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SCIENTIFIC COMMITTEE ON PLANTS **SCP/GMO/165-Final**

OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS ON GENETICALLY MODIFIED HIGH AMYLOPECTIN POTATOES NOTIFIED BY AMYLOGENE HB (NOTIFICATION C/SE/96/3501)

(Opinion adopted by the Scientific Committee on Plants, 18 July 2002)

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A. Title

OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS ON GENETICALLY MODIFIED HIGH AMYLOPECTIN POTATOES NOTIFIED BY AMYLOGENE HB (NOTIFICATION C/SE/96/3501)

B. 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 clone EH92-527-1, derived from cultivar Prevalent, for use in cultivation and starch production, is likely to cause adverse effects on human health and/or the environment.

C. Opinion of the Committee

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 clone EH92-527-1, derived from cultivar Prevalent, for use in cultivation and starch production, is likely to cause adverse effects on human health and/or the environment. The GM potato has modified starch structure (a high amylopectin content) due to the reduced expression of a granule bound starch synthase gene. The Committee has evaluated the molecular analysis of the transgenic line and is satisfied that only the T-DNA region has been transferred and integrated as a single copy insert. From the sequence data provided by the applicant there is no reason to assume that the DNA regions transferred code for toxic and/or allergenic products. Furthermore, in the unlikely event that horizontal transfer of gene sequences would occur between the GM potato and bacteria, the bacteria would not pose any additional risk to human health or the environment. By-products of the starch extraction process are used for other purposes including animal feed. The risk assessment included an analysis of data from appropriate animal feeding studies. This data indicates that after starch extraction the by-products of the GM potato are as safe as those from the non-GM parental line. Compositional analysis has shown that the GM line falls within expected variation for potato, except for the change in starch composition due to the genetic modification. Since potato is vegetatively propagated and the natural exchange of genetic material is only possible with other varieties of potato, there is insignificant risk to the environment of any transgene flow. No adverse effects on plant-associated organisms have been demonstrated or would be expected from cultivation of the modified potato. The Committee is therefore of the opinion that there is no evidence to indicate that the placing on the market of potato clone EH92-527-1, for use in cultivation and starch production is likely to cause adverse effects on human health and the environment.

A. Title

Application for consent to place on the market genetically modified high amylopectin potatoes (Notification C/SE/96/3501)

B. Table of contents

C. Background

Legal framework

Directive 90/220/EEC[1] 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[2]) 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

[1] OJ No L 117 of 8.5.1990. p. 15.
[2] OJ No L 43 of 14.2.1997. p. 1.

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.

Proposed uses

The crop plant, which is the subject of this application, is a GM potato (*Solanum tuberosum* L.), which contains high amylopectin starch. The GM potato, designated line EH92-527-1, is to be cultivated and used for seed production and is intended as a raw product for the starch industry. It will be grown separately from other starch potatoes. The high amylopectin starch will be used to manufacture products for the technical industries, primarily the paper and chemical industries. Utilisation in the food industry will also be considered but this will require compliance with the provisions and procedures of EC Novel Foods and Novel Food Ingredients, which may include, as appropriate, consultation of the Scientific Committee on Food. By-products generated from starch production are to be used according to normal practice i.e. the pulp will be used for feed and the fruit juice and fruit water for irrigation of farmlands or treated as sewage. The notification does not include any progeny derived from crosses between the GM potato clone EH92-527-1 and any other potato varieties. The GM clone will be labeled as genetically modified as will seed potatoes and any starch potatoes which are sold on the open market. When the potatoes are delivered to the factory they will be registered by the grower as being genetically modified and sampled by the receiver, since they require a separate industrial process. Potato pulp delivered from the starch factory will be labeled as originating from GM potatoes.

Description of the product

Potato line EH92-527-1 is derived from cultivar Prevalent 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.

