilseed rape and the respective non-transgenic counterparts was carried out with respect to proximates and fibre compounds, micro-nutients, such as minerals and tocopherols, anti-nutrients such as phytic acid and glucosinolates, and a whole spectrum of amino acids and fatty acids. The overall set of compounds analyzed is in agreement with the key nutrients, anti-nutrients and toxicants recommended by OECD (OECD, 2001).

The analytical data were statistically evaluated by analysis of differences between T45 oilseed rape and its counterpart with analysis of variance (ANOVA). For proximates and fibres the 2000 field trial data revealed lower levels of moisture, total dietary fibre, acid- and neutral detergent fibre, and crude fibre. However, in the 2004 data differences in moisture and fibre content were not observed.

With regard to minerals, ANOVA testing revealed a significant difference in zinc levels between the glufosinate sprayed T45 and the non-transgenic comparator in the 2004 data. Such difference was not observed between the non-sprayed T45 and the non-transgenic comparator. With respect to micronutrients, alpha tocopherol levels were significantly higher in T45 oilseed rape in the 2004 field trials.

Significantly increased levels of the anti-nutrients indole glucosinolates and total glucosinolates were found in both sprayed and non-sprayed T45 oilseed rape in the 2000 and 2004 field trials. However, the absolute difference in glucosinolate levels between the transgenic and non-transgenic oilseed rape seeds samples were small (14-17 %) in comparison with the natural variation. Slight genetic differences between the transgenic and non-transgenic oilseed rape varieties may explain this consistent difference in glucosinolate levels. The total glucosinolate level in T45 oilseed rape was less than 16 micro mol/g seed, a level which is below the threshold glucosinolate content of 25 micro mol/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).

In the analysis of amino acids from samples collected in 2000 an increased amount of tyrosine (15 %) was noted in the glufosinate sprayed T45 oilseed rape. No difference was observed between the non-sprayed transgenic T45 oilseed rape and its comparator variety. Statistical evaluation of the amino acid analyses of 2004 did not reveal any difference between the transgenic and the non-transgenic oilseed rape varieties.

In the ANOVA-testing for fatty acid content, differences between T45 oilseed rape and its comparator were noted with respect to C16:0, C18:0, C18:2, C18:3 and C20:0. These differences were small (about 10 %) except for linolenic acid levels, which were 22 % higher in T45 oilseed rape. In a comparison of the fatty acid profile of T45 oilseed rape with three commercial varieties carried out in 1994 and 1995 no increase of linolenic acid was observed.

Although the GMO Panel noted that the dataset provided in the dossier presented some deficiencies, the assessment of the available dataset does not indicate biologically relevant compositional alterations due to the genetic modification. The GMO Panel is of the opinion that the composition of oilseed rape T45 doesn’t deviate from that of conventional oilseed rape varieties except for the presence of the PAT protein.

3.2.3. Agronomic traits and GM phenotype

The applicant provided information on the agronomic performance and phenotypic characteristics of genetically modified T45 oilseed rape and on the non-genetically modified AC Excel and two commercial oilseed rape varieties (Cyclone and Legend) cultivated at several locations, representing different oilseed

rape growing areas in Canada during 1995, 1996 and 1997. The traits analyzed in these studies were plant height, yield, lodging resistance, disease (blackleg) resistance, plant development and days to maturity. T45 tends to flower and subsequently to mature later than the comparators. These observations do not impact on the safety of oilseed rape T45. The GMO Panel assessed the data provided and considers that T45 oilseed rape agronomic characteristics do not deviate from those of currently grown non-GM oilseed rape, with the exception of the newly introduced trait and maturity.

3.3 Conclusion

Analyses carried out on materials from T45 oilseed rape and its comparators do not provide indication of biologically relevant compositional and agronomical changes. The GMO Panel is of the opinion that the composition of oilseed rape T45 doesn't deviate from that of conventional oilseed rape varieties, except for the introduced transgenic trait.

4. Food/Feed safety assessment

4.1. Issues raised by Member States

Member States raised issues regarding a) the use of a PAT protein produced in bacteria in the safety studies instead of the plant expressed PAT protein, b) the lack of sub-chronic toxicity studies using the whole GM food/feed and c) the allergenicity testing of the newly expressed PAT protein, including bioinformatics analysis.

