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)

(Submitted by the Scientific Committee on Plants, 10 February 1998)

1. TITLE

Outcome of Discussion on Application for the Placing on the Market of AgrEvo glufosinate ammonium tolerant rape (Notification C/UK/95/M5/1).

2. TERMS OF REFERENCE

The Scientific Committee on Plants is asked to consider whether there is any reason to believe that the import of seeds of glufosinate ammonium (GA) tolerant genetically modified (GM) oilseed rape with the aim of processing, is likely to cause any adverse effects on human health and the environment. The opinion offered in this report is based on the importation of seeds only. Cultivation of the crop in Europe would require a different risk assessment by the Committee.

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.

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 on Novel Feeds and Novel Feed Ingredients.

4. PROPOSED USES

Import of seeds of AgrEvo glufosinate ammonium tolerant GM rape for placing on the market for food and feed purposes.

5. DESCRIPTION OF THE PRODUCT

Seeds of spring swede-rape (**Brassica napus** L. ssp. **oleifera**) derived from traditional breeding crosses between non-genetically modified swede-rape and a line resulting from

transformation event Topas 19/2 which has been transformed using plasmid pOCA/Ac containing:

(i) a synthetic **pat** gene coding for phosphinothricin acetyltransferase (PAT) under the regulation of 35S promoter and terminator sequences from Cauliflower Mosaic Virus and

(ii) an **npt II** gene coding for neomycin phosphotransferase II under the regulation of the nopaline synthase promoter and an octopine synthase terminator sequence.

Genetic modification

Seeds of AgrEvo Glufosinate Tolerant GM Spring Oilseed Rape (GTR) varieties were derived by traditional breeding methods from crosses between GM oilseed rape transformation event Topas 19/2 and non-GM oilseed rape cultivars.

GA is a non-systemic, non-selective herbicide that provides effective post-emergence control of many broadleaf and grassy weeds. It controls weeds through the inhibition of glutamine-synthetase (GS), which leads to the accumulation of phytotoxic levels of ammonia in the plant. It is the only enzyme in plants that can detoxify ammonia released by photorespiration, nitrate reduction, and amino acid degradation.

Transformation event Topas 19/2 derived lines are canola rape seed plants that contain a stably integrated gene which encodes phosphinothricin-N-acetyltransferase (PAT). The PAT enzyme catalyses the conversion of L-phosphinothricin (PPT), the active ingredient in GA, to an inactive form, thereby conferring resistance to the herbicide. The **pat** gene is a synthetic version of the gene isolated from **Streptomyces viridochromogenes**, strain Tü 494. The nucleotide sequence has been modified to provide codons preferred by plants without changing the amino acid sequence of the enzyme. The gene was introduced through **Agrobacterium** - mediated transformation of canola microspores. Southern blot analyses show event Topas 19/2 to contain 2 linked copies of the **pat** gene.

Transgenic construct in Topas 19/2

Agrobacterium- mediated transformation was used with the pOCA/AC construct. A synthetic glufosinate gene (pat) with codons preferred by plants was introduced into the disarmed vector and used in the transformation process. For expression, a CaMV promoter (35S) and terminator of the 35-S RNA gene were used. The plasmid also contains an npt II gene driven by the nopaline synthase promoter conferring aminoglycoside resistance to the plants. In addition, an E. coli origin of replication and cos site of bacteriophage lambda are included and the vector has also a tetracycline (tet) resistance gene for selection purposes in bacteria. The tet resistance gene is outside the area that is integrated into the plant.

6. OPINIONS OF THE COMMITTEE

6.1. Molecular/Genetic Aspects

6.1.1. **Transformation Technique:** Based on the information provided, the transformation event with the **pat** gene essentially followed state of the art procedures with **Agrobacterium** - mediated transformation. Available restriction sites and ensuing target DNA fragments for the

final delivery vector construct were exploited without further refining the required components.

6.1.2. **Vector Construct:** The section dealing with the issue on providing information on the degree to which the vector contains sequences whose products or functions are not known concentrates on the region that is designed for integration into the plant. In this construct, the **tet** gene is not designed to integrate into the plant.

