SUMMARY INFORMATION FORMAT (SNIF) FOR PRODUCTS CONTAINING GENETICALLY MODIFIED HIGHER PLANTS (GMHPs)

Potato Clone EH92-527-1

A. GENERAL INFORMATION

1. Details of notification

- (a) Member State of notification: Sweden
- (b) Notification number: C/SE/96/3501
- (c) Name of the product (commercial and other name):

EH92-527-1 is the name of the potato line. A commercial name has not been established.

- (d) Date of acknowledgement of notification: 6 August 1996
- 2. Notifier
 - (a) Name of notifier: Amylogene HB
 - (b) Address of notifier:
 c/o Plant Science Sweden AB, Herman Ehles väg 2-4
 S-268 31 Svalöv, Sweden
 - (c) Is the notifier: Domestic manufacturer [X] Importer []
 - (d) In the case of an import the name and address of the manufacturer shall be given

Not applicable

3. General Description of the product

(a) Name of the recipient or parental plant and the intended function of the genetic modification

The variety Prevalent (of species potato, *Solanum tuberosum*) is the recipient variety of the genetic modification yielding the line EH92-527-1. EH92-527-1 has been modified to contain a starch fraction consisting at least to 98% of amylopectin.

(b) Any specific form in which the product must not be placed on the market (seeds, cut-flowers, vegetative parts, etc.) as a proposed condition of the authorization applied for

The product is not to be placed on the market for food uses of whole potatoes or derived starch.

(c) Intended use of the product and types of users

The potato starch industry and industrial applications are the intended users.

The potato clone will be grown for the production of raw material for the starch industry. The clone will be used for the production of a specific starch quality and will therefore be grown without contamination with other starch potatoes. Cultivation will be partly for production of seed potatoes and partly for production of raw material for the manufacturing of potato starch.

The cultivation of seed potatoes will be for the production of new seed potatoes and for production of certified seed for cultivation of starch potatoes. In the production of seed potatoes the same conditions as used for other seed potatoes will be applied to EH92-527-1.

Seed potato production will be carried out primarily in Sweden, but may also be considered in other parts of the EEG where starch potatoes are grown.

As a by-product of starch production, potato pulp will be used as cattle feed by farmers.

(d) Any specific instructions and/or recommendations for the use, storage and handling, including mandatory restrictions proposed as a condition of the authorisation applied for

The starch potatoes produced will be used for the production of potato starch. The same growing conditions will be applied to EH92-527-1 as are used for other starch potatoes, except that EH92-527-1 potatoes will be handled according to a specifically designed Identity Preservation System. Starch will be extracted from the product and sold to the paper products

industry. As by-products of starch production, potato pulp, fruit juice and fruit water will be produced. The fruit juice will be spread on cultivated land. The fruit water will be spread on cultivated land, or it will be handled as wastewater. Potato pulp will be used as cattle feed. The production areas for EH92-527-1 are identical to the production areas of non-transgenic starch potatoes in the EEG.

Neither whole potatoes nor derived starch will be used as human food.

(e) If applicable, geographical areas within the EEG to which the product is intended to be confined under terms of the authorisation applied for

The product will be grown in areas of Europe where starch potatoes and potato seed are presently grown.

(f) Any type of environment to which the product is unsuited

The product is grown preferable in arable land. Other types of land are generally considered unsuitable.

(g) Any proposed packaging requirements

No special packaging is required for either the potatoes or the derived starch.

(h) Any proposed labelling requirements in addition to those required by law

As part of the Identity Preservation System, EH92-527-1 potatoes, in all stages, will be identified so as to clearly distinguish them from any other potatoes. Seed potatoes are labelled in the same way as other seed potatoes, i. e. with a certification label and/or a plant passport, but with the additional information that the potatoes are genetically modified.

Starch potatoes are handled in bulk and are delivered directly from the grower to the starch factory. Each delivery is immediately sampled and analysed.

(i) Estimated potential demand

An annual production of 50.000 to 75.000 tons of potatoes is estimated to be reached in the next five years.

(j) Unique identification code(s) of the GMO(s)

The unique identifier for potato clone EH92-527-1, BPS-25271-9, has been attributed based on the guidelines for the designation of a unique identifier for transgenic plants developed by the OECD Working Group on the Harmonisation of Regulatory Oversight in Biotechnology.

4. Has the GMHP referred to in this product been notified under part B of directive 2001/18/EC and /or 90/200/EEG?

Yes [X] No []

5. Is the product being simultaneously notified to another member state?

Yes [] No [X]

or

Has the product been notified in a third country either previously or simultaneously?

Yes [] No [X]

6. Has the same GMHP been previously notified for marketing in the community?

Yes [] No [X]

7. Measures suggested by the notifier to take in case of unintended release or misuse as well as measures for disposal and treatment

The Notifier has developed a detailed plan for Identity Preservation that includes tracking of the potato throughout all production stages.

Seed potatoes, which by mistake have been mixed with other seed non-modified potatoes, shall be used for the manufacturing of technical starch. Starch potatoes, which by mistake have been mixed with other non-modified potatoes, shall be used for the manufacturing of technical starch.

Potatoes, which cannot be used for starch production or seed will be destroyed in compost.

By-products from the industrial process (pulp, fruit juice and fruit water) will be handled the same way as the corresponding by-products from non-modified potatoes.