4.2. Evaluation of relevant scientific data

4.2.1. Product description and intended use

Although the seed production of oilseed rape T45 has been discontinued by the applicant, this application concerns the import and processing of T45 oilseed rape for food and feed uses. In the human diet rapeseed is only used after processing into refined vegetable oil. The main by-product from oil processing, the mechanically and/or solvent extracted meal, is used as a protein rich feed for all classes of livestock. The presence of T45 oilseed rape in food and feed products is expected to be restricted to adventitious levels in oilseed rape commodity. Thus, the incidence of oilseed rape T45 in the EU is expected to be limited.

The modification of oilseed rape T45 is intended to improve agronomic performance only and it is not intended to influence the nutritional properties, production processes and overall use of oilseed rape as a crop.

4.2.2. Effects of processing

The effect of temperature on recombinant PAT protein encoded by the pat gene and produced by *E. coli* was assessed by SDS-PAGE and protein staining following incubation for up to 60 minutes at 60°, 75° and 90°C. No degradation of the PAT protein was observed under these temperature conditions. PAT protein was detected by ELISA only in trace amounts in toasted meal from T45 oilseed rape and not detected in blended, degummed, refined, bleached and deodorized oil. Given the toxicological profile and allergenic properties of PAT protein the Panel is of the opinion that no further stability analyses are required.

Taking into account the results of compositional analysis providing no indication of biologically relevant compositional and agronomical changes, the Panel has no reason to assume that the processing characteristics of oilseed rape and derived products from T45 would be different from those of non-GM processed oilseed rape.

4.2.3. Toxicology

4.2.3.1. PAT protein used for safety assessment

Due to the low expression level of the PAT protein in T45 oilseed rape and the difficulties encountered in isolating a sufficient quantity of purified protein from GM oilseeds, protein safety studies were conducted with a PAT protein encoded by the *pat* gene (PAT/*pat* protein) and expressed in *E. coli*. The equivalence of the PAT protein produced by *E. coli* and T45 oilseed rape was shown by Edman degradation, Western analysis, protein mobility in SDS-PAGE, mass spectrometry, and determination of enzymatic activity. All these methods confirmed the equivalence of the bacterial and the plant PAT protein. Based on the identified similarity in structure and function between these proteins, the GMO Panel accepts the use of PAT/*pat* test material derived from *E. coli* for the safety testing of the PAT protein present in T45 oilseed rape.

4.2.3.2. Toxicological assessment of expressed novel protein in T45 oilseed rape

(a) Acute toxicity testing

The applicant provided a single dose toxicity study on female mice intravenously injected with 1 or 10 mg per kg body weight of the PAT/pat protein. Animals were observed for 15 days after dosing and macroscopic examination of internal organs was carried out at necropsy. Even at the relatively high dose of 10 mg/kg body weight, no signs of systemic toxicity were observed.

(b) Degradation in simulated digestive fluids

The PAT/pat protein was used in the *in vitro* digestibility test with simulated gastric fluid containing pepsin. Degradation occurred rapidly (within 30 seconds at pH 2) as demonstrated by Coomassie blue staining of proteins following SDS–PAGE.

Rapid degradation (within seconds) of the 25kDa PAT/pat protein was also demonstrated by Western analysis after incubation in simulated intestinal fluid (pH 7.5) containing pancreatin. During degradation residual fragments of 5 to 14 kDa appeared transiently. These fragments disappeared after 30 seconds of incubation.

The *in vitro* digestion experiments demonstrate that the PAT/pat protein is rapidly degraded in simulated gastric and intestinal conditions.

(c) Bioinformatic studies

Searches for amino acid sequence homology of the PAT/pat protein expressed in T45 oilseed rape with sequences from protein databases indicated significant homology only with other acetyltransferases. There was no sequence homology with known toxic proteins.

4.2.3.3. Toxicological assessment of new constituents other than proteins

Since no new constituents other than the above mentioned PAT protein were expressed in T45 oilseed rape, and there were no indications of alterations of endogenous compounds levels, a toxicological assessment for new constituents is not applicable.

