Opinion of the Scientific Committee on Plants regarding submission for placing on the market of genetically modified high Amylopectin potato cultivars apriori and apropos notified by Avebe (Notification C/NL/96/10) - SCP/GMO/044 - (Opinion adopted on October 2, 1998)

1. TITLE

Application for consent to place on the market genetically modified high amylopectin potatoes (Notification C/F/95/12-01/B)

2. TERMS OF REFERENCE

The Scientific Committee on Plants is asked to consider whether there is any reason to believe that the placing on the market of the potato cultivars Apriori and Apropos, for use as any other industrial cultivars for starch production excluding further breeding with other cultivars, is likely to cause adverse effects on human health and on the environment.

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 potato 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. The present opinion relates to the assessment provided for under Directive 90/220/EEC, all applications relating to the placing on the market of this potato and its derived products intended for food use purposes must also comply with the provisions and procedures of EC Novel Foods and Novel Food Ingredients mentioned above, which may include, as appropriate, consultation of the Scientific Committee on Food.

4. PROPOSED USES

The cultivars which are the subject of this application are potatoes (**Solanum tuberosum** L.) with high amylopectin starch. The potatoes are to be used for the extraction and utilisation of the modified starch for purposes including food use. The potatoes themselves are not to be sold as a consumer product but the starch is to be used as any other potato starch. The high amylopectin starch crops will be grown separately from other potato lines to ensure no mixing of starch types. The users will be breeders, farmers and the potato starch industry. It should also be noted that by-products from potato starch processing are likely to be used as animal

feed. Part 3 of the application states that AVEBE is limiting its request for approval to the two varieties Apriori and Apropos,without derived varieties.

5. DESCRIPTION OF THE PRODUCT

Cultivars Apriori and Apropos are derived from cultivar Karnico by genetic modification. The genetic modification involves antisense inhibition of the gene encoding granule bound starch synthase protein (**gbss**) which is responsible for amylose biosynthesis. The starch produced has little or no amylose and consists of branched amylopectin, which modifies the physical properties of the starch.

6. OPINIONS OF THE COMMITTEE

6.1. Molecular/Genetic Aspects

6.1.1. Transformation Technique: Plasmid DNA was introduced into the potato lines by **Agrobacterium**-mediated gene transfer technology. This is standard technology for potato transformation. The application makes it clear that DNA outside the T-DNA regions are incorporated into the GMOs.

6.1.2. Vector Constructs: The potato cultivars were transformed using plasmid pKGBA50, derived from pBIN19. The plasmid includes **ori**V (vegetative origin of replication), **ins**B (insertion sequence Â– part of transposase), **npt**III (=aphIII=3Â'5" aminoglycoside phosphotransferase type III from **Streptococcus faecalis** [function is bacterial antibiotic resistance]), **trf**A (transcription repression factor), M13 **ori** (phage replication origin from phage M13), t- **nos** (terminator of nopaline synthase), **gbss** (antisense orientation - in sense orientation the **gbss** gene encodes for granule bound starch synthase enzyme), p- **gbss** (**gbss** promoter), **npt**II (neomycin phosphotransferase type II [function is plant kanamycin resistance]), p- **nos** (promoter of nopaline synthase), **Col**E1 **ori** (origin of replication in **E. coli**), **tet**R (repressor of tetracycline resistance), **tra**J (transfer gene-relaxosome protein).

6.1.3. Transgenic Constructs in the Genetically Modified Plants: The entire vector sequence of plasmid pKGBA50 and not simply the T-DNA region is incorporated into the GM potato. Four and six copies of bacterial (vector) DNA are incorporated into Apriori (tBK50-13) and Apropos (tBK50-66), respectively. Of these bacterial sequences two and three copies of vector DNA could be under the control of relevant plant regulatory sequences since the others are between T-DNA border sequences. Since vector sequences have been integrated into the GM potatoes there is the possibility of horizontal gene transfer.

Of the thirty-six possible open reading frames (ORFs) integrated into the GM potatoes, six encode known complete proteins. These are **ins**B, **npt**III, **trf**A, **npt**II, **tet**R and **tra**J. With regard to the other possible ORFs database searches indicate that the majority encode incomplete, known bacterial genes or are internals within an ORF. However, some ORFs correspond to unknown genes. Database searches give no indication that any ORF encodes a toxic or environmentally hazardous product. However, the fact remains that the functions of potential products of specific ORFs are unknown.

The genetic integration is stable as determined over several generations of vegetative propagation.

6.2 Safety Aspects

6.2.1. Potential for Gene Transfer : The starch granule, which is retained intact during initial extraction from the tuber, contains little protein or contaminating DNA and does not reflect any hazards posed by the intact plant. However, small and damaged tubers and the by-products of starch extraction are routinely used as animal feed thus DNA may be available for transfer.

Among the thirty-six ORFs recognised by the Company, six (**npt**II **, npt**III **, tet**R **, ins**B **, tra**J **, trf**A) code for known complete proteins. Two are bacterial marker genes coding for different isoforms of the enzyme neomycin phosphotransferase, which confer resistance to aminoglycoside antibiotics. It is theoretically possible that DNA from the various by-products of starch extraction containing a resistance marker gene could transform an intestinal bacterium and, in the case of **npt**II **,** recombination could bring the gene under the control of a bacterial promoter. However, kanamycin and neomycin have limited clinical value. Introduction of the **npt**II gene would not increase the existing risks to any significant extent since the **npt**II gene and the resistance to these antibiotics are already common in nature. More concern is raised over the presence of **npt**III, which remains under the control of a bacterial promoter. It is reported that this gene can additionally confer resistance to (or tolerance of) amikacin which is of greater clinical significance. Amikacin is a reserve antibiotic of value in the treatment of nosocomial infections involving Gram-negative organisms resistant to gentamycin and tobramycin. The development of amikacin resistance amongst the gut flora of livestock via horizontal gene transfer from the GM plants could act as a reservoir from which resistance in humans could develop.