B. NATURE OF THE GMHP CONTAINED IN THE PRODUCT

INFORMATION RELATING TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

8. Complete name

- (a) Family name: Solanacae
- (b) Genus: Solanum
- (c) Species: *tuberosum*
- (d) Subspecies: tuberosum
- (e) Cultivar/breeding line: Prevalent
- (f) Common name Scientific and other denominations: Potato

9. (a) Information concerning reproduction

(i) Mode(s) of reproduction

Reproduction can be executed vegetatively by tubers as well as sexually by botanical seed.

(ii) Specific factors affecting reproduction, if any

Due to frost sensitivity the survivability of the tubers is influenced the winter temperature.

(iii) Generation time

The generation period is one year.

(b) Sexual compatibility with other cultivated or wild plant species

The transgenic clone/cultivar is compatible with other cultivated potato varieties as well as with true seedling plants produced by hybridization between potato varieties, including their vegetative progenies. All these belong to the species *Solanum tuberosum*. *Solanum tuberosum* is not compatible with wild related species in Europe, *S. nigrum* and *S. dulcamara*. Hybridisation with wild relatives present in Europe is not possible, due to efficient incompatibility barriers.

10. Survivability

(a) Ability to form structures for survival or dormancy

Potatoes survive as tubers or as seed. As the tubers are generally frost sensitive their survivability is dependent on temperature. They may survive the winter in the soil in most parts of Europe, but seldom in Scandinavia north of the 57th latitude. The survivability is also limited by cultivation practices such as ploughing, harrowing and application of herbicides and by competition from other crops in the crop rotation.

(b) Specific factors affecting survivability, if any

Botanical seed over winter regardless of temperature. Their survival depends on cultivation practices and crop rotation. Normally, seedling plants are eliminated by ploughing, harrowing, herbicides and competition in crop rotation. When plants spread outside cultivated areas, they are usually eliminated by competition from the natural flora. The potato plants do not usually thrive in this environment. This is also true for potato plants originating from tubers, which might have been spread from cultivated areas. In northern Europe they occur exclusively in arable land, while in southern Europe they may also be found as escapes outside arable land.

11. Dissemination

(a) Ways and extent of dissemination

Dissemination may be caused by handling of the crop (tubers and botanical seed) or by wind and insects (pollen).

(b) Specific factors affecting dissemination, if any

Spread of tubers is mainly caused by human activities, but may also be caused by animals. Potato fruits are toxic (glycoalkaloids), making dissemination by animals very unlikely. Dissemination of botanical seed may take place as a consequence of handling of foliage. The dissemination of tubers and seed is mainly restricted to the actual field where the crop is grown, but may also to some extent happen outside that area. Pollen is mainly spread by insects over short distances but may occur over longer distances at a very low frequency. Wind dissemination is negligible.

12. Geographical distribution of the plant

Potatoes are grown throughout Europe, starch potatoes in Eastern Europe, the Nordic countries, Germany, Holland, Belgium and France. The original natural habitat of potatoes is in South America.

13. In the case of plant species not normally grown in the Member State(s), description of the natural habitat of the of the plant, including information on natural predators, parasites, competitors and symbionts

Potatoes are grown throughout Europe, starch potatoes in Eastern Europe, the Nordic countries, Germany, Holland, Belgium and France. The original natural habitat of potatoes is in South America.

14. Potentially significant interactions of the plant with other organisms where it is usually grown, including information on toxic effects on humans, animals and other organisms

The main toxic or anti-nutritional substances in potatoes are glycoalkaloids and nitrates. Glycoalkaloids which in high concentrations are toxic, are found in harmful amounts mainly in the above ground parts of the plant - stems, leaves and fruits. In the tubers of cultivated potato varieties, the content is usually low, below 100 mg per kilogram fresh weight. It happens, however, that higher, occasionally much higher concentrations are found as a consequence of stress. Therefore authorities and controlling agencies in many countries have established a maximum glycoalkaloid content of 200 mg per kilogram fresh weight in consumption potatoes. All new potato varieties are assessed at a level well below that limit.

Nitrates are found in the entire plant and are considered anti-nutritional, especially for babies. Therefore plant breeder's aim at very low contents in new potato varieties.

People must be considered a part of the ecosystem where potatoes are grown. Man is probably the organism most intensely interacting with the potato crop, as potatoes are a significant part of the diet in large parts of the world. The only part of the plant, which is consumed, is the tubers. The experience of individuals (e.g. "do not eat green potatoes") along with restrictions mentioned above should create a sufficient protection.

Potatoes are also commonly used as feed throughout the world. Wild animals mammals and birds) occasionally feed on potatoes exposed in the field or in potato clamps. As is the case for humans, a high content of glycoalkaloids is toxic and poisoning may occur.

Insects like aphids (*Myzus persicea, Aphis nasturtii, A. frangulae* and others), leaf hoppers (*Empoasca* spp) and the Colorado beetle (*Leptinotarsa decemlineata*) are well known parasites in potato cultivation, as are nematodes (*Globodera* spp, *Ditylencus* spp, *Paraditylencus* spp, *Tricodorus* spp and *Paratricodorus* spp). Normal contents of glycoalkaloids in leaves and stems do not appear to be toxic for those animals. On the other hand it has been shown that larva of the klick beetle (*Agriotes* spp) avoid potatoes with high contents. At a very high content of glycoalkloids it also seems that the Colorado beetle and

leaf hoppers are repelled, while aphids and cyst nematodes are apparently not affected.