4.2.4. Toxicological assessment of the whole GM food/feed

As no indication of biologically relevant compositional and agronomical changes was identified for seeds from T45 oilseed rape except for the presence of the PAT protein, the Panel did not see the need for further animal safety studies with the whole food/feed (EFSA, 2006).

4.2.5. Allergenicity

The strategies used when assessing the potential 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 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 (CAC, 2003; EFSA, 2006).

4.2.5.1. Assessment of allergenicity of the newly expressed proteins

The *pat* gene originates from *Streptomyces viridochromogenes,* a soil microorganism that is not known to be allergenic. The PAT/pat protein was subjected to bioinformatics analysis. The results of sequence homology search for identical sequences of at least 8 contiguous amino acids showed no similarities between known allergens and the PAT/pat protein expressed by T45 oilseed rape. An additional search for overall similarity indicated no sequence similarity with known allergens. Furthermore, PAT/pat is not stable in an acidic environment and it is rapidly degraded under simulated gastric and intestinal conditions. Based on these results the GMO Panel considers that the newly expressed PAT/pat protein is not likely to be allergenic.

4.2.5.2. Assessment of allergenicity of the whole GM plant

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. However, given that no biologically relevant agronomic and compositional changes were identified (with the exception of the introduced trait), no increased allergenicity is anticipated for T45 oilseed rape. The Panel is aware of two case studies indicating that children with atopic dermatitis reacted to protein extracts from Brassica rapa and napus seeds when topically exposed (Poikonen et al., 2006; Puumalainen et al., 2006). Nevertheless oilseed rape is not considered a common allergenic food.

Given the low expected exposure to T45 oilseed rape proteins through refined oil consumption and pollen dispersal (the production of T45 oilseed rape has been discontinued and the scope of this application does not include cultivation), the Panel is of the opinion that no further information on T45 allergenicity is needed.

4.2.6. Nutritional assessment of GM food/feed

The comparative, compositional and agronomic analyses of T45 oilseed rape and its non-GM comparators showed no biologically relevant agronomic and compositional changes in T45 oilseed rape. Therefore the GMO Panel concludes that no nutritional studies are needed (Guidance document - EFSA, 2006).

However the applicant provided a 42-day broiler feeding study to evaluate the nutritional performance of T45 oilseed rape. Three groups of 140 birds consisting of 14 pens (7 pens/gender) with 10 animals each were fed diets containing T45 oilseed rape sprayed with glufosinate or non-sprayed with the target herbicide, or conventional (non-GM) oilseed rape, respectively. The inclusion rate of oilseed rape meal in the starter, grower and finisher diets was always approximately 10 %. No statistically significant differences in body weight gain, feed consumption, feed conversion or weights of chilled carcass, abdominal fad pad, breast, thigh, leg or wing weights among treatment groups were found. This study confirms the nutritional equivalence of T45 oilseed rape containing diet in comparison with a conventional diet in broiler chickens.

4.2.7. Post-market monitoring of GM food/feed

The risk assessment concluded that no data have emerged to indicate that T45 oilseed rape is any less safe than its non-GM comparators. In addition, no biologically relevant agronomic and compositional changes were identified in T45 oilseed rape. Therefore, in line with the Guidance document (EFSA, 2006), the GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

4.3. Conclusion

No toxicity of the PAT/pat protein was observed in a single dose acute toxicity study in mice using intravenous injection. In addition, the PAT/pat protein is rapidly degraded under simulated gastric and intestinal conditions. The PAT/pat protein shows no homology with known toxic proteins and/or allergens. Furthermore, PAT proteins have been extensively assessed in previous opinions of the EFSA GMO Panel and found to be safe (EFSA 2006c, 2007). No concerns were raised regarding the safety of the PAT proteins.

The comparative analyses showed no biologically relevant agronomic and compositional changes in T45 oilseed rape, except for the introduced trait. The GMO Panel is of the opinion that T45 oilseed rape is as safe as its non GM counterparts and that the overall allergenicity of the whole plant is not changed through the

genetic modification. In a 42-day broiler feeding study the nutritional equivalence of T45 oilseed rape meal and conventional oilseed rape containing diets was confirmed. The GMO Panel considers that no additional animal safety or nutritional study is needed.

