Opinion of the Scientific Committee on Plants regarding submission for placing on the market of Glufosinate tolerant swede rape transformation event GS 40/90 notified by the agrevo company (notification C/DE/96/05) (Opinion expressed by SCP on 14 July 1998)

1. Title

Application for consent to place on the market herbicide protected oilseed rape expressing the **pat** gene conferring tolerance to glufosinate-ammonium (phosphinotricin) (Notification C/DE/96/05).

2. Terms of reference

The Scientific Committee on Plants (SCP) is asked to consider whether there is any reason to believe that the placing on the market of oilseed rape line (**Brassica napus** L. ssp. **oleifera**) transformant Falcon GS 40/90, with the purpose to be used as any other oilseed rape is likely to cause any adverse effects on human health and on the environment.

In this context the SCP is invited to consider, in particular, 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 that an assessment has 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 to the environment connected with the release of the GMOs. For genetically modified plants, the assessment must be based on information outlined in Annex II B of Directive 90/220/EEC and take into account the proposed uses of this product.

Following the entry into force of the Regulation on Novel Foods and Novel Food Ingredients (EC N°. 258/97) on 15 May 1997, in order for this oilseed rape and its derived products to be placed on the market for food purposes, the requirements of the Regulation will have to be satisfied. Such Regulation does not exist on Novel Feeds and Novel Feed Ingredients.

Glufosinate-ammonium herbicide has not so far been authorised for direct application onto oilseed rape plants except for desiccation purposes. This issue comes under the scope of other Community legislation, such as Directive 91/414/EEC.

4. Proposed uses

The GM **Brassica napus** will be used for exactly the same uses as are currently, and may in future be, allowed for conventionally bred varieties. The most significant use is for field growing for food, animal feed and industrial purposes.

5. Description of the product

The product consists of inbred lines of the winter oilseed rape (**Brassica napus)** transformant Falcon GS 40/90 which has been transformed using plasmid pHoe6/Ac containing a synthetic **pat** gene coding for phosphinotricin acetyltransferase under the regulation of 35S promoter and terminator sequences from Cauliflower Mosaic Virus. The product includes any progeny derived from crosses of Falcon GS 40/90 with any traditionally bred oilseed rape.

6. Opinions of the committee

6.1 Molecular/Genetic Aspects

6.1.1. Transformation technique: According to the information provided, the construct was introduced in haploid microspores of cultivar Falcon by **Agrobacterium tumefaciens**-mediated transformation. After selection of secondary embryos in the presence of glufosinate and treatment with colchicine tolerant diploid shoots were selected.

6.1.2. Vector construct: Falcon GS 40/90 was produced with plasmid pHoe6/Ac, the sequence of which has been provided. This plasmid contained between the left and right border T-DNA partial sequence from Ti-plasmid pTiT37, the CaMV 35S promoter, the coding sequence of a synthetic **pat** gene, the 35S terminator of CaMV, T-DNA partial sequence of the Ti-plasmid pTiAch5. Sequence outside the borders contained: the streptomycin/spectinomycin adenyltransferase gene from **E. coli** plasmid R538-1, **Col**E1 replication region from **E. coli,** a portion derived from **Agrobacterium tumefaciens** Ti plasmid, **Ori**V and **Ori**T regions from **E. col**i RK2 plasmid and a portion derived from **Agrobacterium tumefaciens** Ti plasmid Ach5.

6.1.3. Transgenic construct in the GM plant: Southern and segregation analyses demonstrate that Falcon GS 40/90 has integrated the sequence at two independent loci. It is shown that the insert is stable and follows standard Mendelian inheritance.

The important issue of demonstrating the vector sequences around the left and right border has been addressed. Data obtained from Southern and PCR analysis with series of markers, including **Sm/Sp** gene, show that vector sequences outside of the borders have not been integrated into the oilseed rape genome.

6.2 Safety aspects

6.2.1. Potential for gene transfer:

Antibiotic resistance gene Â– On the basis of the information provided, no antibiotic resistance genes are present in GM plant.

pat gene - The gene is under the control of a plant promoter which is not functional in bacteria.

Consequently, in the unlikely event of gene transfer from the transgenic oilseed rape to intestinal bacteria, expression of the **pat** gene could not occur. Even if it is assumed that, due to genetic recombination events, the gene would be expressed in intestinal microorganisms or in animal cells (the probability of which is remote), no negative effects are to be expected since the only known substrate of phosphinotricin acetyltransferase (PAT) is the herbicide glufosinate-ammonium.