Just like other plants there are many microorganisms, viruses and viroids interacting with the potato plant. Well known pathogenic fungi are for example potato late blight (*Phytophtora infestans*), black scurf (*Rhizoctonia solanii*), potato wart disease (*Synchythrium endobioticum*), early blight (*Alternaria solani*), powdery scab (*Spongospora subterrana*), skin spot (*Polyscytalum pustulans*), silver scurf (*Helminthosporium solani*), grey mold (*Botrytis cinerea*), watering wound rot (*Pythium ultimum*), wilt (*Verticillium* spp) and storage rots (*Phoma foveata* and *Fusarium* spp). According to available literature a high content of glycoalkaloids does not hinder an attack by those disease fungi with a possible exception of *Fusarium* rot.

Among pathogenic bacteria, the most common ones are black leg (*Erwinia carotovora* ssp *carotovora*, *Erwinia carotovora* ssp *atroseptica*, and *Erwinia chrysanthemi*) and common scab (*Streptomyces scabies*), while in Europe brown rot (*Pseudomonas solanaceareum*) and ring rot (*Corynebacterium sepedonicum*) are quarantine diseases. None of the pathogenic bacteria seem to be affected by high glycoalkaloid contents.

There are many viruses that attack the potato plant. Economically most important are potato leaf roll virus (PLRV), potato virus Y (PVY), potato virus A (PVA), potato virus X (PVX), potato virus S (PVS), potato virus M (PVM), tobacco rattle virus (TRV) and potato mop top virus (PMTV). Among viroids the potato spindle tuber viroid (PSTV) is the most important one. The only report regarding glycoalkaloid influence on virus infection concerns PVY.

15. Phenotypic and genetic traits

The Prevalent potato variety grows with a tall, dense and well covering foliage, green stems, round-oval tubers with yellow skin and flesh, red violet light sprouts and flowers, high yielding capacity, high starch content, and resistance to wart disease, cyst nematodes (*Globodera rostochiensis*) race Ro1, late blight (*Phytophtora infestans*) race specific, and potato virus A.

The recipient clone (variety Prevalent) is identified by its morphological characteristics according to the official variety description published by the seed certification authorities.

The modified line EH92-527-1 is phenotypically equivalent to Prevalent.

INFORMATION RELATING TO THE GENETIC MODIFICATION

16. Description of the methods used for the genetic modification

Transformation of recombinant DNA has been executed with *Agrobacterium tumefaciens*. A binary vector system has been utilised, in which T-DNA containing the genes to be transferred is found on one plasmid, while DNA-mobilising functions are found on a modified Ti-plasmid. Transformation has been made to cut leaf tissue, and was followed by treatment with Claforan (500 mg/l) in order to kill *A. tumefaciens*. Shoots were regenerated using kanamycin (50 mg/l) as a selection agent. *A. tumefaciens* is regarded as eradicated, since subsequent cultivation without any selection agent does not generate any bacteria growth.

17. Nature and source of the vector used

Agrobacterium tumefaciens strain LBA4404 with Ti-plasmid pAL4404 was used for transformation of potatoes. In pAL4404 T-DNA and onkogenic traits have been deleted. The binary vector, which was used as a carrier of traits transformed to plant tissue, originates from pBIN 19. pBIN19 may be multiplied in *E. coli* as well as in *A. tumefaciens*. It is limited to the left and to the right by border sequences from pTiT37. Outside the T-DNA border sequences there is a kanamycin resistance gene, which can be used for selection in bacteria. That gene is not transferred to the plant.

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 phosphotransferaseII coding sequence (*nptII*; 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.

18. Size, source {name of donor organism(s)} and intended function of each constituent fragment of the region intended for insertion

Agrobacterium tumefaciens strain LBA4404 containing Ti-plasmid pAL4404 was used for transformation of potato. In pAL4404 the T-DNA and the oncogenic traits are deleted. The binary vector pHoxwG, which functioned as a carrier of

the traits that have been transferred to plant tissue is derived from pBIN19. pHoxwG can be propagated both in *E. coli* as well as *A. tumefaciens* and contains a T- DNA that is limited by the right and left border sequences from pTiT37. Outside the T-DNA border sequences there is a gene for kanamycin resistance that makes selection in bacteria possible. This gene, however, is not transferred to the plant. The T-DNA on pHoxwG is identical to pBIN19 except DNA segments inserted by the applicant and is described more in detail below.

The T-DNA of pBIN19 has according to sequence data (Genbank acc. U09365) a size of 3213 base pairs and is limited by border sequences from pTiT37, origin Within these borders there is a kanamycin Agrobacterium tumefaciens. resistance gene that can be expressed in plant tissue, and also a multiple cloning site from M13mp19. The kanamycin resistance gene is of *nptII*-type and originates from Tn5, which can be isolated from various species of bacteria such as *Escherichia coli*. This gene is regulated by a nopaline-synthase promoter for expression in plant tissue and is terminated by a polyadenylationsequence from the nopaline-synthase gene of origin A. tumefaciens. In potato the nopalinesynthase promoter expresses a succeeding gene (here *nptII*) in leaf tissue and to some degree in tuber tissue. The T-DNA border sequences are used to introduce foreign DNA into plant chromosomes. The *nptII*-gene with nopaline-synthase DNA segments is used for selection of transformed plant tissue. A multiple cloning site, origin M13mp19, is used in order to be able to clone genes of interest for introduction into plant chromosomes. A gene that is to modify the starch composition has been inserted into the multiple cloning site. This gene consists of the gbss-promoter (987 bp), which is isolated from potato, and a polyadenylationsequence from the nopaline-synthase gene (252 bp), origin Agrobacterium tumefaciens. The promoter gives strong expression in tubers, pollen and root tips. This has been determined by expressing a marker gene (uidA) in transgenic potato. Between these two DNA-segments, a segment of the gbss-gene has been inserted in reversed orientation in relation to the promoter. This DNA-segment constitutes in the gene construct pHoxwG 1,945 base pairs of the potato gbss-gene. In the tuber the gene construct pHoxwG is to inhibit the expression of the endogenous gbss-gene and thereby reduce the amount of amylose in the tuber.