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 the genetic characterisation of the construct, the nature of the insert DNA has been investigated in the application. The important issue of demonstrating that vector sequences around the left and right border has been addressed with supplementary information. Hybridisation analysis with vector encoded markers (**tet, trf** genes) some distance away from the borders suggest that these specific sequences have not been transferred. This conclusion was reached by Southern hybridisation analysis and not by **direct** DNA sequence analysis. However, failure to detect such vector marker genes by Southern analysis can be related to the hybridisation conditions and parameter employed in the assay. Failure to detect a signal should be considered against this background.

6.2. Safety Aspects

6.2.1. **Potential for Gene Transfer:** The relevance of antibiotic resistance genes present in GMOs to the human or animal welfare depends on their ability to interfere with the use of antibiotics as therapeuticals. The probability of this event occurring, on the other hand, depends on whether these genes can be transferred to intestinal bacteria in natural conditions, express themselves, and whether there exists a sufficient selective pressure to favour the resistant bacteria.

It appears that the only process by which the gene transfer from plant GMOs to bacteria could occur would be bacterial transformation by free DNA released from the plant in the digestive tract. As the so called **pat**-gene specific sequences could be detected by PCR even after 60 minutes in simulated digestion trials using gastric juices from various animals and glufosinate tolerant oilseed rape (page 145 of the original dossier), the possibility of functional DNA release from plant GMOs cannot be excluded. The extent of the ability to natural transformation among intestinal bacterial species and strains is not known, although as a phenomenon natural bacterial transformation seems to be more frequent than hitherto recognised, and also intestinal pathogens might be transformable. The expression of transformed DNA requires the presence of a bacterial promoter and ribosome binding site. Keeping these facts in mind, the following conclusions were made on the risks of antibiotic resistance markers associated with glufosinate tolerant rape.

This GM rape plant contains the neomycin resistant marker gene **nptII** under the **Agrobacterium tumefaciens** Ti-plasmid-derived opine promoter. This promoter is only expressed in plants. It is theoretically possible that DNA containing this gene would transform an intestinal bacterium, and that in this bacterium a recombinational rearrangement of DNA

would result in a situation where the **nptII** gene would be under a bacterial promoter and be expressed. Even in this case, the likelihood of interference with chemotherapy is remote. Kanamycin/neomycin is usually used only topically or parenterally. Sometimes it is used to disinfect the bowel from gram-negative bacteria before surgical operations, and occasionally in veterinary medicine to treat intestinal infections. Kanamycin resistant bacteria are relatively common in nature, and the introduction of this particular resistance gene in its present form would not increase the already existing risks in any significant way.

The **pat** 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.

6.2.2. **Safety of Gene Products/Metabolites (Food and Feed Aspects):**

Safety of gene products: PAT protein is ubiquitous in nature but represents only 0.005% of protein in finished canola meal. It is heat and acid labile and the enzyme activity is always destroyed in toasted canola meal.

Additional **in vitro** tests demonstrated the inactivity of PAT and **npt II** in gastric juice of farm animals and it is hypothesised that it does not survive ingestion. **In vivo** tests performed on broiler chickens fed canola in their diets confirmed the safety established in rats with purified protein.

Data concerning the chemical analysis demonstrated substantial equivalence with control seeds for major nutrients including protein, oil, amino acids, fatty acids, tocopherols and sterols. The content of glucosinolates and erucic acid in glufosinate tolerant rape was identical with that of control seeds. Sequence comparisons show that PAT protein does not have homology to known allergens. Acute toxicity tests on PAT protein in rats showed no negative effects.

These results lead the Committee to conclude that there is no significant risk to human and livestock following ingestion of the GM seeds.

Residue assessment: The metabolism of glufosinate ammonium in transgenic plants, carrying the pat gene, has been thoroughly studied. The gene enables the plant to rapidly metabolise the herbicidal active moiety into a non-toxic metabolite, N-acetyl-L-glufosinate. The metabolism studies on genetically modified canola (rape) showed a rapid conversion of glufosinate to N-acetyl-glufosinate. In the immature canola 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 a minor metabolite.

