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Opinion of the Scientific Committee on Plants regarding the Glufosinate tolerant, hybrid rape derived from genetically modified parental lines (MS8 x RF3) notified by plant genetic systems (notification C/B/96/01) (Submitted by the Scientific Committee on Plants, 19 May 1998)

1. TITLE

Outcome of Discussion on Application for the Placing on the Market of Plant Genetic Systems glufosinate ammonium tolerant rape (Notification C/B/96/01).

2. TERMS OF REFERENCE

The Scientific Committee on Plants is asked to consider:

(1) Whether there is any reason to believe that the placing on the market of hybrid seed of swede rape (consisting of crossings of parentals derived from the genetically modified swede rape lines MS8 and RF3) with the purpose to be used as any other swede rape is likely to cause any adverse effects on human health and the environment.

(2) Whether there is any reason to believe that the potential transfer of the herbicide resistance 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.

3. BACKGROUND

Directive 90/220/EEC requires an assessment to be carried out before a product containing or consisting of genetically modified organisms (GMOs) can be placed on the market. The aim of the assessment is to evaluate any risks to human health and the environment connected with the release of the GMOs. For genetically modified plants, the assessment must be based on the information outlined in Annex II B of Directive 90/220/EEC and take account of the proposed uses of the product.

Following the entry into force of the Regulation on Novel Foods and Novel Food Ingredients (EC No. 258/97) on 15 May 1997, in order for this rape seed and its derived products to be placed on the market for food purposes, the requirements of the Regulation will have to be satisfied. Such a regulation does not exist for Novel Feeds and Novel Feed Ingredients.

Glufosinate ammonium herbicides have not so far been authorized for direct application onto rape plants in the EU except for desiccation purposes. This issue comes under the scope of other legislation, such as Directive 91/414/EEC.

4. PROPOSED USES

- Growing and multiplication of parental lines seed for breeding material and for placing hybrid seed on the market
- Field cropping of hybrid swede rape for seed production to be used for feed, food, and industrial uses of non-living processed products arising from the product (including honey).
- Import of seed from non-EU countries for processing for food, feed and industrial uses of non-living processed products arising from the product.

5. DESCRIPTION OF THE PRODUCT

The product is a specific hybrid system in oilseed rape (*Brassica napus* L. spp.oleifera) with the following components:

- i) The male sterile oilseed rape line MS8 Bn (DBN230-0028) and all progeny derived through traditional breeding crosses with non transgenic rape (*Brassica napus* L. spp. oleifera) . Line MS8 has been transformed using plasmid pTHW107 and contains a *barnase* gene (origin *Bacillus amyloliquefaciens*) coding for a ribonuclease expressed only in the tapetum cells during anther development that leads to lack of viable pollen and male sterility, and a *bar* gene (bialaphos resistance, origin *Streptomyces hygrosopicus*) coding for phosphinotricin acetyl transferase as a selectable marker for tolerance to herbicides based on glufosinate ammonium. The *bar* gene is driven by a plant promoter that is active in all green tissues of the plant.
- ii) The fertility restoration line RF3 Bn (DBN212-0005) and all progeny derived through traditional breeding crosses with non transgenic rape (*Brassica napus* L. spp. oleifera). Line RF3 has been transformed using plasmid pTHW118 and contains a *barstar* gene (origin *Bacillus amyloliquefaciens*) coding for a inhibitor of *barnase* expressed only in the tapetum cells during anther development , that leads to restoration of fertility after crossing to the male sterility line, and a *bar* gene (bialaphos resistance, origin *Streptomyces hygrosopicus*) coding for phosphinotricin acetyl transferase as a selectable marker for tolerance to herbicides based

on glufosinate ammonium. The *bar* gene is driven by a plant promoter that is active in all green tissues of the plant.

iii) The hybrid seeds from traditional crossings between parental lines derived from MS8 and RF3.

6. OPINIONS OF THE COMMITTEE

6.1. Molecular/Genetic Aspects

6.1.1. Transformation Technique: Based on the information provided, the DNA was introduced in MS8 and RF3 by *Agrobacterium tumefaciens* mediated transformation, a standard technology. The dossier provides satisfactory information on that regenerated transgenics and their progeny were free from *Agrobacterium tumefaciens*.

