NOTIFICATION FOR PLACING THE POTATO CLONE EH92-527-1, BEING GENETICALLY MODIFIED FOR INCREASED CONTENT OF AMYLOPECTIN, ON THE MARKET

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Foreword

Potato starch is a very common product of potatoes. Normally potato varieties with a very high starch content are used for starch production, but also table potatoes may be used for this purpose. The annual production of potato starch in the EU varies between 1,5 - 2 million tons depending on yearly variation in production. The amount of starch is equivalent to 7,5 - 10 million tons of potatoes. The total starch production in the EU amounts to around 7,5 million tons. Maize is the most common source of starch, followed by wheat and potato.

The different starch sources produce starches with different properties. Besides differences in starch granule sizes there are also differences in occurrence of associated proteins and other characters. In all sources the normal starch consists of two molecules, amylose and amylopectin, approximately in the proportions 1:3. Amylose is a linear un-branched molecule with a size of 200 - 10 000 glucose units. Amylopectin is a branched molecule with a size of over 1 million glucose units.

Maize types producing pure amylopectin starch, i.e. waxy maize, have been available since the 1940's. The same type of starch can also be produced from barley, sorghum and rice. Due to the fact that maize starch consists of only one type of starch molecule, its properties are changed, and for a number of uses it's superior to normal starches. Therefore amylopectin starches are utilized in a number of applications in food products as well as in the technical sector such as paper manufacturing.

So far the different types of maize and other crops which produce starch of amylopectin type are mutants. In some cases mutations have occurred spontaneously and in other cases they have been induced by plant breeders. As the potato is a tetraploid, recovery of mutants is extremely difficult. On the other hand methods for chemical separation of potato starch have been used up to the 1980's. The amylopectin starch produced this way from potato starch has mainly been used for food products. In many respects potato starch is superior to other starches. One can expect that variants of potato starch, such as amylopectin starch, will be unique.

The Swedish Starch Producers is a small company among starch producers. On a yearly basis the company sells about 80 000 tons of potato starch products. The company is world leading in special potato starch products. The company strives as much as possible to work with speciality products in order to maintain its leading position on the market. In order to accomplish this goal, it's necessary that the starches produced and marketed are unique and/or that the modifications executed are unique.

In 1987 the Swedish Starch Producers began a cooperative project with Svalöf Weibull AB within Amylogene HB, a jointly owned R&D subsidiary. The purpose of the cooperation was to create unique starch qualities in potatoes using gene technology. This cooperation has resulted in the present notification. The potato variety developed produces amylopectin starch with a very high purity. Parallel to the breeding work adapted methods for starch extraction and possible uses for the starch have been investigated. Within the technical field products have been developed to be used in the paper and chemical industries. In the field of food products only limited development work has been carried out. Therefore such products will not be ready for marketing in the near future.

The present potato clone is intended as a raw product for the starch industry. The clone will be used for the production of a special quality starch and will therefore be grown separated from other starch potatoes. The potatoes will be grown for production of the raw product for starch extraction as well as for seed production.

Seed potatoes will be grown for the production of basic as well as certified seed. The seed production and utilization will be subject to the same requirements and rules as all other seed potatoes. Seed production will primarily be carried out in Sweden, but may also be considered in other parts of the EES where starch potatoes are grown.

Starch potatoes will be grown exclusively for production of potato starch. The requirements for cultivation will be the same as for all other starch potatoes except that cultivation and handling will be strictly separated from other starch potatoes. Starch will be extracted as well as potato pulp, fruit juice and fruit water. The fruit juice will be spread on the fields. The fruit water will also be spread on the fields or processed as sewage. During 1998 the potato pulp will be spread on the fields. In the future, when such a permit has been granted, the intention is to use the pulp as livestock feed. The cultivation area will be the same as for starch potatoes in the EES.

The starch produced from the potatoes will be used for the production of starch products for the technical industries, primarily the paper and chemical industries. In the future utilization in food industry may also be considered. Such use, however, has to be preceded by tests and approvals according to the requirements for food products.