5. Environmental risk assessment and monitoring plan

5.1. Issues raised by the Member States

Questions were raised regarding (1) the need for a complete environmental risk assessment of the event even though oilseed rape T45 is no longer commercialized; (2) the risk associated with hybridisation with wild relatives. Further comments were raised with respect to the environmental monitoring plan regarding in particular a more detailed general surveillance plan.

5.2. Evaluation of relevant scientific data

5.2.2. Environmental risk assessment

The scope of applications on oilseed rape T45 is for food and feed uses, import and processing of oilseed rape T45 and all derived products and does not include cultivation of the crop in the EU. Considering the intended uses of oilseed rape T45, excluding cultivation purposes, the environmental risk assessment is concerned with indirect exposure through (1) manure and faeces from the gastrointestinal tracts of mainly animals fed on the GM oilseed rape and with (2) accidental release into the environment of GM seeds during transportation and processing.

The applicant has stated in the applications, that it has discontinued the sale of oilseed rape T45 derived lines by its retailers and deregistered as of 2003 all oilseed rape T45 lines that it has produced with the exception of line LL2393 that was still for sale in 2005 until inventory is exhausted. According to the applicant, stock of all other oilseed rape T45 lines has been recalled from distribution and destroyed, the applicant commits not to commercialize the event in the future and the import will therefore be restricted to adventitious levels in commodity oilseed rape. Thus, the incidence of oilseed rape T45 in the EU is expected to be limited.

The scope of both applications exclude cultivation, therefore concerns regarding the use of glufosinate-ammonium herbicides on oilseed rape T45 apply only to imported and processed oilseed rape seeds that may have been treated with those glufosinate-ammonium herbicides in the countries of origin. The regulation and risk assessment of the residues of these herbicides is within the scope of Directive 91/414/EEC concerning the placing of plant protection products on the market (EC, 1991).

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

Oilseed rape *Brassica napus* is predominantly self-pollinated but out-crossing can range from 12 to 55% (see Rabow 1987 and Cuthbert 2001 in (Legere, 2005)). It also produces large quantities of small seeds. These seeds are very robust and can remain viable in soil for many years (OECD, 2003; Lutman *et al.*, 2005). Oilseed rape also exists as a weed in other crops and can colonise semi-natural habitats in Europe. Unintended seed dispersal and gene flow via pollen thus pose the potential for exposure to conventional

varieties and several wild relatives across Europe (Devos *et al.*, 2004; Pessel, 2001). Gene flow in oilseed rape is exacerbated by seed losses at harvest, the possible induction of secondary seed dormancy, and the formation of persistent seed banks (Legere, 2005). There is no evidence that seeds engineered to contain glufosinate-ammonium tolerance or tolerance to other herbicides have enhanced dormancy (Hails *et al.*, 1997; Lutman *et al.*, 2005). Feral plants are likely to occur wherever oilseed rape is cultivated and transported (Chèvre, 2000) and GM oilseed rape is no exception (Hall, 2000; Aono *et al.*, 2006; Yoshimura *et al.*, 2006).

However, the herbicide tolerant trait can only be regarded as providing a selective advantage for the GM oilseed rape trait where and when glufosinate-ammonium herbicides are applied (EFSA, 2006). Glufosinate-ammonium herbicides are rarely used to control weeds in conventional oilseed rape or in crops grown in rotation with it. It is primarily used as a desiccant in these crops. It is also not commonly used to control plants in semi-natural or natural habitats. Without the complementary herbicide application, the traits will have a neutral effect on the fitness of the potential hybrids (Crawley, 1993; Crawley *et al.*, 2001; Snow, 1999) .

The field data provided in the applications (conducted over three years and thirty locations) do not show increased invasiveness or enhanced weediness and fitness, except in the presence of glufosinate-ammonium herbicides. In addition to the data presented by the applicant, the GMO Panel is not aware of any scientific report of increased spread and establishment of oilseed rape T45 and any change in survival capacity, including overwintering. Crawley et *al.* (Crawley, 1993; Crawley *et al.*, 2001) have examined the likelihood of herbicide resistant oilseed rape becoming invasive in 10 different natural habitats over a 10-year period and found no evidence of increased potential of invasiveness. Snow *et al.* (1999) created BC_3 plants from *B. napus* with transgenic resistance to glufosinate-ammonium herbicides and weedy *B. rapa*, backcrossing to *B. rapa*. Herbicide resistance segregated in a 1:1 ratio in BC_3 plants. No significant difference in survival or fecundity between transgenic and non-transgenic plants of the BC_3 generation (Snow, 1999).