INFORMATION RELATING TO THE GMHP

19. Description of the trait(s) and characteristics which have been introduced or modified

By means of a recombinant nptII-gene the plant has obtained resistance to kanamycin. By way of the recombinant gbss-gene the plant has obtained a reduced content of amylose in tuber tissue.

20. Information on the sequences actually inserted/deleted/modified

(a) Size and structure of the insert and methods used for its characterization, including information on any parts of the vector introduced in the GMHP or any carrier or foreign DNA remaining in the GMHP

The insert in GM line EH92-527-1 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 Tn5 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. DNA sequences towards the left border have been deleted during integration. This was confirmed by DNA sequencing of the inserted sequence.

An open reading frame analysis based on the actual inserted sequence in EH92-527-1 has been carried out. Eighteen ORFs are found and searched against databases, 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 that 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 Tn5 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 Several polyclonal antibodies were developed using known allergens. 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. Data are provided demonstrating that the ORF4 polypeptide is not present in potato leaves from EH92-527-1. The genetic integration was shown to be stable as determined over several generations of vegetative propagation. The consistently low level of amylose is evidence of the stability of the inserted DNA.

- 1. The choice of method for transformation. *Agrobacterium tumefaciens* produces chromosomal transformation. For the transformation of organelles, it is necessary to use other techniques like a particle cannon to introduce DNA.
- 2. The integrated copy of the selected DNA has been ascertained by hybridizing to the total DNA from the transformed clone.
- 3. The integrated copy has given the intended effect, i.e. it inhibits the expression of a chromosomal gene.
- 4. There was no detection of the DNA belonging to the region outside the T-DNA when hybridizing with the total DNA from the transformed clone.

(b) In case of deletion, size and function of the deleted region(s)

Not applicable

(c) Location of the insert in the plant cells (integrated in the chromosome, chloroplast, mitochondrion, or maintained in a non-integrated form) and methods for its determination

The presence of an integrated copy of the selected DNA has been shown by "Southern blotting". The recombinant gbss-gene has inhibited the expression of the chromosomal endogene gbss-gene, and the modified trait has been shown to be stable for several tuber generations. Along with the fact that the vector does not contain functions for replication in plant cells, this excludes that the selected DNA is present in a non-integrated form. Examination of clean chloroplast DNA with "Southern blotting" has not revealed any integration of DNA from the transformation vector pHoxwG.

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The integrated copy has given the intended effect, i.e. it inhibits the expression of a chromosomal gene.

There was no detection of the DNA belonging to the region outside the T-DNA when hybridizing with the total DNA from the transformed clone.

(d) Copy number and genetic stability of the insert

Evidence is provided that the GM potato clone has one integrated copy of the T-DNA at one locus. Southern blot and sequence analysis shows 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.

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The stability of the integrated DNA over consecutive tuber generations has not been determined. However, when transforming with the antisense technique, aiming at a down-regulation of an endogenous gene, there are comparatively few transformation events, which result in a complete inhibition of the particular gene. This indicates that the specific transformation event, its structure and localization in the genome, plays a very decisive role for complete inhibition. Therefore, it is very unlikely that an unstable insert would result in a stable inhibition of amylose production over several consecutive generations. (e) In case of modifications other than insertion or deletion, describe function of the modified genetic material before and after the modification as well as direct changes in expression of genes as a result of the modification

Not applicable

21. Information on the expression of the insert

(a) Information on the expression of the insert and the methods used for its characterization

Kanamycin resistance has been determined by the survival of the organism on medium containing kanamycin. The concentration of the NPTII protein has been measured in plant tops. A reduction of the amount of GBSS-protein has been determined by polyacrylamide electrophoresis. A reduction of the amylose content has been determined by iodine staining of starch granules and with spectrophotometry.

Extraction and characterization of starch from tubers of the potato clone have been conducted. A pilot plant has been used for the production of starch. In the pilot plant the same technique is used as in a full-scale production of starch. Subsequently full-scale production has also been used to determine the processability of the product.

Characterization was made by the following methods:

- The chain length profile of branched starch molecules by gel permeation chromatography.
- The blue value was determined by staining with iodine and measuring the amount of absorbance.
- Degree of branching by 500mhz ¹H-NMR
- Stability of water solution by measuring of the storage module and the absorption during storage.
- Swelling behaviour.

The results of the starch characterization show that the starch of the potato clone produced contains less than 2% amylose.

Experiments with iodine staining and spectrophotometric analysis have shown a reduction of amylose content mainly in tubers and root tips, but it is also indicated that the starch composition in leaves may be affected. NptII is supposed to be expressed in all parts of the plant although the expression pattern in the potato plant of the promoter (nos-promoter) used for expression of the nptII-gene has not been sufficiently investigated. There are not any parts of the plant where the expression of the nptII-gene can be completely excluded.

(a) Parts of the plant where the insert is expressed (e.g. roots, stem, pollen, etc.)

Experiments with iodine staining and spectrophotometric analysis have shown a reduction of amylose content mainly in tubers and root tips, but it is also indicated that the starch composition in leaves may be affected.