Source documentation made available to the Committee:

1. Amylogene HB. Notification for placing the potato clone EH92-527-1, being genetically modified for increased content of amylopectin, on the market - 4 May 1998.
2. SCP - First request of 4 February 1999 to the notifier for clarification, Document SCP/GMO/137.
3. Amylogene HB, Response of 6 May 1999 to SCP regarding the first request to the notifier for clarification of 4 February 1999, Document SCP/GMO/147
4. SCP – Second request of 18 May 1999 to the notifier for clarification, Document SCP/GMO/151.
5. Amylogene HB, Response of 20 September 1999 regarding the second request to the notifier for clarification of 18 May 1999, Documents SCP/GMO/ 179 & 180.
6. Amylogene HB, Further response of 22 October 1999 regarding the second request to the notifier for clarification of 18 May 1999, Documents SCP/GMO/ 193 & 194.
7. SCP – Third request of 29 October 1999 to the notifier for clarification, Document SCP/GMO/204 Rev.1.
8. Amylogene HB, Response of 26 November 1999 regarding the third request to the notifier for clarification of, SCP/GMO/215.

9. SCP – Fourth request of 27 January 2000 to the notifier for clarification, Document SCP/GMO/238-Rev.1
10. Amylogene HB, First response of 2 May 2000 to SCP regarding the fourth request to the notifier for clarification of 27 January 2000, SCP/GMO/248.
11. Amylogene HB, Second response of 17 July 2000 to SCP regarding the fourth request to the notifier for clarification of 27 January 2000, SCP/GMO/266 & 267.
12. SCP – Fifth request of 20 July 2000 to the notifier for clarification, Document SCP/GMO/269.
13. Amylogene HB, First response of 20 September 2000 regarding the fifth request to the notifier for clarification of 20 September 2000, Documents SCP/GMO/ 274 & 275.
14. Amylogene HB, Second response of 25 January 2001 regarding the fifth request to the notifier for clarification of 20 September 2000, Documents SCP/GMO/ 290.
15. Amylogene HB, Third response of 31 January 2001 regarding the fifth request to the notifier for clarification of 20 September 2000, Documents SCP/GMO/ 291.
16. SCP – Sixth request of 1 February 2001 to the notifier for clarification, Document SCP/GMO/292.
17. Amylogene HB, Response of 5 March 2001 regarding the sixth request to the notifier for clarification of 1 February 2001, Documents SCP/GMO/ 293.
18. SCP – Seventh request of 12 March 2001 to the notifier for clarification, Document SCP/GMO/294.
19. Amylogene HB, Response of 28 March 2001 regarding the sixth request to the notifier for clarification of 1 February 2001, Documents SCP/GMO/ 295.
20. SCP – Eighth request of 28 March 2001 to the notifier for clarification, Document SCP/GMO/296.
21. Amylogene HB, Response of 3 July 2001 regarding the eight request to the notifier for clarification of 28 March 2001, Documents SCP/GMO/ 301.
22. SCP – Ninth request of 13 November 2001 to the notifier for clarification, Document SCP/GMO/302.
23. Amylogene HB, Response of 19 April 2002 regarding the eight request to the notifier for clarification of 28 March 2001.

D. Scientific background on which the opinion is based

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 clone EH92-527-1, derived from cultivar Prevalent, for use in cultivation and starch production, is likely to cause adverse effects on human health and/or the environment. The GM potato has modified starch structure (a high amylopectin content) due to the reduced expression of a granule bound starch synthase gene. The Committee has evaluated the molecular analysis of the transgenic line and is satisfied that only the T-DNA region has been transferred and integrated as a single copy insert. From the sequence data provided by the applicant there is no reason to assume that the DNA regions transferred code for toxic and/or allergenic products. Furthermore, in the unlikely event that horizontal transfer of gene sequences would occur between the GM potato and bacteria, the bacteria would not pose any additional risk to human health or the environment. By-products of the starch extraction process are used for other purposes including animal feed. The risk assessment included an analysis of data from appropriate animal feeding studies. This data indicates that after starch extraction the by-products of the GM potato are as safe as those from the non-GM parental line. Compositional analysis has shown that the GM line falls within expected

variation for potato, except for the change in starch composition due to the genetic modification. Since potato is vegetatively propagated and the natural exchange of genetic material is only possible with other varieties of potato, there is insignificant risk to the environment of any transgene flow. No adverse effects on plant-associated organisms have been demonstrated or would be expected from cultivation of the modified potato. The Committee is therefore of the opinion that there is no evidence to indicate that the placing on the market of potato clone EH92-527-1, for use in cultivation and starch production is likely to cause adverse effects on human health and the environment.