Magnitude of residues in glufosinate tolerant rape seed : Many trials were conducted in various Canadian regions during 1992 and 1993 with different application rates. In no case were residues of the metabolite MPP found at levels above the limit of determination; residues of parent glufosinate were found only in one trial; N-acetyl-glufosinate was found in samples of 5 trials up to 0.14 mg/kg. The residue behaviour in tolerant oilseed rape processed

fraction was also studied in a trial conducted in Canada. No detectable residues were found in crude/refined/refined bleached/refined bleached deodorised/oil. N-acetyl-glufosinate was found in soapstock at a concentration of up to 0.1 mg/kg after application at an exaggerated rate only.

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 for 28 consecutive days 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%) at 3 dose levels. 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 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 imported seed of tolerant swede rape. In food of animal origin derived from livestock animals fed with feedstuffs after application of glufosinate herbicide in tolerant rape, no residues above the limit of determination can be expected. In Canada, the MRL for glufosinate-derived residues in tolerant canola is covered under the existing MRL of 3.0 mg/kg for canola from desiccation use (information by the applicant). 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).

6.2.3. **Substantial Equivalence:** Compositional analysis on seed harvested from trials at a number of locations within Canada in several successive years provided data on oil content, fatty acid composition (including erucic acid content) and glucosinolate levels. Those for the seed from the genetically modified plants fall within the range for non-GM control varieties.

For food purposes the product is likely to be highly processed so that both the genetic material introduced into the transgenic plant and its protein products would be absent from the refined product.

On the basis of substantial equivalence, it can be concluded that refined products from plants derived from this glufosinate tolerant plant would be safe for food use.

6.3. Environmental Aspects

6.3.1. **Potential for Genetic Transfer:** Since this rape will not be grown in the EC but imported as seed, the only potential release is by spillage during transport for processing. Any subsequent germination and establishment of feral plants probably on road verges will not have selective advantage in the absence of glufosinate ammonium and are unlikely to have any impact on the environment. 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. Spillage during handling and transit by rail may require control by alternative herbicide by users who would not usually have access to agricultural advice and the labelling information. Modified spring rape seed does not show any enhanced dormancy characteristics and is unlikely to survive a northern European winter.

In the unlikely event of spilled seed becoming established, spring rape exhibits a variable level of outcrossing through insect and wind pollination. There may be a low frequency of hybridisation with related species (e.g. other **Brassicae**) but poor vigour and high sterility of hybrids will limit spread. Overall the risk of genetic escape is considered small particularly in view of the low risk of release of the seed into the environment.

6.3.2. **Treatment of Volunteers:** Since this rape will not be cultivated in the EC, there is not a problem of volunteers in following crops.

6.3.3. **Safety to Non-Target Organisms:** In the unlikely event of becoming established, the risks to non-target or beneficial species associated with the plants are considered to be very low. Foraging honeybees are not affected and there is no difference in susceptibility to disease and insect pests between transformed and untransformed plants.

6.3.4. **Resistance and Tolerance Issues:** The risk of establishment of tolerance in other species is considered to be remote as a result of the low risk of genetic release.

7. OVERALL ASSESSMENT

The Commission requested the Scientific Committee on Plants "to consider whether there is any reason to believe that the import of seeds of AgrEvo Glufosinate tolerant GM oilseed rape with the aim of processing is likely to cause any adverse effects on human health and the environment". In the assessment of the dossier against the criteria set out in Directive 90/220/EEC, the Committee has reached the following conclusion:

- The Committee after examining and considering the existing information and data provided in the AgrEvo dossier, against the background of available knowledge in the areas concerned, considers that there is no evidence indicating that the seeds of AgrEvo glufosinate ammonium tolerant genetically modified oilseed rape, to be imported and processed in the manner indicated, are likely to cause adverse effects on human or animal health and the environment.