22. Information on how the GMHP differs from the recipient plant in

(a) Mode(s)and/or rate of reproduction

No different than parent plant

(b) Dissemination

No different than parent plant

(c) Survivability

No different than parent plant

(d) Other differences

The results of the starch characterization show that the starch of the potato clone produced contains less than 2% amylose in the starch fraction and thereby more than 98% amylopectin. On a side by side comparison in field trials significant differences to the parental variety were found in vitamin C, sucrose and fructose levels being higher. However all levels are within ranges found in parental variety.

23. Potential for transfer of genetic material from GMHP to other organisms

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.

24. Information on any harmful effects on human health and the environment, arising from the genetic modification

There are no information or data indicating that EH92-527-1 represents any more of a risk to human health or the environment than the parental variety Prevalent. This is based on field trials over 9 years including official trials, compositional analysis and analysis of the genetic insert and its expression.

25. Information on the safety of the GMHP to animal health, where the GMHP is intended to be used in animal feedstuffs, if different from that of the recipient/parental organism(s)

Pulp produced as a by-product of the extraction process 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 ruminal fluid broth containing enzymes, bacteria and protozoans (refer to Annex 40).

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 that 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.

26. Mechanism of interactions between the GMHP and the target organisms (if applicable), if different from that of the recipient/parental organism(s)

Not applicable

27. Potentially significant interactions with non-target organisms, if different from the recipient or parental organism(s)

In potatoes normally existing toxic and anti-nutritional substances are glycoalkaloids and nitrates. The interaction with other organisms has been described and it is shown that the impact of these substances have not been

changed as a consequence of the modification. On that account, the modified potato clone, EH92-527-1, does not affect other organisms in any way different than what the unmodified recipient cultivar, Prevalent.

28. Description of detection and identification techniques for the GMHP, to distinguish it from the recipient or parental organism(s)

EH92-527-1 potatoes and the derived starch may be distinguished from all other potatoes through Iodine staining of cut potatoes and visual inspection of surface coloration and of starch granules and microscopy inspection of granule coloration.

PCR may be used to identify the specific event, EH92-527-1.

INFORMATION ON THE POTENTIAL ENVIRONMENTAL IMPACT FROM THE RELEASE OF THE GMHP

29. Potential environmental impact from the release or placing on the market of GMOs (Annex II, D2 of Directive 2001/18/EC), if different from a similar release or placing on the market of the recipient or parental organism(s)

There are no differences between EH92-527-1 and the parental variety Prevalent that will result in a potential environmental impact.

This is based on field trials over 9 years including official trials, compositional analysis and analysis of the genetic insert and its expression.

30. Potential environmental impact of the interaction between the GMHP and target organisms (if applicable), if different from that of the recipient or parental organism(s)

Not applicable

31. Possible environmental impact resulting from potential interaction with nontarget organisms if different from that of the recipient or parental organism(s)

(a) Effects on biodiversity in the area of cultivation

EH92-527-1 will be grown in the same parts of Europe where starch potatoes are presently grown and using the identical farming techniques. The demonstrated equivalency of the EH92-527-1 clone to the parental variety Prevalent means that the growing of EH92-527-1 represents a no greater risks to biodiversity than does the parental variety or other starch potatoes.

(b) Effects on biodiversity in other habitats

Cultivated starch potatoes no not grow well outside of the immediate cultivated area. Pollen movement from EH92-527-1 is like the parental variety and moves only very short distances, if at all. Considering this and the equivalency of EH92-527-1 to the parental variety, the effects on biodiversity in other habitats is no different than from other starch potatoes.

(c) Effects on pollinators

The specific change of starch composition in EH92-527-1 is manifested in pollen but amylopectin is normally found in pollen and not unique and therefore neither is the exposure to pollinators.

(d) Effects on endangered species

The equivalency of EH92-527-1 is based on field trials and in part on interaction with other species further in part on a compositional analysis of significant components. No components of the parental variety that may affect endangered species have been altered. EH92-527-1 represents no greater risk to endangered species than does the parental variety Prevalent.

C. INFORMATION RELATING TO PREVIOUS RELEASES

32. History of previous releases notified under Part B of the Directive 2001/18/EC and under Part B of Directive 90/220/EEC by the same notifier

(a) Notification number

Please refer to Attachment A for a list of prior notifications under 90/220/EEG.

(b) Conclusions of post-release monitoring

Potato plants have been found in the fallow following cultivation of modified potatoes and in the subsequent 3 years without potatoes. This situation is no different than that which exists with Prevalent. It is therefore concluded that the modified clone does not differ from the recipient clone with respect to survivability.

(c) Results of the release in respect to any risk to human health and the environment (submitted to the competent authority according to Article 10 of Directive 2001/18/EC)

No risk to human health or the environment resulted from any of the listed releases. Reports have been submitted to the competent authority and fields have been inspected by competent authority.

The general agronomic characteristics of EH92-527-1 were no different than those for the parental variety Prevalent. All aspects of EH92-527-1 in the field production stages were identical to the parental variety. No negative impacts on the environment were observed during any field trials or production. The characteristics of EH92-527-1 and significant equivalency to the parental variety do not introduce any risks to human health or the environment.

33. History of previous releases carried out inside or outside the Community by the same notifier

(a) Release country

All in Sweden

(b) Authority overseeing the release

Jordbruksverket S-551 82 Jönköping, Sweden

(c) Release site

Please refer to Attachment A for a list of all release sites.