1. Molecular/Genetic Aspects

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.

1.2. Vector Constructs: The potato cultivar Prevalent was transformed with the plasmid pHoxwG, which is derived from the vector pBIN19. The vector backbone sequence is the same as pBIN19 of which the complete sequence exists. The backbone contains two pTiT37 fragments, origin *Agrobacterium tumefaciens*; a pRK2 fragment with *trfA* coding sequence (promotes plasmid replication); a fragment with neomycin phosphotransferaseIII coding sequence (*nptIII*; confers resistance to antibiotics of the aminoglycoside group); a fragment with origins similar to transposable element *is1*; a pRK2 fragment with *oriV* region (plasmid origin of replication); a pRK2 fragment with *traJ* coding sequence (promotes plasmid transfer by conjugation) and *oriT* region (origin of transfer by conjugation); a sequence similar to a ColE1 fragment; a pRK2 fragment with *tetR* coding sequence. All DNA fragments are of prokaryotic origin and associated with prokaryotic control elements. All DNA fragments have been detected in microorganisms in the natural environment. Complete genes code for no known toxic compounds.

The T-DNA region contains a pTiT37 fragment with right border sequence including the 5' untranslated part of a nopaline synthase gene (functional as a promoter in plants); a pTiT37 fragment including left border sequence; a Tn*5* fragment with *nptII* coding sequence; a Ti-plasmid fragment; a pTiT37 fragment including the 3' untranslated part of a nopaline synthase gene (*nospA*) functional as a polyadenylation sequence in plants; a M13mp19 fragment with polylinker sequences; a genomic *gbss* fragment from potato functional as a plant promoter; cloning remainders from M13mp19 (origin phage M13) and pJRD184 (synthetic sequence); a genomic *gbss* fragment from potato inserted in antisense orientation relative to the promoter.

1.3. Transgenic Constructs in the Genetically Modified Plants: The notifiers provide a detailed description of the actual insert in GM line EH92-527-1. It includes a pTiT7 fragment with right border sequence including the 5' untranslated region of a nopaline synthase gene (*Pnos*) functional as a promoter in plants; a Tn*5* fragment with *nptII* coding sequence; a Ti plasmid fragment; a pTiT37 fragment including the 3' untranslated part of a nopaline synthase gene; a M13mp19 fragment with polylinker sequences; a genomic *gbss* fragment (*gbss* promoter); cloning remainders of M13 and pJD184; a genomic *gbss* fragment in antisense orientation; a genomic *gbss* fragment inverted to sense orientation during integration (revealed by sequencing). DNA sequences towards the left border have

been deleted during integration. Evidence is provided that the GM potato clone has one integrated copy of the T-DNA at one locus. Southern blot analyses indicate that no vector backbone sequences are linked to the T-DNA. This includes the absence of the *nptIII* gene which could encode for resistance to an important antibiotic, amikacin.

An open reading frame analysis of the insert in EH92-527-1 has been carried out. Eighteen ORFs are found, eleven of which have no significant homologies to known coding regions. The only ORF having a complete coding region for a known protein is the *nptII* gene. A list of ORFs with a coding region of more than 50 amino acids which are present in EH92-527-1 is provided. They include, in addition to *nptII:*

- ORF4 in which the first 50 amino acids are homologous to the bleomycin resistance protein (126 amino acids) of Tn*5* and 68 internal amino acids are homologous to ornithine cyclodeaminase (354 amino acids) of *Agrobacterium tumefaciens*.
- ORF6 in which the first 98 amino acids are homologous to gene III protein of phage M13 (no known regulatory elements are attached to this ORF).
- ORF10 in which two stretches of amino acids of the ORF are homologous with the potato *gbss*.
- ORF13 in which the first 25 amino acids are homologous to a polymerase of the Rice Ragged Stunt Virus but which would not yield a functional protein with polymerase properties.
- ORFs16 and 17 show homologies with potato *gbss* but would not produce a functional protein.