Examples of possible fields of applications for starch products based upon EH92-527-1 are:

Stabilisation of dispersions

In the chemical industry large amounts of dispersions (solutions) are produced in which substances are dispersed in water. In order to make the dispersion durably stable a number of chemicals, including starch, are used as stabilizors. The dry content in such dispersions is normally 10-15%. Using starch products based upon potato amylopectin starch it seems possible to increase the dry content up to 15-25% without loosing stability. The consequence will be that considerably less water will be moved during transport from the chemical industry to the user of the dispersion.

The paper industry

In the paper industry starch products are used to give strength in paper. By using starch products based upon potato amylopectin starch it will be possible to use larger amounts of recycled fibres in the process, maintaining the strength of the paper. Additionally it will be possible to improve the de-watering in the paper machine and consequently to save energy in the drying of the paper. The use of amylopectin starch in certain systems seems to improve the internal strength of the paper, making it possible to use inferior raw materials or more filler (clay or chalk) without loosing strength.

Using oxidized potato amylopectin starch in coating trials we have seen possibilities to work with higher dry substance contents in the coating colour, without loosing viscosity properties as compared to conventional oxidized potato starch. The consequence will be energy saving as well as quality advantages.

In clone EH92-527-1 a gene coding for the production of one enzyme has been blocked by a reversing part of the gene. The specific enzyme controls the production of amylose. Also a marker gene has been added, making it possible to detect genetically modified plants. The gene technology work has not been aiming at any other modifications of the clone as compared to the mother variety. This means that the cultivation including fertilisation and application of herbicides and fungicides will not differ from what is common practice for the mother clone.

During 1998 starch extraction will be carried out at the starch factory in Jämjö, county of Blekinge in Sweden. Cultivation will be localized in the normal starch potato area, and will be carried out according to common practice. The handling of potatoes, secondary products and starch will follow the same procedures as for normal starch production, except that potatoes and starch will be kept strictly separated from other potatoes and starch.

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Part 1.

A. GENERAL INFORMATION

1. Name and address of the notifier

Amylogene HB, c/o Svalöf Weibull AB, S-268 81 Svalöv, Sweden

2. Names of responsible scientists, their qualifications and experience

Lennart Erjefält, fil lic, geneticist. Head of Potato Department, Svalöf Weibull AB. Plant Breeder at the Potato Department, Swedish Seed Association 1973-1979, Svalöf AB 1980-1992, and Svalöf Weibull AB 1993-, since 1980 as responsible head of department.

Jüri Känno, agronomist. Head of Agricultural Department, Lyckeby Stärkelsen. Involved in seed production and variety assessment of the company since 1973. Head of department since 1993.

Both of the above have been engaged in the activities of Amylogene HB regarding genetically modified potatoes during the last 9 and 5 years, respectively. The first attempts at gene constructions were made in 1988, transformations have been made since 1989, greenhouse cultivation has been carried out since 1990, field trials since 1991, and seed production in the field since 1994. Amylogene HB is the one company in Sweden with the greatest experience regarding activities involving genetically modified plants.

3. Name of the project

Modified starch quality in potatoes through the increase of amylopectin content.

B. INFORMATION RELATING TO THE RECIPIENT ORGANISM

1. Name and taxonomy

a)	**family**	*Solanaceae*
b)	**genus**	*Solanum*
c)	**species**	*tuberosum*
d)	**subspecies**	*tuberosum*
e)	**variety/cultivar**	Prevalent
f)	**common name**	potato

2.a) Information concerning reproduction

i) Reproduction can be vegetatively with tubers as well as sexually with botanical seed.

ii) Due to frost sensitivity the survival of tubers depends upon winter temperature.

iii) Generation time 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*. In northern Europe they occur exclusively in arable land, while in southern Europe they may also be found as escapes outside arable land. *Solanum tuberosum* is not compatible with wild related species in Europe, *S. nigrum* and *S. dulcamara* (Eijlander and Stiekma, 1994; Conner, 1994).