(d) Aim of the release

Observations on stability of agronomic characteristics, general susceptibility to diseases and pests, control of identity and seed production as well as test production of starch.

(e) Duration of the release

The duration of all releases was 4-5 months, which is consistent with the typical growing season of starch potatoes.

(f) Aim of post-releases monitoring

To verify absence of potato plants emerging from groundkeepers or botanical seed, and in case such plants are found to remove and destroy them.

(g) Duration of post-releases monitoring

1 year fallow after each release + 3 years without potato cultivation

(h) Conclusions of post-releases monitoring

Potato plants have been found in the fallow following cultivation of modified potatoes and in the subsequent 3 years without potatoes. This situation is no different than that which exists with Prevalent. It is therefore concluded that the modified clone does not differ from the recipient clone with respect to survivability.

(i) Results of the release in respect to any risk to human health and the environment

The general agronomic characteristics of EH92-527-1 are no different than those for the parental variety Prevalent. All aspects of EH92-527-1 in the field production stages are identical to the parental variety. No negative impacts on the environment were observed during any field trials or production. The characteristics of EH92-527-1 and significant equivalency to the parental variety do not introduce any risks to human health or the environment.

D. INFORMATION RELATING TO THE MONITORING PLAN – IDENTIFIED TRAITS, CHARACTERISTICS AND UNCERTAINTIES RELATED TO THE GMO OR ITS INTERACTION WITH THE ENVIRONMENT THAT SHOULD BE ADDRESSED IN THE POST-COMMERCIALISATION MONITORING PLAN

An Environmental Risk Assessment was prepared in accordance with guidance documents and the general principles of risk assessment. This document identified those areas related to EH92-527-1 that differ from the parent variety that could, under certain circumstances, represent risks to human health and the environment. The Monitoring Plan contains elements that incorporate "general surveillance for unanticipated or unforeseen adverse effects" and "case-specific" monitoring for those areas identified in the Environmental Risk Assessment.

General surveillance will consider areas related to (1) growth characteristics, (2) general characteristics of the plants as to height, shape and colour, (3) susceptibility to disease and pests and (4) any changes in animals within and in proximity to the fields. The case-specific monitoring will focus on (1) any significant detrimental changes in the composition of the tubers and (2) a change in the presence of the bleomycin resistant protein associated with ORF4.

Attachment A: Information Relating to Previous Releases Notified under Part B of Directive 90/220/EEC

Year	Notification No.	Authorizing Country	Location	Duration (Dates)	Purpose	Size
1993	Dnr 22-4314/92	Sweden	Teckomatorp	26 April-27 Sept.	Observation trial	30 plants
1994	Dnr 22-4363/93	Sweden	Teckomatorp	27 April-7 Oct.	Observation trial	75 plants
1994	Dnr 22-4363/93	Sweden	Händene	16 May-22 Sept.	Seed production & Observation	0.1 ha
1994	Dnr 22-4363/93	Sweden	N. Sunderbyn	15 June-22 Sept.	Seed production & Observation	21,000 plants
1995	Dnr 22-28/95	Sweden	Häljarp	10 May-4 Oct.	Official trial	250 m2
1995	Dnr 22-28/95	Sweden	Fjälkinge	18 May-13 Oct.	Official trial and Starch production	0.5 ha
1995	Dnr 22-28/95	Sweden	Händene	13 May-1 Oct.	Seed production & Observation	0.2 ha
1995	Dnr 22-28/95	Sweden	Habo	27 May-9 Oct.	Seed production & Observation	1.3 ha
1995	Dnr 22-28/95	Sweden	N. Sunderbyn	12 June-14 Sept.	Seed production & Observation	1.3 ha
1996	Dnr 22-530/96	Sweden	Häljarp	15 May-3 Oct.	Official trial	250 m2
1996	Dnr 22-530/96	Sweden	Skepparslöv	17 May-14 Oct.	Official trial	250 m2
1996	Dnr 22-530/96	Sweden	Fjälkinge	7 May-9 Oct.	Official trial and Starch production	1.35 ha
1996	Dnr 22-530/96	Sweden	Habo	5 June-3 Oct.	Seed production & Observation	3.5 ha
1996	Dnr 22-530/96	Sweden	Flaskebo	6 June-10 Oct.	Seed production & Observation	3.5 ha
1996	Dnr 22-530/96	Sweden	Händene	15 June-3 Oct.	Seed production & Observation	1.0 ha
1996	Dnr 22-530/96	Sweden	N. Sunderbyn	10 June-19 Sept.	Seed production & Observation	2.5 ha
1996	Dnr 22-530/96	Sweden	Tegsnäset	15 June-24 Sept.	Seed production & Observation	2.0 ha
1997	Dnr 22-1782/97	Sweden	Axeltofta	14 May-3 Oct.	Official trial	200 m2
1997	Dnr 22-1782/97	Sweden	Ronneby	26 May-25 Sept.	Official trial	270 m2
1997	Dnr 22-1782/97	Sweden	Skepparslöv	12 May-14 Oct.	Official trial	270 m2