The Company concludes, on the basis of disk diffusion studies with a single strain of **E. coli**, that the **npt**III gene does not confer significant resistance to amikacin. Results are expressed only relative to the resistance shown to kanamycin/neomycin. Given the importance of amikacin, risk assessment requires a more thorough investigation using appropriate target species (e.g. **Pseudomonas, Aeromonas** spp.). Tests should use the internationally recognised Minimum Inhibitory Concentration (MIC) method to establish the extent to which any resistance to amikacin develops.

Given the potential for horizontal gene transfer it also important that the risks associated with such events are fully assessed by the Company. This applies to **tet**R **, ins**B **, tra and trf**A in addition to **npt**III and the potential products of ORFs, which have no known homologies.

6.2.2. Safety of Gene Products: The CompanyÂ's safety evaluation is restricted to an oral toxicity study made with whole potatoes. No data are provided for those by-products (for animal feed) in which any foreign DNA or protein would be concentrated. This study with whole potatoes revealed no significant differences in animal performance or histopathological effects when GM and non-GM potatoes were fed, in sub-chronic tests (90 days) to rats. The Committee is of the opinion that the sub-chronic feeding tests with rats fed with raw GM potatoes indicates an apparent lack of toxicity which may, by extrapolation, also apply to animal feed containing concentrated by-products, since the concentration of these products is relatively low (max 7.5%). However, hazards and toxicological implications of the transfer of genes which confer resistance to antibiotics such as amikacin cannot be assessed on the basis of this type of feeding trial. Therefore the Committee considers the statement of the Company concerning the safety of products following (accidental) consumption by humans and animals as insufficiently proven.

6.2.3. Substantial Equivalence: Several components and features of the GM potatoes have been assessed for substantial equivalence with respect to the fresh product, the starch derived from it and the by-products of the starch extraction process (protein concentrate [Protamyl] and fruit juice [Protamylasse]). In general the quantitative data provided are inadequately supported by statistical analyses. The company, by introduction of the **gbss** in antisense orientation, successfully changed the composition of the starch produced. This intentional change in granule and starch structure has nutritional implications and, in this respect, the modified potato cannot be judged as nutritionally equivalent. However, the purpose underlying the introduction of the test of "substantial equivalence" was to ensure that changes other than those controlled by the introduced trait did not occur. The glycoalkaloid content of the fresh GM tubers appears to fall within the natural variation for potato. Starch yield also appears unchanged. The Company also states that the GM potatoes are no more frost resistant than the non-GM parent. Resistance to viruses, **Phytophthora**, nematodes, and **Synchytrium** are claimed but no data are presented. There are, however, some slight morphological changes, which are attributed to somaclonal variation.

With respect to the by-products of the starch extraction process the amino acid composition of the protein concentrate (Protamyl) produced from waste product appears the same for GM and non-GM potatoes but adequate statistical treatment and data on ranges of natural variation are lacking. This is also the case for some of the differences observed in many measured parameters e.g. the glycoalkaloid content in protein by-products and fruit juice (Protamylasse) derived from GM and non-GM lines.

6.3 Environmental Aspects

6.3.1 Potential for Gene Transfer/Escape

The natural exchange of genetic material is only possible with other varieties of potato Solanum tuberosum. No natural genetic exchange has been found with wild relatives, Solanum nigrum and Solanum dulcamara. Very low frequency exchange has been detected with Solanum nigrum under forced hybridisation. Therefore the chances for successful hybridisation between transformed potatoes and other Solanum species are very unlikely. Genetic spread is assessed as limited to cross-pollination with other potatoes.

In view of the presence of a bacterial promoter linked to the nptIII gene, there is the potential for genetic transfer to microbes in the soil and other environments and expression in such backgrounds. There are no data provided to assess this risk or to relate it to the natural occurrence of resistance to relevant antibiotics in microbial populations in the soil.

Dissemination is by tuber and seed over a limited distance. Potatoes have difficulty becoming established outside cultivated fields.

6.3.2 Treatment of Volunteers: Small tubers will be left in the ground after harvest (groundkeepers) and may give rise to volunteer plants in the next crop. These will be killed by frost, drought and standard agricultural practice in following crops.

6.3.3 Safety to Non-Target Organisms: There are no data on safety of the modified crops to non-target organisms, e.g. pollinators. Only mammalian toxicity data is provided. It is currently not possible to undertake a risk assessment of the environmental impact of these modified potatoes.

7. OVERALL ASSESSMENT

The Committee is of the opinion that insufficient risk assessment has been carried out with respect to specific genes or gene elements (some of unknown function) incorporated into the GM lines under the control of bacterial promoters. This is particularly the case for the nptIII gene, which confers resistance to amikacin, a clinically important antibiotic. Without an adequate risk assessment of the potential consequences of horizontal gene transfer from the GM plants to humans, animals and the environment, the Committee considers that it is not possible to fully assess the safety of the transgenic potato lines in question under Directive 90/220/EEC.