3. 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.

Botanical seed overwinter 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 and can not be considered as a harmful ecological factor. This is also true for potato plants originating from tubers which might have been spread from cultivated areas.

b. Specific factors affecting survival, if any

Sensitivity of tubers to frost
Agricultural practices (cultivation practices including use of herbicides)
Competition with other crops in crop rotation

Competition with the natural flora

4. Dissemination

a. Means and extent of dissemination

Dissemination of tubers and botanical seed is normally limited to the area of cultivation. Tubers can also be spread during transportation and handling at other locations. According to available publications and our own studies pollen dissemination is limited to around 3 m, with a maximum distance of 10 m (Tynan et al., 1990; Dale et al., 1992; Conner, 1993; McPartlan & Dale, 1994; Conner & Dale, 1996). Deviating results presented by Skogsmyr (1994) are rejected by Conner & Dale (1996) for sound scientific reasons. Skogsmyr´s data were totally based on PCR analyses with nptII primers. They were not verified with (e.g.) gene expression studies, and no controls for detection of false positive reactions were reported.

b. Specific factors affecting dissemination, if any

Dissemination of tubers, botanical seed and potato foliage is mainly caused by man while carrying out transports, handling and cultural practices. A limited amount of dissemination may also be caused by animals, especially large birds. Such dissemination of botanical seed, however, is practically excluded, as the seeds are contained in very poisonous fruits. Dissemination of pollen is executed almost extensively by insects (Osvald, 1965). Also Skogsmyr (1994) practically excluded pollen dissemination executed by other agents than insects. Wind dissemination is considered marginal.

5. The geographical distribution of the plant

Potatoes are found in agricultural areas throughout Europe. Only in southern Europe there is a very limited occurrence as a weed outside cultivated fields. Wild related species, *Solanum nigrum* and *S. dulcamara*, are found throughout Europe, but efficiant incompatibility barriers prevent hybridization between those and *S. tuberosum* (Eijlander & Stiekma, 1994; Conner, 1994).

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

Potatoes are normally grown in the Member State(s).

7. Potentially significant interactions of the plant with other organisms than plants in the ecosystem where it is normally 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 breeders 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 (Olsson & Jonasson, 1990, 1992 and 1995). At a very high content of glycoalkloids it also seems that the Colorado beetle (Sinden et al., 1980, 1986 and 1988) and leaf hoppers (Tingey et al., 1978) are repelled, while aphids (Tingey, 1984) and cyst nematodes (*Globodera* spp) (Forrest & Coxon, 1980; Grassert & Lellbach, 1987) 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 (Deahl et al., 1973; Frank et al., 1975; Gopth et al., 1969; Morrow & Caruso, 1983; Olsson, 1987; Umaerus & Umaerus, 1986), with a possible exception of *Fusarium* rot (Thalman et al., 1984).

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 (Frank et al., 1975; McKee, 1959; Paquin & Lachance, 1964; Umaerus & Umaerus, 1986).

There are many viruses which 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. Umaerus & Umaerus, 1986, indicate that such influence is not present.

All references cited above, except Olsson & Jonasson, 1995, are summarized in ”Glykoalkaloider i potatis” (Glycoalkaloids in potatoes). This is a literature review made by Dr Kerstin Olsson, Svalöf Weibull AB, at the request of and supported by ”Stiftelsen Lantbruksforskning” (The Foundation for Agricultural Research, LRF) 1993.

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C. INFORMATION RELATING TO THE GENETIC MODIFICATION

1. Description of the methods used for the genetic modification.

Transformation of potato with recombinant DNA was made using *Agrobacterium tumefaciens*. In this case a binary vector system was used where the T-DNA, containing the genes that are to be transferred, is found on one plasmid while the DNA mobilising functions are found on a modified Ti-plasmid (Hoekema et al, 1983). Leaf discs from potato were transformed after which *A. tumefaciens* was eliminated using Claforan (500mg/l). Shoots have then been generated under selection on kanamycin containing medium (50mg/l). *A. tumefaciens* can be considered eliminated since the culturing of shoots on non-selective medium does not lead to bacterial growth.