1997	Dnr 22-1782/97	Sweden	Halltorp	16 May-early Oct.	Starch production & Observation	2.8 ha
1997	Dnr 22-1782/97	Sweden	Bergkvara	16 May-early Oct.	Starch production	8.0 ha
1997	Dnr 22-1782/97	Sweden	Ljungbyholm	16 May-early Oct.	Starch production	4.0 ha
1997	Dnr 22-1782/97	Sweden	Ljungbyholm	16 May-early Oct.	Starch production	4.0 ha
1997	Dnr 22-1782/97	Sweden	Ljungbyholm	17 May-early Oct.	Starch production	4.4 ha
1997	Dnr 22-1782/97	Sweden	Ljungbyholm	16 May-early Oct.	Starch production	2.8 ha
1997	Dnr 22-1782/97	Sweden	Ljungbyholm	16 May-early Oct.	Starch production	1.3 ha
1997	Dnr 22-1782/97	Sweden	Ljungbyholm	16 May-early Oct.	Starch production	2.1 ha
1997	Dnr 22-1782/97	Sweden	Mörbylånga	20 May-early Oct.	Starch production	4.5 ha
1997	Dnr 22-1782/97	Sweden	Mörbylånga	15 May-3 Oct.	Starch production	2.5 ha
1997	Dnr 22-1782/97	Sweden	Söderåkra	24 May-early Oct.	Starch production	1.5 ha
1997	Dnr 22-1782/97	Sweden	Vassmolösa	15 May-3 Oct.	Starch production	2.0 ha
1997	Dnr 22-1782/97	Sweden	Vassmolösa	16 May-early Oct.	Starch production	2.3 ha
1997	Dnr 22-1782/97	Sweden	Ramdala	17 May-early Oct.	Starch production	1.6 ha
1997	Dnr 22-1782/97	Sweden	Ronneby	20 May-early Oct.	Starch production	4.0 ha
1997	Dnr 22-1782/97	Sweden	Ronneby	26 May-early Oct.	Starch production	3.2 ha
1997	Dnr 22-1782/97	Sweden	Skara	20 May-20 Sept.	Seed production & Observation	6.5 ha
1997	Dnr 22-1782/97	Sweden	Skara	7 May-8 Sept.	Seed production & Observation	7.4 ha
1997	Dnr 22-1782/97	Sweden	Lidköping	19 May-20 Sept.	Seed production & Observation	7.7 ha
1997	Dnr 22-1782/97	Sweden	Lindärva	7 May-5 Sept.	Seed production & Observation	7.0 ha
1997	Dnr 22-1782/97	Sweden	Umeå	12 June-21 Sept.	Seed production & Observation	1.5 ha
1997	Dnr 22-1782/97	Sweden	Granö	4 June-25 Sept.	Seed production & Observation	2.0 ha
1997	Dnr 22-1782/97	Sweden	N. Sunderbyn	4 June-21 Sept.	Seed production & Observation	2.3 ha

1998	Dnr 22-2519/98	Sweden	Axeltofta	7 May-1 Oct.	Official trial	175 m2
1998	Dnr 22-2519/98	Sweden	Skepparslöv	11 May-19 Oct.	Official trial	216 m2
1998	Dnr 22-2519/98	Sweden	Ramdala	14 May-7 Oct.	Official trial	202 m2
1998	Dnr 22-2519/98	Sweden	Sölvesborg	4 May-15 Oct.	Starch production	20 ha
1998	Dnr 22-2519/98	Sweden	Sölvesborg	2 May-13 Oct.	Starch production	2 ha
1998	Dnr 22-2519/98	Sweden	Sölvesborg	20 May-14 Oct.	Starch production	1.75 ha
1998	Dnr 22-2519/98	Sweden	Mörrum	10 May-10 Oct.	Starch production	4.5 ha
1998	Dnr 22-2519/98	Sweden	Åhus	15 May-14 Oct.	Starch production	12 ha
1998	Dnr 22-2519/98	Sweden	Sölvesborg	20 April-28 Sept.	Starch production	6 ha
1998	Dnr 22-2519/98	Sweden	Fågelmara	10 May-12 Oct.	Starch production	2 ha
1998	Dnr 22-2519/98	Sweden	Ramdala	17 May-14 Oct.	Starch production	2 ha
1998	Dnr 22-2519/98	Sweden	Lyckeby	5 May-5 Oct.	Starch production	1.1 ha
1998	Dnr 22-2519/98	Sweden	Fågelmara	10 May-8 Oct.	Starch production	5 ha
1998	Dnr 22-2519/98	Sweden	Ljungbyholm	10 May-3 Oct.	Starch production	4 ha
1998	Dnr 22-2519/98	Sweden	Färjestaden	10 May-5 Oct.	Starch production	3 ha
1998	Dnr 22-2519/98	Sweden	Ramdala	10 May-10 Oct.	Starch production	8 ha
1998	Dnr 22-2519/98	Sweden	Mörbylånga	20 May-13 Oct.	Starch production	1.5 ha
1998	Dnr 22-2519/98	Sweden	Mörbylånga	10 May-18 Oct.	Starch production	5 ha
1998	Dnr 22-2519/98	Sweden	Jämjö	20 May-15 Oct.	Starch production	9 ha
1998	Dnr 22-2519/98	Sweden	Kallinge	11 May-5 Oct.	Starch production	4 ha
1998	Dnr 22-2519/98	Sweden	Jämjö	10 May-8 Oct.	Starch production	6 ha
1998	Dnr 22-2519/98	Sweden	Färjestaden	20 May-12 Oct.	Starch production	4.5 ha
1998	Dnr 22-2519/98	Sweden	Mörbylånga	10 May-4 Oct.	Starch production	2.9 ha

