Opinion regarding submission for placing on the market of Glufosinate tolerant oilseed rape transformation event liberator PHOE 6/AC notified by the Hoechst schering AGREVO COMPANY [NOW AVENTIS CROPSCIENCE] (Notification C/DE/98/6) (Opinion adopted by written procedure following the SCP meeting of 30 November 2000)

1.Title

OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS REGARDING SUBMISSION FOR PLACING ON THE MARKET OF GLUFOSINATE TOLERANT OILSEED RAPE TRANSFORMATION EVENT LIBERATOR PHOE 6/AC NOTIFIED BY THE AGREVO COMPANY [NOW AVENTIS CROPSCIENCE] (NOTIFICATION C/DE/98/6)

(Opinion adopted by written procedure following the SCP meeting of 30 November 2000)

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 Liberator pHoe6/Ac, 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 an agricultural nature.

3. Background

Directive 90/220/EEC [1] 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 [2] , 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 [3].

4. Opinion of the Committee

Question:

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 Liberator pHoe6/Ac, 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.

Opinion of the Committee:

The Committee is of the opinion that there is no evidence to indicate that the placing on the market for cultivation purposes of oilseed rape line (*Brassica napus* L. ssp. *oleifera*) transformant Liberator pHoe6/Ac and varieties derived from this line by conventional crossing with rapeseed lines other than genetically modified ones, is likely to cause adverse effects on human and animal health and the environment.

With respect to potential transfer of the herbicide resistant gene to wild *Brassica*, the information currently available indicates that herbicide tolerant volunteers would probably be canola plants and not wild *Brassica* relatives. The Committee recommends that the introduction of herbicide tolerant oilseed crops should be accompanied by an agreed code of practice for field management and a specific research programme to detect the occurrence of herbicide tolerant volunteers and weeds.

Technical background on which the opinion is based:

4.1 Proposed uses

The GM *Brassica napus* will be used for exactly the same uses as the current non-GM variety, and may in the future be allowed for conventionally bred varieties. The most significant use of the field grown crop is for food, animal feed and industrial purposes.

4.2 Description of the product

The product consists of inbred lines of the winter oilseed rape (*Brassica napus*) transformant Liberator pHoe6/Ac which has been transformed using plasmid pHoe6/Ac containing a synthetic *pat* gene coding for phosphinotricin acetyltransferase, derived from the bacterium *Streptomyces viridochromogenes* strain Tu 494 under the regulation of the 35S promoter and a terminator sequence from Cauliflower Mosaic Virus (CaMV). The transformation event is coded Liberator pHoe 6/Ac. The product will be Liberator pHoe6/Ac and its progeny produced by conventional breeding techniques.

4.3 Molecular/Genetic Aspects

4.3.1 . *Transformation technique*

According to the information provided, the construct was introduced in pieces of hypocotyl fragments of the oilseed rape cultivar Liberator by *Agrobacterium tumefaciens*-mediated transformation. Regenerant shoots were selected in the presence of glufosinate. Selfing of this plant led to the transformation line homozygous for the introduced gene.

4.3.2 *Vector construct*

Liberator pHoe6/AC 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. Sequences outside the borders contained: the streptomycin/spectinomycin adenyltransferase gene from *Escherichia coli* plasmid R538-1, ColE1 replication region from *E. coli*, a portion derived from *Agrobacterium tumefaciens* Ti plasmid, OriV and OriT regions from *E. coli* RK2 plasmid.

4.3.3 *Transgenic construct in the GM plant*

Southern and segregation analyses demonstrate that Liberator pHoe6/Ac has integrated the sequence at one locus. It is shown that the insert is stable through at least four generations 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 a series of markers, including Sm/Sp gene, show that vector sequences outside of the borders have not been integrated into the oilseed rape genome.

4.4 Safety aspects

4.4.1 *Potential for gene transfer*

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

pat gene - According to the present knowledge, gene transfer from the transgenic plant, under the control of CaMV 35S promoter, to intestinal bacteria, is very unlikely. Even if it is assumed that, due to genetic recombination events, the gene would be expressed in intestinal micro-organisms 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.

4.4.2 *Safety of gene products/metabolites*

pat gene - The protein product of the phosphinotricin acetyltransferase gene is not present in humans, animal intestinal micro-organisms 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 of a stretch of 8 amino acids shows that the PAT protein does not have homology to known allergens. The expression of *pat* is much lower in old pods than in the green parts of the plant as demonstrated by activity measurements. The degradation of the PAT protein has been extensively studied in animals (dog, pig and cattle) and in simulated human gastric fluid. The protective effects of other proteins were to some extent taken into account by using crude extracts of leaves expressing PAT protein. Degradation of the PAT protein was shown in all cases to be pH-dependent being most efficient at pH 1.5. PAT is greater than 60% active in the pH range from 7 to 10 after exposure for 3 min. At pH values of 4, PAT is inactive after exposure for 30 min. The fact that no acute or subchronic effects following ingestion of PAT protein by rats (male or female), or chicken fed with a diet containing 15% of rape 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-phytotoxic metabolite, the N-acetyl-L-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 detection (< 0.05 mg/kg).

The residue behaviour in tolerant oilseed rape processed fraction was studied in a trial conducted in Canada. In the cake no glufosinate residues were detectable but for residues of N-acetyl-glufosinate a mean concentration factor of 3.1 for untoasted meal and 3.7 for toasted meal were determined. No detectable residues of glufosinate, N-acetyl-glufosinate and MPP were found in crude/refined/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 of 15 : 85% which represents the terminal residues in relevant animal feed. 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 [4] for oilseed rape from desiccation uses and are not of toxicological concern taking into account the acceptable daily intake for humans (ADI) of 0-0.02 mg/kg bw [5] for glufosinate-ammonium, N-acetyl-glufosinate and 3-[hydroxy(methyl) phosphinoyl]-propionic acid, alone or in combination recommended by WHO 1999 [IPCS, WHO/PCS/00.2: FAO Plant Production and Protection Paper 153, 1999].