In summary, there is no evidence that the herbicide tolerance trait introduced by genetic modification results in increased invasiveness of any crop species, except in the presence of the glufosinate-ammonium herbicides. Thus escaped plants and genes dispersed to other oilseed rape plants would not create additional agronomic or environmental impacts (Ammitzboll *et al.*, 2005; Crawley, 1993; Crawley *et al.*; 2001; EFSA, 2004; EFSA, 2005). The GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from this GM oilseed rape will not differ from that of conventional oilseed rape varieties. This conclusion is in line with previous scientific opinions of the GMO Panel on other glufosinate tolerant GM oilseed rape (EFSA, 2004; EFSA, 2005; EFSA, 2006).

5.2.1.2. Potential for gene transfer

A prerequisite for any gene transfer/dispersal is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer of DNA, or vertical gene flow via seed dispersal and cross-pollination. Considering the scope of the applications and the physical characteristics of oilseed rape seeds (see section 5.2.1.1), a possible pathway of dispersal is from (1) occasional oilseed rape plants originating from indirect exposure through manure and faeces from the gastrointestinal tracts of animals fed on the GM oilseed rape or (2) from accidental release into the environment of GM seeds during transportation and processing for food or feed uses (including germination from an oilseed rape seed bank previously established by accidental release). However, the imported grain will be processed prior to feeding to animals and so it is very unlikely that viable seeds will be ingested.

(a) Plant to bacteria gene transfer

Based on present scientific knowledge and elaborated in more detail elsewhere (EFSA, 2004; EFSA, 2007) gene transfer from GM plants to microorganisms under natural conditions is extremely unlikely, and its establishment would occur primarily through homologous recombination in microorganisms.

In the case of accidental release and establishment of oilseed rape T45 in the environment, exposure of microorganisms to transgenic DNA derived from GM oilseed rape would take place during natural decay of GM plants material and/or pollen in the soil of areas where GM plants establish. Food and feed products derived from the GM oilseed rape plants could contain transgenic DNA. Therefore microorganisms in the digestive tract of humans and animals may be exposed to transgenic DNA.

The *pat* gene is known to be ubiquitous in soil microbial populations. Taking into account the origin and nature of the *pat* gene and the lack of selective pressure in the intestinal tract, the likelihood that horizontal gene transfer would confer selective advantages or increased fitness of microorganisms is very limited. For this reason it is very unlikely that the *pat* gene from oilseed rape T45 would become transferred and established in the genome of microorganisms in the environment or in the digestive tracts of humans or domestic animals. 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 new traits would be introduced into microbial communities.

(b) Plant to plant gene transfer

Oilseed rape can establish as feral populations in Europe and cross with other *Brassica*-relatives especially *Brassica rapa* and disperse genes through these related species (OECD, 1997; Chèvre, 2000; Ford et *al.*, 2006; Heenan, 2007; Jenczewski *et al.*, 2003; Landbo, 1997; Wilkinson *et al.*, 2003). Thus spilled seeds could result in GM plants that might establish and survive, outcross and disperse genes to compatible plants. However, if gene flow into the environment occurs, the event would only show enhanced fitness in the presence of the glufosinate-ammonium herbicides (Crawley, 1993; Crawley *et al.*, 2001; EFSA, 2004; Warwick, 2004). There is also no evidence that glufosinate-tolerance enhances dormancy (Lutman et *al.*, 2005; Hails *et al.*, 1997). However, there are some indications that glyphosate-tolerance can persist in feral *Brassica rapa* in Canada (Warwick, 2007). As stated under section 5.2.1.1, glufosinate-ammonium herbicides are not widely used in arable farming systems in the EU. The GMO Panel is thus of the opinion that hybrids with other oilseed rape varieties or wild relatives would not show any enhanced fitness and would behave as conventional plants.