ORF4, due to its association with ORF 1 (*nptII* gene) could be expressed at the RNA level. Since bleomycin is a chemotherapeutic used in cancer treatment the SCP requested further information on the potential for transcription and translation of ORF4. RT-PCR was used to demonstrate transcription of ORF4 in both leaves and tubers of the GM line but not in the parental control. ORF1 and ORF4 were transcribed to the same RNA, although a stop codon exists before ORF4. ORF4 is out of reading frame with the preceding *nptII* gene. This makes it extremely unlikely, but not impossible, that ORF4 is expressed in the GM potato plant as a polypeptide. Sub-cloning of ORF4 into a bacterial vector containing the wildtype promoter associated with the *ble* gene did not support growth of the bacteria on either bleomycin or zeocin as antibiotics. This, together with the fact that 60% of the *ble* gene incorporated into the GM potato plant is missing, provides acceptable evidence that a functional bleomycin protein is not present. This does not preclude the possibility that expression of ORF4 results in the production of a novel polypeptide in the plant, although database searches do not indicate significant homologies between ORF4 amino acid sequences and known allergens. Several polyclonal antibodies were developed using synthetic peptides derived from the ORF4 sequence. In Western blots (ECL Plus ^{TM} detection system), immunoaffinity purified polyclonal sera detected purified recombinant ORF4 polypeptide produced in *E coli*. The sensitivity of detection was at least 0.35ng of protein. Western blots of proteins extracted from leaves of line EH92-527-1 and its parental control revealed no antigenic reaction when probed with immunoaffinity purified sera for ORF4, but positive reactions occurred with leaf protein extracts to which recombinant ORF4 protein was added. The data indicate that although ORF4 transcript is detectable in the transgenic line there is no corresponding translation into a protein, confirming expectations from the molecular characterisation of ORF4 and its association

with ORF1. The genetic integration was shown to be stable as determined over several generations of vegetative propagation.

2. Safety Aspects

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. 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.

The presence in the GM potato plants of a functional *nptII* gene which confers resistance to kanamycin and neomycin, raises the possibility that DNA from the various by-products of starch extraction containing a resistance marker gene could transform an intestinal bacterium. Recombination could bring the gene under the control of a bacterial promoter. However, kanamycin and neomycin have limited clinical value. Given that resistance to these antibiotics is widespread in microbes in the natural environment, no additional selective advantage would be gained in the unlikely event of transfer of the *nptII* gene from the GM potato. Furthermore, *nptII* gene fragments cannot be detected in fruit juice and water by PCR. Any potential risks of gene transfer to soil bacteria caused by utilising these by-products as fertiliser are therefore negligible.

As described in section 6.1.3, analysis of open reading frames present in the GM potato line indicates that for ORF 4 the first 50 amino acids are homologous to the bleomycin resistance protein of Tn*5*. Bleomycin is a chemotherapeutic used in cancer treatment. However, the notifier has shown that a functional bleomycin resistance protein is not expressed from ORF4 and that ORF4 does not transcribe and translate a protein which is detectable immunochemically.

2.2. Safety of By-Products of Starch Production: Pulp produced as a by-product of the extraction process represents a source of any potential contaminant which will be fed primarily to ruminants. The notifiers tested degradability of NPTII protein in ruminal fluid. Quantities of protein used in the test were within the range of expected practical levels (0.1 ng/ml, 1.0 ng/ml) but tests also included much higher concentrations (100 ng/ml). Data provided indicate that even the highest concentration of NPTII protein was degraded within a very short time period in a fermentation broth containing enzymes, bacteria and protozoans.

Pulp derived from the GM potato line was also used in a heifer feeding trial. Groups of 16 animals were fed for up to 8 weeks with a diet which included pulp produced from GM or non-GM potato material. The pulp constituted slightly more than 30% of the total feed calculated on a dry weight basis. There were no conspicuous differences in feed intake between animals fed on pulp derived from GM or non-GM potatoes. No statistically significant differences in heifer weight gain were detected. No effects of pulp derived from the GM potato line were observed on animal health and intestinal functions.