2. Nature and source of the vector used.

Agrobacterium tumefaciens strain LBA4404 containing Ti-plasmid pAL4404 was used for transformation of potato (Ooms et al, 1981). In pAL4404 the T-DNA and the oncogenic traits are deleted. The binary vector which functioned as a carrier of the traits that have been transferred to plant tissue is derived from pBIN19 (Bevan 1984). pBIN19 can be propagated both in *E. coli* as well as in *A. tumefaciens* and contains a T-DNA that is limited by the right and left border sequences from pTiT37 (Zambryski et al., 1980). Outside the T-DNA border sequences there is a gene for kanamycin resistance which makes selection in bacteria possible. This gene, however, is not transfered to the plant. The T-DNA on pBIN19 is described more in detail below.

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

The T-DNA of pBIN19 has according to sequence data (Genbank acc. U09365) a size of 3 213 base pairs and is limited by border sequences from pTiT37. Within these borders there is a kanamycin resistance gene, that can be expressed in plant tissue, and also a multiple cloning site from M13mp19 (See annex 3). The kanamycin resistance gene is of *nptII*-type and originates from Tn5 (Beck et al., 1982) 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 polyadenylation sequence from the nopaline-synthase gene, both originating from *A. tumefaciens*. In potato the nopaline-

synthase promoter drives the expression of a succeeding gene (here *nptII*) in leaf tissue (De Block, 1988) and to some degree in tuber tissue (Yang et al., 1989). 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 is used in order to be able to clone genes of interest for introduction into plant chromosomes. In order to modify the starch composition a gene has been inserted into the multiple cloning site, resulting in the plasmid pHoxwG. This gene consists of the *gbss*-promoter (987 bp), which is isolated from potato, and a polyadenylation sequence from the nopaline-synthase gene (252 bp). The *gbss*-promoter gives strong expression in tubers, pollen and root tips. This has been determined by the expression of a marker gene (*uidA*) in transgenic potato. Between these two regulatory DNA-segments, a 1.945 base pair segment of the potato *gbss*-gene has been inserted in reversed orientation in relation to the promoter. In the tuber this gene construct is to inhibit the expression of the endogenous *gbss*-gene and thereby reduce the amount of amylose in the tuber.
GBSS = "granule bound starch synthase" EC 2.4.1.11

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D. INFORMATION RELATING TO THE GENETICALLY MODIFIED PLANT

1. *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 (see D.3.b). This has been determined by analyzing the starch composition of the tubers (see Annex 6).

2. *Information on the sequences inserted / deleted*

a) The size and structure of the insert and methods used for its characterization, including information on any parts of the vector introduced in the genetically modified plant or any carrier of foreign DNA remaining in the genetically modified plant.

The size of the DNA-segment present in pHoxwG is calculated to be 6 634 bp (see C.3). The structure of the inserted T-DNA-segment is described in C.3. where non-coding cloning remainders are also included. The presence of transformed DNA-segments has been confirmed by ”Southern-blotting” (see Annexes 3 and 4). Analyses with ”Southern-blotting” also indicate that no unintended regions of the vector have been transferred (see Annex 21). PCR-analysis has shown that the DNA-segment inserted is not larger than it was intended, and also that the size and structure of the integrated parts correspond to the description of the T-DNA present in pHoxwG (see Annex 18). However, it should be noted that the left border and adjacent sequences of the T-DNA seems not to have been integrated. The structure of the part of the T-DNA that contains the gbss antisense gene could not be determined due to unspecific DNA fragments produced by the actual transformed clone as well as by non-transformed Prevalent.

b) In case of deletion of DNA-segment: size and function of deleted segments

Elimination of DNA-segment(s) is not the purpose of the project.