6.1.2. Vector Constructs: Line MS8 was produced with plasmid pTHW107. This plasmid contained between the left and right borders: the tapetum cell-specific promoter PTA29 from *Nicotiana tabacum*; the *barnase* gene from *Bacillus amyloliquefaciens*; part of the 3' non-coding region (3' nos) of the nopaline synthase gene of *Agrobacterium tumefaciens*; the PssuAra promoter from *Arabidopsis thaliana*; the *bar* gene identical to the gene isolated from *Streptomyces hygrosopicus* except for the N-terminal two codons; the 3' untranslated sequence of the TL gene 7 of *Agrobacterium tumefaciens*. Sequences outside the border contained: colE1 replication region from *Escherichia coli*; pVS1 replication region isolated from *Pseudomonas*; a fragment of plasmid R751 from *Klebsiella aerogenes* comprising the streptomycin/spectinomycin (Sm/Sp) resistance gene with its own promoter and with insertion of the *barnase* with its own promoter.

Line RF3 was produced with plasmid pTHW118. This plasmid was identical to the one described before except for having the *barstar* gene in place of the *barnase* gene.

Information included in the dossier on genetic transfer capabilities of the vector and the frequency of mobilisation of the vector is deduced from the already published properties of the vector and not based on a direct experimental evaluation. Based on available information on the properties of the vector, the conclusions reached appear to be appropriate.

6.1.3. Transgenic Construct in the Genetically Modified Plant: In both MS8 and RF3 the DNA has integrated in a single genetic locus, based on Southern and segregation analyses. Based on phenotypic and molecular techniques it is shown that the genes are stable and follow standard Mendelian inheritance. In parental MS8 the inserted DNA has been characterized and shown to consist of a single copy of T-DNA insert. In parental line RF3 there is a T-DNA copy arranged in an inverted repeat structure with a second, incomplete T-DNA copy. The second copy includes a functional part of promoter PTA29, the coding region of *barstar*, the 3' nos and a non-functional part of promoter PssuAra. Detailed

analysis of the insertion site in the plant genome have been carried out. Regions flanking the T-DNA have been characterized. Sequences outside the T-DNA borders of the vector are not present. There is no indication of insertion of T-DNA in a functional gene. Based on Southern blots as well as detailed PCR analyses it is confirmed that no sequences from the backbone of plasmids pTHW107 or pTHW118, including the marker genes for Sp/Sm antibiotic resistance, are present in the plants. Overall, the molecular analysis are carefully done and satisfactory.

Trangene expression and cryptic expression are addressed with appropriate techniques (Northern blot). In line MS8 the *bar* gene expression is detected in leaves and flower buds but not in dry seeds. In line RF3, *bar* gene expression is detected in leaves and flower buds but not in dry seeds or pollen. *Barnase* gene expression in MS8 (specific to tapetum cells) was under the level of detection. *Barstar* gene expression in RF3 was only detectable in flower buds. Evidence for the absence of cryptic gene expression (using sense probes in Northern blots) is provided.

The PAT protein activity (the product of the *bar* gene that confers tolerance to herbicide) in tissues other than green parts of the plant was assessed by enzymatic assays. No PAT activity above background was detected in seeds from progeny of MS8 or RF3 transformants.

The Committee acknowledges that the dossier contains appropriate information on PCR detection methods for identification of these plants .

6.2. Safety Aspects

6.2.1. Potential for Gene Transfer: There are no antibiotic resistance genes present in this GMO.

The *bar* gene is under the control of a plant promoter which is not functional in bacteria. Consequently, its expression in the unlikely event of transformation would not occur. Even if, due to genetic recombination, the gene would be expressed in intestinal micro-organisms or human or animal cells, the probability of which is remote, no negative effects are expected because the only known substrate of phosphinothricin acetyltransferase (PAT) is the herbicide glufosinate ammonium.

The *barnase* and *barstar* genes are both under the control of plant promoters and consequently not expressed in bacterial cells. Even if, due to genetic recombination, the gene would be expressed in intestinal micro-organisms, the probability of which is remote, no negative effects are expected because ribonucleases and inhibitors are ubiquitous among bacteria, including those present on the digestive tract.

6.2.2. Safety of the gene products/metabolites (food and feed aspects):
Safety of gene products. The PAT protein can be detected in very low amounts in dry seed (0.1 µg/ mg seed protein). Because virtually no

protein is present in the oil extracted from the plants, the risk for human consumption are non-existent. The amounts of PAT present in seed-meal fed to animals would be too low to cause even theoretical concern. The low amount of PAT protein in vegetative tissues of the plants is an additional guarantee of safety in case of occasional consumption of the green parts of the plant by farm or wild animals. The low levels of PAT protein and the weight of evidence available elsewhere concerning the safety of PAT leads the Committee to conclude that there is no significant risk to humans or livestock following ingestion of the gene product.