6.2.2. Safety of gene products/metabolites

Safety of gene products:

pat gene - The protein product of the phosphinotricin acetyltransferase gene is not present in humans, animal intestinal microorganisms or in traditional food and feed plants. The nucleotide sequence has been modified to provide codons preferred by plants without changing the amino acid sequence of the protein. Sequence comparison shows that the PAT protein does not have homology to known allergens. Only the expression of PAT protein should be considered. The expression of **pat** is much lower in the seed than in the green parts of the plant as demonstrated by activity measurements. The degradation of PAT protein has been extensively studied in gastric juices from a variety of livestock species and humans. The protective effects of other proteins were to some extent taken into account by using crude extracts of leaves. Degradation of PAT protein was shown in all cases to be pH-dependent being most efficient at pH 1.5, while at pH values > 5.0 up to half of the PAT protein could be recovered after 15 minutes (the longest period measured). Similar evidence was presented for the survival of the gene itself in the digestive tract. No evidence of the transformation of competent **E. coli** could be demonstrated. The fact that no acute or chronic effects following ingestion of the PAT protein could be demonstrated, together with the food and feed use being restricted to seed and oil with low or non-existent PAT content, indicates the essential safety of the **pat** gene.

Residue assessment: The metabolism of glufosinate-ammonium has been thoroughly studied in transgenic plants carrying the **pat** gene. The gene enables the plant to metabolise rapidly the herbicidal active moiety into a non-toxic metabolite, the N-acetyl-L-glufosinate. The metabolism studies on GM oilseed rape showed a rapid conversion of glufosinate to N-acetyl-glufosinate. In the immature oilseed rape plant the principal residue identified was N-acetyl-glufosinate followed by glufosinate with lesser quantities of 3-methylphosphinico-propionic acid (MPP). In seeds and hulls MPP was the major metabolite and the N-acetyl-glufosinate the minor metabolite.

Magnitude of residues in glufosinate tolerant rape seed and oil: The residue behaviour of glufosinate-ammonium in tolerant winter oilseed rape was investigated in about 20 supervised field residue trials at different application rates in the Northern European region. In seed the combined residues of glufosinate and the N-acetyl-glufosinate ranged between < 0.05 mg/kg and 0.15 mg/kg; the level of metabolite MPP was below the limit of determination (< 0.05 mg/kg).

The residue behaviour in tolerant oilseed rape processed fraction was studied in a trial conducted in Canada. No detectable residues were found in crude/refined/refined bleached/refined bleached and deodorised oil.

Magnitude of residues in food of animal origin: Ruminant and poultry feeding studies were conducted to determine the magnitude of glufosinate-derived residues in the tissues and milk of dairy cows and in the tissues and eggs of chicken hens which were dosed with a mixture of parent glufosinate and the metabolite N-acetyl-glufosinate in a ratio which represents the terminal residues in relevant animal feed (15%/85%). No detectable residues were found in meat, milk or eggs at the dose calculated to represent the highest residues in livestock feed under Good Agricultural Practice and taking into account the potential use of glufosinate-ammonium as a herbicide in several tolerant crops.

Conclusion on residue assessment: On the basis of the available data a maximum of 0.2 mg/kg of residues of glufosinate-ammonium and its metabolites, N-acetyl-glufosinate and 3-methylphosphinico-propionic acid, expressed as glufosinate free acid equivalents can be calculated for oilseed rape grown under European conditions. The residues will be covered under existing national MRLs for oilseed rape from desiccation uses. The US EPA uses a Reference Dose of 0.02 mg/kg b.w. in the human dietary risk assessment for glufosinate-derived residues in commodities from non-transgenic plants as well as from transgenic plants (Federal Register Vol. 62, No. 24, p. 5333, 1997).

In food of animal origin from livestock animals fed with feedingstuffs after the application of glufosinate-ammonium to tolerant rape, no residues are expected above the limit of determination.

6.2.3. Substantial equivalence

The composition of whole seed, seed meal, and extracted oil from the original and GM plant lines was analysed. Data generated on oil, on crude fibre, on ash, on protein content and on fatty acid composition, on the content of glucosinolates, sterols and tocopherols indicated no changes related to the process of genetic modification. In case of seed meal the observed differences in oil content are considered to be due to the production process. The SCP therefore concluded that the GM plants may be considered substantially equivalent to their unmodified counterparts, except for the inserted traits.