c) Location of the insert in the plant cells (integrated in chromosomes, chloroplasts, mitochondria, 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” (see Annex 4). The recombinant gbss-gene has inhibited the expression of the chromosomal endogene gbss-gene (see Annex 11), 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 pure chloroplast DNA with ”Southern blotting” has not revealed any integration of DNA from the transformation vector pHoxwG (see Annex 19). An attempt to examine the mitochondrial DNA has been carried out. The purified

mtDNA, however, was contaminated with chromosomal DNA in all studies, making hybridization for examination of non-intended integration of DNA from the transformation vector pHoxwG in mitochondria impossible. The studies described in various annexes and in the literature indicate that no such unintended DNA-integration into the mitochondria has taken place;

1. The choice of method for transformation. *Agrobacterium tumefaciens* produceschromosomal transformation (Zambryski, 1992; Hohn et al., 1991). For the transformation of organelles, it is necessary to use other techniques like a particle canon to introduce DNA (Klein et al., 1992). Of course it should be mentioned that there is a report on transformation of chloroplasts, in which *A. tumefaciens* was involved (Venkateswarlu et al., 1991). In that study, however, the transformation vector contained chloroplast DNA intended to give homologous recombination. The results shown in the article could be questioned because the hybridization with 15-20 μg chloroplast DNA showed only a very weak band.
2. The integrated copy of the selected DNA has been ascertained by hybridizing with the total DNA from the transformed clone (see Annex 4).
3. The integrated copy has given the intended effect, i.e. it inhibits the expression of a chromosomal gene (see Annex 11).
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 (see Annex 21).

d) Copy number of the insert

Results from Southern blotting indicate one inserted copy, for the region examined.

3. Information on the expression of the insert

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

Kanamycin resistance has been determined by the survival of the organism on medium containing kanamycin. A reduction of the amount of GBSS-protein has been determined by polyacrylamide electrophoresis (see Annex 11). A reduction of the amylose content has been determined by iodine staining of starch granules (see Annex 12) and with spectrophotometry according to Hovenkamp-Hermelink et al. (1988).

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. The technique is described in Annex 17.

Characterization was made by the following methods (Annex 6):

- The chain length profile of branched starch molecules by gas chromatography.
- The blue value was determined by staining with iodine and measuring the amount of absorbance.
- Degree of branching by 500mhz ^{1}H-NMR

- Stability of water solution by measuring of the storage module and the absorbtion during storage.
- Swelling behavior.

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

b) Parts of the plant where the insert is expressed (e.g. roots, stems or pollen)

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. However, the expression pattern in the potato plant of the promoter (nos-promoter) used for expression of the nptII-gene has not been sufficiently investigated in this respect to give an adequate answer. Thus, there are not any parts of the plant where the expression of the nptII-gene can be completely excluded (see Annex 12).

4. Information on how the genetically modified plant differs from the recipient plant in:

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

Comparative observations regarding flowering frequencies, fruit setting and tuber formation in the clone EH92-527-1 and the recipient variety Prevalent have not revealed any differences. It is therefore assumed that there are no differences between the two clones with respect to mode or rate of reproduction. Besides this, observations have been compiled from practical cultivation trials in 1993, 1994, 1995 and 1996 (see Annexes 10, 13, 14, 15 and 20).

b) dissemination

Field trials have shown that there are no differences between the recipient clone and EH92-527-1 in pollen production, fruit setting and tuber formation (see Annexes 2, 10, 13, 14, 15 and 20).

c) survivability

In the field trial carried out in 1995 frost tolerance was studied after natural frosts before foliage maturity. No difference in frost tolerance between the recipient clone and EH92-527-1 could be recorded. Tubers submitted to artificial frosts in the laboratory did not show any behavior deviating from those of the recipient clone. They were affected identically (see Annex 10).