2.3. Substantial Equivalence: Comprehensive data sets are provided for field experiments carried out in 1996, 1997 and 1998. Dry matter, protein, ash, fibre, digestible fibre, fat, starch and sugar, chlorogenic acid, glycoalkaloid, vitamin C, nitrate and mineral ion contents have been quantified in tubers of the GM line and in the non-GM parent control. Compositional analyses are also provided for the potato pulp (crude protein, ash, fibre, digestible fibre, mineral ion content), fruit juice and fruit water (dry matter, acidity, mineral ion content) derived from the starch extraction process. Some differences in tuber composition are reported e.g. glycoalkaloid levels are lower, and vitamin C levels higher, in the GM line compared with the non-GM parent. However, values obtained fall within the ranges reported for potato tubers and there were no increases in any compounds which could be regarded as toxic or anti-nutritional.

From official trials carried out in Sweden there is no evidence that insects associated with the transgenic clone are more or less abundant compared with the non-GM parent. The GM clone is no more susceptible or resistant to aphids, leafhoppers, larvae, worms, snails or potato cyst nematodes than the parent variety. Similarly there is no evidence for modified susceptibility to *Phytophthora, Erwinia, Alternaria* and the viruses PVY, PLRV, PMTV and TRV.

On the basis of these findings it is accepted that, with the exception of the modified starch structure, the GM clone is substantially equivalent.

3. Environmental Aspects

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 detected with the potato's wild relatives *Solanum nigrum* and *S. dulcamara.* Very low frequency exchange has been found with *Solanum nigrum* under artificial and forced hybridisation. Therefore the chances of successful hybridisation between transformed potatoes and other *Solanum* species is considered to be very unlikely. No data is available on potential transfer to other *Solanum* species e.g. *S. eleagnifolium.* However since the chance of any successful transfer is considered to be remote and would convey no selective advantage to any hybrid, the potential risk is considered to be extremely low. Any genetic spread is assessed as limited to cross-pollination with other cultivated potatoes.

The modified potato contains an *nptII* gene for kanamycin resistance with the potential for transfer from plant material to microbes in the soil. However, considering the likelihood of degradation of cell DNA during autolysis in any plant material left in the soil and the natural occurrence of kanamycin resistance in soil bacteria, any additional contribution from potential transfer to soil microbes is considered to be insignificant.

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

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 (where winter temperatures are low enough), drought and standard agricultural practice in following crops.

3.3. Safety to Plant-Associated Organisms: No data is provided on the safety of the modified crops to plant-associated organisms. However, the results of growing trials suggest neither greater susceptibility nor greater resistance to pests and diseases than non-modified potato lines. In view of this and the equivalent composition of the modified potato plant, it is considered that no adverse effects on plant-associated organisms would be expected from cultivation of the modified potato.

4. OVERALL ASSESSMENT

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 clone EH92-527-1, derived from cultivar Prevalent, for use in cultivation and starch production, is likely to cause adverse effects on human health and/or the environment. The GM potato has modified starch structure (a high amylopectin content) due to the reduced expression of a granule bound starch synthase gene. The Committee has evaluated the molecular analysis of the transgenic line and is satisfied that only the T-DNA region has been transferred and integrated as a single copy insert. From the sequence data provided by the applicant there is no reason to assume that the DNA regions transferred code for toxic and/or allergenic products. Furthermore, in the unlikely event that horizontal transfer of gene sequences would occur between the GM potato and bacteria, the bacteria would not pose any additional risk to human health or the environment. By-products of the starch extraction process are used for other purposes including animal feed. The risk assessment included an analysis of data from appropriate animal feeding studies. This data indicates that after starch extraction the by-products of the GM potato are as safe as those from the non-GM parental line. Compositional analysis has shown that the GM line falls within expected variation for potato, except for the change in starch composition due to the genetic modification. Since potato is vegetatively propagated and the natural exchange of genetic material is only possible with other varieties of potato, there is insignificant risk to the environment of any transgene flow. No adverse effects on plant-associated organisms have been demonstrated or would be expected from cultivation of the modified potato. The Committee is therefore of the opinion that there is no evidence to indicate that the placing on the market of potato clone EH92-527-1, for use in cultivation and starch production is likely to cause adverse effects on human health and the environment.

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[3] Scientific Committee on Animal Nutrition
[4] Scientific Committee on Food