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

4.4.3 *Substantial equivalence*

Composition data for whole seeds from 3 years' trial harvests of Liberator pHoe6/Ac and appropriate comparators have been provided. These trials had been carried out either for variety testing or breeding in Germany, except for one trial in the United Kingdom. Near-infrared analysis of the contents of oleic acid (data from one season), erucic acid (data from one season), protein (data from two seasons), oil (data from three years), and glucosinolates (data from three years) revealed no conspicuous or consistent differences between transgenic varieties of Liberator pHoe6/Ac and their non-GM comparators. In addition, gas chromatography analysis of fatty acid composition (data from two years) revealed no differences.

Analysis was carried out on pilot-processed fractions of either transgenic or non-transgenic oilseed rape harvested in Canada (data from one season). Desolventised meal produced by oil extraction of press cake was analysed for macro-components, anti-nutrients, and amino acid composition. Blending crude oil was analysed for mineral composition and fatty acid composition, including erucic acid, in addition to chlorophyll, sterols, and tocopherols. The only conspicuous difference was that chlorophyll in crude oil extracted GM plants was higher than in the non-GM comparators. However, the chlorophyll could have originated from contaminated green (instead of yellow) seeds and is not considered relevant. For both GM and non-GM sources, oil fractions analysed after bleaching and de-odourising were equivalent.

From the data provided, it can be concluded that genetically modified oilseed rape varieties derived from Liberator pHoe6/Ac are substantially equivalent to their conventional counterparts except for the introduced traits.

In addition, nutritional equivalence has been assessed in a feeding trial with broilers, which grow rapidly. In this trial, 180 (6x30) chickens received diets containing 15% of either genetically modified rape seed or control rape seed for 42 days. Birds were checked for feed intake and body weight, while their carcasses were checked for weights of carcass, breast meat, and abdominal fat pads. A statistically significant difference was observed for feed intake and body weight gain, which was approximately five per cent less for broilers fed genetically modified rape seed during the first 17 days of feeding. This statistically significant difference was not observed, however, in the following period (18-42 days). No difference was found in carcass characteristics and mortality. The effects observed during the first period of the study are therefore not considered to be of concern.

4.5 Environmental aspects

4.5.1 *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 use 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 herbicides 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.

4.5.2 *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 'stack' genes within the same plant. Any derived plants with multiple herbicide tolerance would be difficult to control with broad-spectrum herbicides.

4.5.3 *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 or commercial release (Canada).

4.5.4 *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 and elsewhere (e.g., post-release monitoring in Canada) to identify and assess the long term implications for management practice of any genetic movement by pollination between transgenic oilseed rape crops and related plant species.

4.6 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 Liberator pHoe6/Ac 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. There is no evidence to indicate that the placing on the market of line transformant Liberator pHoe6/Ac oilseed rape, with the purpose to be used as any other oilseed rape is likely to cause adverse effects on human or animal health and on the environment.

2. 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. Based on the presently available information, herbicide-tolerant volunteers that may appear would probably 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. It is recommended 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 and elsewhere. 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.

5. Documentation made available to the Committee

1. A dossier comprising:

- The objection of the Member States authorities;
- A table summarising these objections;
- The statement of the German authorities;
- A letter from Hoechst Schering AgrEvo GmbH (now Aventis CropScience) dated 26 October 1998;
- The dossier submitted by Hoechst Schering AgrEvo GmbH;
- Additional information submitted by Hoechst Schering AgrEvo GmbH in response to Member States comments and objections.

2. Question to notifier (doc. SCP/GMO/206-rev1), raised by the SCP, 28.1.1999.

3. Response to questions 1, 2 and 3 (SCP/GMO/206-rev.1) raised by the SCP, submitted by by Aventis CropScience, 27. 1.2000 (Doc. SCP/GMO/235).

4. Further answer to questions (SCP/GMO/206-rev.1) raised by the SCP, submitted by Aventis CropScience, 3. 5.2000, (Doc. SCP/GMO/247).

5. Response to questions 4 and 5 (doc. SCP/GMO/206-rev1), submitted by Aventis CropScience, 12. 9.2000, (Doc. SCP/GMO/273).

6. Additional information from the notifier, submitted by Aventis CropScience, 11.10.2000, (Doc. SCP/GMO/281).

7. Specific data on compositional analysis that have been compiled during the breeding process, submitted by Aventis CropScience, 6.11.2000, (Doc. SCP/GMO/287).

6. Acknowledgements

The Committee wishes to acknowledge the contributions of the working group that prepared the initial draft opinion:

GMO Working group: Prof. O'Gara (Chairman) and Committee members: Prof. Davies, Dr. Delcour-Firquet, Dr. Hans, Prof. Hardy, Prof. Karenlampi, Mr. Koepp, Dr Kuiper, Prof. Silva-Fernandes, Dr. Speijers and invited experts: Dr. Aumaitre, Dr. Chesson, Prof. Moseley, Prof. Vighi and Dr. von Wright.

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[1] OJ N° L 117 of 8. 5.1990, p. 15.

[2] OJ N° L 43 of 14. 2.1997, p. 1.

[3] OJ N° L 230 of 19. 8.1991, p. 1.

[4] Maximum residue limits.

[5] Body weight.