1998	Dnr 22-2519/98	Sweden	Ljungbyholm	14 May-13 Oct.	Starch production	7 ha
1998	Dnr 22-2519/98	Sweden	Ronneby	20 May-5 Oct.	Starch production	2.5 ha
1998	Dnr 22-2519/98	Sweden	Kallinge	20 May-9 Oct.	Starch production	5 ha
1998	Dnr 22-2519/98	Sweden	Kallinge	23 May-3 Oct.	Starch production	2 ha
1998	Dnr 22-2519/98	Sweden	Ramdala	20 May-8 Oct.	Starch production	4.3 ha
1998	Dnr 22-2519/98	Sweden	Mörbylånga	2 May-2 Oct.	Starch production	8 ha
1998	Dnr 22-2519/98	Sweden	Bergkvara	10 May-5 Oct.	Starch production	3 ha
1998	Dnr 22-2519/98	Sweden	Ramdala	23 May-1 Oct.	Starch production	1.5 ha
1998	Dnr 22-2519/98	Sweden	Fågelmara	20 May-7 Oct.	Starch production	3.8 ha
1998	Dnr 22-2519/98	Sweden	Jämjö	10 May-6 Oct.	Starch production	4.5 ha
1998	Dnr 22-2519/98	Sweden	Vassmolösa	16 May-3 Oct	Starch production	2.5 ha
1998	Dnr 22-2519/98	Sweden	Ljungbyholm	10 May-8 Oct.	Starch production	5 ha
1998	Dnr 22-2519/98	Sweden	Lyckeby	13 May-10 Oct.	Starch production	4.5 ha
1998	Dnr 22-2519/98	Sweden	Mörbylånga	3 May-8 Oct.	Starch production	4 ha
1998	Dnr 22-2519/98	Sweden	Mörbylånga	10 May-10 Oct.	Starch production	6 ha
1998	Dnr 22-2519/98	Sweden	Mörbylånga	10 May-7 Oct.	Starch production	5.5 ha
1998	Dnr 22-2519/98	Sweden	Ronneby	10 May-13 Oct.	Starch production	5 ha
1998	Dnr 22-2519/98	Sweden	Ronneby	10 May-8 Oct.	Starch production	4 ha
1998	Dnr 22-2519/98	Sweden	Vassmolösa	7 May-5 Oct.	Starch production	3.5 ha
1998	Dnr 22-2519/98	Sweden	Trekanten	18 May-8 Oct.	Starch production	6 ha
1998	Dnr 22-2519/98	Sweden	Lyckeby	20 May-2 Oct.	Starch production	2 ha
1998	Dnr 22-2519/98	Sweden	Mörbylånga	8 May-7 Oct.	Starch production	7 ha
1998	Dnr 22-2519/98	Sweden	Halltorp	5 May-4 Oct.	Starch production	5 ha

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1998Dnr 22-2519/98SwedenSölvesborg27 April-5 Oct.Starch production1998Dnr 22-2519/98SwedenVollsjö1 May-29 Sept.Seed production & Observation1998Dnr 22-2519/98SwedenSkara17 May-1 Oct.Seed production & Observation1998Dnr 22-2519/98SwedenSkara14 May-28Seed production &	1 ha
1998Dnr 22-2519/98SwedenVollsjö1 May-29 Sept. Seed production & Observation1998Dnr 22-2519/98SwedenSkara17 May-1 Oct.Seed production & Observation1998Dnr 22-2519/98SwedenSkara14 May-28Seed production & Observation	5 ha
1998 Dnr 22-2519/98SwedenSkara17 May-1 Oct.Seed production & Observation1998 Dnr 22-2519/98SwedenSkara14 May-28Seed production &	7 ha
1998 Dnr 22-2519/98 Sweden Skara 14 Mav-28 Seed production &	4 ha
Sept. Observation	5 ha
1998 Dnr 22-2519/98 Sweden Händene 22 May-25 Seed production & Sept. Observation	8 ha
1998 Dnr 22-2519/98 Sweden N. Härene 16 May-28 Seed production & Sept. Observation	11.5 ha
1998 Dnr 22-2519/98 Sweden Lidköping 18 May-23 Seed production & Sept. Observation	
1998 Dnr 22-2519/98SwedenSkallmeja15 May-20Seed production &Sept.Observation	7.5 ha
1998 Dnr 22-2519/98SwedenEntorp15 May-20Seed production & Observation	7.5 ha 7.2 ha

1998	Dnr 22-2519/98	Sweden	Boxholm	27 May-5 Oct.	Seed production & Observation	8.9 ha
1998	Dnr 22-2519/98	Sweden	Skara	19 May-9 Oct.	Seed production & Observation	6 ha
1998	Dnr 22-2519/98	Sweden	Granö	6 June-24 Sept.	Seed production & Observation	1.5 ha
1998	Dnr 22-2519/98	Sweden	Granö	6 June-27 Sept.	Seed production & Observation	1.7 ha
1998	Dnr 22-2519/98	Sweden	Flurkmark	18 June-22 Sept.	Seed production & Observation	1.5 ha
1999	Dnr 22-1087/99	Sweden	3 sites	5 May-18 Oct.	Yield trial & Observation	600 m2
1999	Dnr 22-1087/99	Sweden	75 sites	20 April-Oct.	Starch production	319 ha
1999	Dnr 22-1087/99	Sweden	12 sites	2 May-early Oct.	Seed production & Observation	57.3 ha
2000	Dnr 22-1019/00	Sweden	24 sites	17 April-Oct.	Starch production	136.2 ha
2000	Dnr 22-1019/00	Sweden	7 sites	9 May-end Sept.	Seed production & Observation	25.6 ha
2001	Dnr 22-1019/00	Sweden	7 sites	11 May-22 Oct.	Seed production & Observation	4.9 ha