The ribonuclease and its inhibitor encoded by *barnase* and *barstar* genes, respectively, are not detected in dry seeds. These proteins represent activities widely occurring among common bacteria and with no foreseeable risks associated with them.

Residue assessment: The Committee considers that the basic statements of the assessment of glufosinate-derived residues in tolerant rape contained in the "Opinion of the Scientific Committee on Plants regarding the genetically modified, glufosinate tolerant rape notified by the AgrEvo company (Notification C/UK/95/M5/1) are also applicable to this submission.

6.2.3. Substantial equivalence: Compositional analyses were carried out on seed harvested from the original plant lines, their hybrids, and lines developed from them by multiple backcrosses to different oilseed rape varieties from field trials in Europe and North America, where locally adapted commercial varieties were used as controls. Data included oil content, glucosinolate levels, fatty acid profiles including erucic acid content, vitamin E and mineral content. The ranges for all genetically modified lines fell within the range for the non-modified lines grown in each trial.

6.3. Environmental Aspects

6.3.1. Potential for gene transfer/gene escape: The risk of genetic escape from modified crop plants will depend on dispersal and cross-pollination with other plants of the same or different species. Successful hybrid formation depends not only on the sexual compatibility of the recipient species (whether the same or related wild species) but the two species must flower simultaneously, share the same insect pollinator (if insect pollinated) and be sufficiently nearby for the transfer of viable pollen. The consequences of successful transfer will depend on the sexual fertility of the hybrid progeny, vigour and the fertility of subsequent generations or their ability to propagate vegetatively.

Oilseed rape as a crop is capable of both self-pollination (70%) and cross-pollination (30%) and is mainly pollinated by wind and attracted insects. Comparative data on substantial equivalence, germination, establishment, plant phenotype and parameters of normal agronomic performance suggest that transgenic rape will not behave differently from

untransformed plants in their ability for genetic transfer or dispersal. Available evidence shows no differences in their ability to outpollinate between transformed and untransformed rape plants.

While rape crops will naturally hybridise with other cultivars in the vicinity there may be a very low level of natural crossing with related species particularly *Brassica rapa* and *B.juncea* under field conditions. Forced hybridisation has been demonstrated with *Raphanus raphanistrum* and *Sinapis arvensis*. Any viable progeny will have no competitive advantage in the absence of selection by herbicide containing glufosinate-ammonium.

The risk assessment assumes that transfer will occur at a low level. The relevant question is whether this can be contained by risk management and whether it is an environmental or agronomic problem.

Available evidence from the scale of release to date suggests that volunteers can be controlled by agronomic practice (cultivation and the use of an alternative broad spectrum herbicide) provided that adequate monitoring procedures are in place to identify spillage, dispersal and any subsequent volunteers. Normal management methods for wild *Brassicae* including cultivation, rotation and alternative herbicide should be maintained.

The dispersal of transgenic rape seed should not be significantly different from that of untransformed plants. There is no evidence that transformed plants which germinate in adjacent uncropped habitats will have any significant ecological advantage in the absence of herbicide containing glufosinate-ammonium. Rape is a poor competitor and is not regarded as an environmentally-hazardous colonising species. Modified rape is no more invasive than unmodified plants and can be controlled by the combination of cultivation and the use of alternative non-selective herbicides. Potential transgenic exchange is unlikely to lead to establishment as a result of reduced viability of any hybrid plants and competition.

6.3.2. Treatment of volunteers: Although non-transgenic rape as well as transgenic rape can be volunteers in following crops, current agricultural practices (including cultivation, rotation, selective herbicides and isolating production fields of different rapeseed types) are able to control both modified and unmodified volunteer rape plants. As a result of seed loss through pod shattering before harvest, transportation of seed out of fields (e.g. in combines) and spillage during transport, volunteers can be expected. In non-cropped areas these should be controlled by the combination of cultivation and the use of alternative non-selective herbicides. Caution is advised over the potential enhancement of establishment through glufosinate impact on field margins which should be monitored and dealt with locally.



The inclusion of different transgenic rape with tolerance to alternative herbicides in the same or nearby rotations should be avoided in order to

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prevent any potential for outbreeding which could accumulate or 'stack' genes within the same plant. Any derived plants with multiple herbicide tolerance would be particularly difficult to control other than by cultivation.

6.3.3. Safety to non-target organisms: Few studies have been conducted on the safety of modified rape to other organisms. No adverse effects were noted in pollinating honey and bumble bees and effects on seed-eating birds and grazing mammals are not expected. No differences are reported in insect pest or disease susceptibility between transformed and untransformed rape in either glasshouse or field trials.