6.3 Environmental Aspects

6.3.1 Potential for gene transfer/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 to occur. 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 oilseed rape will not behave differently from untransformed plants in its ability for genetic transfer or dispersal. Available evidence shows no differences in their ability to outpollinate between transformed and untransformed oilseed rape plants.

While oilseed 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 **Brassica 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 and wild **Brassicae** can be controlled by agronomic practice (cultivation and the use of an alternative 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 oilseed rape should not be different from that of untransformed plants. There is no evidence that transformed plants that germinate in adjacent uncropped habitats will have any significant ecological advantage in the absence of herbicide containing glufosinate-ammonium. Oilseed rape is a poor competitor and is not regarded as an environmentally-hazardous colonising species. Modified rape plants are 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 oilseed rape as well as transgenic oilseed rape can be volunteers in following crops, current agricultural practices (including cultivation, rotation, selective herbicides and isolating production fields of different oilseed rape types) are able to control both modified and unmodified volunteer oilseed 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 appropriate means. Caution is advised over the potential enhancement of establishment through glufosinate impact on field margins, which should be monitored and dealt with locally.

Care should be taken with the inclusion of different transgenic oilseed rape with tolerance to alternative herbicides in the same or nearby rotations in order to prevent any potential for outbreeding, which could accumulate or Â‘stackÂ’ genes within the same plant. Any derived plants with multiple herbicide tolerance would be difficult to control with broad-spectrum herbicides.

6.3.3. Safety to non-target organisms: Few studies have been conducted on the safety of modified oilseed rape to other organisms. No adverse effects were noted in pollinating honey and bumblebees 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 oilseed rape in either glasshouse or field trials.

6.3.4. Resistance and tolerance issues: Transgenic oilseed rape is a relatively new crop in Europe. Since the cropped area to date is limited no extensive field data are available yet on the impacts of the commercial introduction of these new crops on agriculture and the wider environment including the potential transfer of herbicide tolerance to related wild species and other oilseed rape crops. In order to manage the introduction of herbicide tolerant modified crops, the SCP recommends the active development by industry of a cohesive code of practice for field management supported by a strong education programme to promote best practice by farmers. This should involve the active support of the notifier. The SCP wishes to be kept informed by the notifier of any difficulties in implementing the code of practice. The SCP also wishes to be kept informed of the results of various monitoring and research studies underway in several Member States to identify and assess the long term implications of any genetic movement by pollination between transgenic oilseed rape crops and related plant species for management practice.

7. Overall assessment

The Commission requested the Scientific Committee on Plants to consider whether the placing on the market of oilseed rape line (**Brassica napus** L. ssp. **oleifera**) transformant Falcon GS 40/90 with the purpose to be used as any other oilseed rape is likely to cause any adverse effects on human health and on the environment. The SCP was also 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. In the assessment of the dossier provided against the criteria set out in Directive 90/220/EC, the SCP has reached the following conclusions:

1. The SCP, after examining and considering the existing information and data provided in the dossier, against the background of available knowledge in the areas concerned, considers that there is no evidence to indicate that the placing on the market of line transformant Falcon GS 40/90 oilseed rape, with the purpose to be used as any other oilseed rape is likely to cause adverse effects on human health and on the environment.

2. The SCP is also of the opinion that the potential transfer of the herbicide resistance gene to wild **Brassica** relatives is a new issue in Europe in view of the limited scale of release to date. The SCP has examined the available evidence from monitoring and research programmes to date. After evaluating all the information available to the SCP, it was concluded that herbicide tolerant volunteers that may appear would be canola plants and not wild **Brassica** relatives. Such herbicide-tolerant volunteers could be controlled in subsequent crops by conventional agricultural methods other than by the use of glufosinate-ammonium. The SCP recommends that the introduction of herbicide tolerant crops should be accompanied by:

i) an agreed code of practice for field management of the particular modified crop involving the active participation of the notifier to promote best practice by farmers.

ii) a research programme with an agreed design and implementation plan to detect the occurrence and the establishment of herbicide tolerant volunteers and weeds under field conditions in the EU.

The SCP should be kept informed of the results of monitoring and research studies in Member States. These data will serve to identify and assess the longer-term implications of any gene transfer between transgenic oilseed rape and wild relatives under commercial scale conditions.