However, feral plants may establish and become successful weeds, in certain farming systems, at ports and along railways and roads (Saji *et al.*, 2005; Yoshimura *et al.*, 2006). Such plants can be managed by the use of other herbicides and/or adequate mechanical practices (Devos *et al.*, 2004; Warwick *et al.*, 2004). The GMO Panel advises that appropriate management systems should be in place to minimise accidental loss and spillage of transgenic oilseed rape during transportation, storage, handling in the environment and processing into derived products (EFSA, 2004; EFSA, 2005; EFSA, 2006).

5.2.1.3. Potential interactions of the GM plant with target organisms

This point was not considered an issue by the Member States or by the GMO Panel considering the intended uses of T45 oilseed rape, excluding cultivation, and the absence of target-organisms.

5.2.1.4 Potential interactions of the GM plant with non-target organisms

This point was not considered an issue by the Member States or by the GMO Panel considering the intended uses of T45 oilseed rape, excluding cultivation, and consequently the low level of exposure to the environment.

5.2.1.5. Potential interaction with the abiotic environment and biogeochemical cycles

This point was not considered an issue by the Member States or by the GMO Panel considering the intended uses of T45 oilseed rape, excluding cultivation, and consequently the low level of exposure to the environment.

5.2.2. Monitoring

The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC 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 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 applications do not cover cultivation. No potential risks requiring the establishment of a case-specific monitoring plan were identified in the environmental risk assessment.

Monitoring 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, 2006; EFSA, 2006). The potential exposure to the environment of oilseed rape T45 would be related to indirect exposure through manure and faeces from the gastrointestinal tracts mainly of animals fed on the GM oilseed rape and with accidental release into the environment of GM seeds during transportation and processing. In this respect, the applicant states that monitoring will be conducted as part of already existing monitoring procedures for import of currently available commercialised GM oilseed rape.

The general surveillance plan proposed by the applicant includes i) the description of an approach involving operators (federations involved in oilseed rape import and processing), reporting to the applicants, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment, and ii) a coordinating system newly established by EuropaBio for the collection of the information recorded by the various operators. The applicant will submit a general surveillance report on annual basis and a final report at the end of the consent. In case of confirmed adverse effects, the applicant will immediately inform the European Commission and the Member States.

The GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicant is in line with the intended uses of oilseed rape T45 since the environmental risk assessment does not cover cultivation and identified no potential adverse environmental effects. The GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan. The GMO Panel advises that appropriate management systems should be in place to minimise accidental loss and spillage of transgenic

oilseed rape during transportation, storage, handling in the environment and processing into derived products (EFSA, 2004; EFSA, 2005; EFSA, 2006).

5.3. CONCLUSION

The scope of the applications includes food and feed uses, import and processing of oilseed rape T45 and all derived products and excludes cultivation. Considering the intended uses of oilseed rape T45, the environmental risk assessment is concerned with indirect exposure through manure and faeces from the gastrointestinal tracts mainly of animals fed on the oilseed rape T45 and with accidental release into the environment of oilseed rape T45 seeds during transportation and processing.

If accidental spillage and subsequent release into the environment of oilseed rape T45 seeds occurs, oilseed rape T45 plants will only be fitter in the presence of glufosinate-ammonium herbicides which are not currently used on cultivated oilseed rape or in most areas where the GM oilseed rape might be spilled. Therefore the GMO Panel is of the opinion that the likelihood of the spread and establishment of oilseed rape T45 is very low and that unintended environmental effects due to this GM oilseed rape will be no different from that of conventional oilseed rape varieties. Furthermore the scope of the monitoring plan provided by the applicant is in line with the intended uses of oilseed rape T45 since this does not include cultivation.

The GMO Panel is aware that, due to the physical characteristics of oilseed rape seeds and methods of transportation, accidental spillage is unavoidable. The GMO Panel advises that appropriate management systems should be in place to minimise accidental loss and spillage of transgenic oilseed rape during transportation, storage, handling in the environment and processing into derived products (EFSA, 2004; EFSA, 2005; EFSA, 2006).

CONCLUSIONS AND RECOMMENDATIONS

The GMO Panel was requested to carry out a scientific risk assessment of the oilseed rape T45 for food and feed uses, import and processing of oilseed rape T45 and all derived products.

The GMO Panel is of the opinion that the molecular characterisation provided for the transformation event T45 is sufficient for the safety assessment. The bioinformatic analysis of the inserted DNA and flanking regions does not raise any safety concern. The expression of the genes introduced by genetic modification has been sufficiently analysed and the stability of the genetic modification has been demonstrated over several generations. The GMO panel considers that the molecular characterization does not indicate any safety concern.

The comparative analyses showed no biologically relevant agronomic and compositional changes in T45 oilseed rape. The GMO Panel is of the opinion that T45 oilseed rape is as safe as its non GM counterparts and that the overall allergenicity of the whole plant is not changed through the genetic modification.

A 42-day feeding study with broilers did not indicate differences in the nutritional value of T45 oilseed rape versus the non-GM comparator.

The application EFSA-GMO-UK-2005-25 is for food and feed uses, import and processing of oilseed rape T45 and all derived products. The application EFSA-GMO-RX-T45 covers the continued marketing of existing food

additives and feed materials produced from oilseed rape T45. There is therefore no requirement for scientific information on possible environmental effects associated with the cultivation of the GM oilseed rape T45 in the EU. The GMO Panel is of the opinion that the likelihood of the spread and establishment of oilseed rape T45 is very low and that unintended environmental effects due to this GM oilseed rape will be no different from that of conventional oilseed rape varieties. The scope of the monitoring plan provided by the applicant is in line with the intended uses of oilseed rape T45. The GMO Panel advises that appropriate management systems should be in place to minimise accidental loss and spillage of transgenic oilseed rape during transportation, storage, handling in the environment and processing into derived products (EFSA, 2004, EFSA, 2005, EFSA, 2006).

In conclusion, the GMO Panel considers that information available for oilseed rape T45 addresses the comments raised by the Member States and considers it unlikely that oilseed rape T45 will have any adverse effect on human and animal health or on the environment in the context of its proposed uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of United Kingdom (FSA), dated 4 November 2005, concerning a request for placing on the market of oilseed rape T45 in accordance with Regulation (EC) 1829/2003.

2. Acknowledgement letter, dated 30 November 2005, from EFSA to the Competent Authority of the United Kingdom (ref. SR/KL/jq (2005) 1357).

3. Letter from EFSA to applicant, dated 05 December 2006, with request for clarifications under completeness check (ref SR/CP/svh (2006) 1865506).

4. Letter from applicant to EFSA, dated 28 February 2007, providing EFSA with an updated version of the application EFSA-GMO-UK-2005-25 submitted by Bayer CropScience under Regulation (EC) No 1829/2003:

   Part I – Technical dossier
   Part II – Summary
   Part III – Cartagena Protocol
   Part IV – Labelling and Unique Identifier
   Part V – Samples and Detection
   Part VI – Additional information for GMOs

5. Letter from EFSA to applicant, dated 13th April 2007, delivering the 'Statement of Validity' for application EFSA-GMO-UK-2005-25, oilseed rape T45 submitted by Bayer CropScience under Regulation (EC) No 1829/2003 (ref. SR/KL/CP/shv (2007) 2077834).

6. Letter from EFSA to applicant, dated 29 August 2007, with request for clarifications/additional information (ref. SR/AC/svh (2007) 2343149).

7. Letter from applicant to EFSA, dated 10 October 2007, providing additional information upon EFSA request.

8. Letter from EFSA to applicant, dated 06 December 2007, with request for clarifications/additional information (ref. SR/KL/shv (2007) 2557543).

9. Letter from applicant to EFSA, dated 18 December 2007, providing additional information upon EFSA request.

10. Letter from the European Commission, dated 18 June 2007 (received 29 June 2007), concerning the request for renewal of the authorization for continued marketing of existing food additives and feed materials produced from oilseed rape T45 in accordance with Regulation (EC) 1829/2003, submitted by Bayer Crop Science (ref. SANCO/E1/SG/al/D(2007) 510460).

11. Letter from EFSA to applicant, dated 7 September 2007, delivering the 'Statement of Validity' for application EFSA-GMO-RX-T45, oilseed rape T45 submitted by Bayer CropScience under Regulation (EC) No 1829/2003 (ref. SR/KL/eb (2007) 2368185).

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