5. Genetic stability of the insert

The transgenic potato clone has been vegetatively multiplied by way of cuttings and tubers. With such multiplication during several generations the transformed characters have been found to be stable. This has been studied by analysing the composition of tuber starch from EH92-527-1 during three consecutive years. The amylose content has been assessed by spectrophotometry (Hovenkamp-Hermelink et al., 1988). Characteristics of the analyzed starch have shown a stabile profile,

amylose content being less than 8% in all experiments. The spectrophotometric method that has been used may over-estimate the content of amylose. The method using gel permeation chromatography (GPC) described in Annex 6 is considered to give a better estimate of the true amylose content. According to this method the amount of amylose is less than 2% of the total starch content. Consequently, the spectrophotometric data should only be considered as indicating the relative differences in amylose content between normal Prevalent and EH92.527-1.

Table 1. The amylose content (% of total starch) in EH92-527-1 determined according to Hovenkamp-Hermelink et al. (1988)

Potato clone	Greenhouse 1992	Field trial 1993	Field trial 1994	Field trial 1995
EH92-527-1	7.5	7.6	5.2	*
Prevalent	26.0	19.0	22.2	22.1

* In 1995 no analysis was made with this method. The starch analysed was with the GPC-method.

Stability of the transformation is shown in Table 1 for three consecutive years. Corresponding data obtained with gel permeation chromatography are included in annex 6. Here no presence of amylose could be shown during the years 1993 - 1995.

The stability of the integrated DNA over consecutive tuber generations has not been determined. However, when transforming with the antisense technique, aiming at down-regulation of an endogenous gene, there are comparatively few transformation events which result in a complete inhibition of the particular gene (Kuipers et al., 1995). This indicates that the specific transformation event, its structure and localisation 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.

6. Potential for transfer of genetic material from the genetically modified plant to other organisms

Genetic material can be transferred to other potato varieties by pollen, which could result in hybrids containing the genetic modification. Considering the poor competitive ability of potato seedlings there is a very small risk that hybrid plants should become established in the succeeding crops.

There is no risk for transfer to other plants than the common potato, as compatibility barriers prevent hybridization with wild related species (Conner, 1993; Dale et al., 1992; McPartlan & Dale, 1994; Tynan et al., 1990).

7. Information on any toxic or harmfull effects on human health or the environment arising from the genetic modification

In 1996 the transgenic clone, EH92-527-1, and the recipient cultivar Prevalent, have been grown in three parallel trials in Svalöv, Kristianstad and Fjälkinge. Each trial had four replicate plots of EH92-527-1 and four replicate plots of

Prevalent. Samples were taken from each plot and analyzed for glycoalkaloid, chlorogenic acid and nitrate content. A total of 12 samples from each of EH92-527-1 and Prevalent were analyzed.

Assessed amounts are given as parts per dry matter. A simple analysis of variance using location and clones as variates has been carried out. In general both the replicates and clone x location-interaction were non-significant. The variance was therefore carried over to the remainder variance in order to maximize degrees of freedom.

Regarding traits, where significant differences between clones were indicated, a covariance analysis was also carried out, using yield as a covariant. Yield was used as covariant because most characters studied are influenced by the yield levels in the individual trials. A statistical analysis of yield differences between the clones is reported in Part 3 of this notification. The statistical program STATGRAPHICS, Manugistics Inc. was used for planning of the experiment and for the analysis of variance. The results of the analysis are shown in the tables below as averages of all replicates per clone and as locality and clone differences. Where applicable the level of significance after the covariance analysis is indicated within brackets ( ). A complete description of the design of the experiment is reported in Part 3.

Table 2. Results of samples and statistical analysis of chlorogenic acid and nitrate. (Complete statistical data are found in Annex 8.)

Trial	Chlorogenic acid (μmol/100g)		Nitrate (mg/kg)	
	Prevalent	EH92-527-1	Prevalent	EH92-527-1
1	49	65	92	70
1	45	62	43	129
1	91	70	80	66
1	90	63	83	172
2	105	88	195	429
2	105	84	169	336
2	115	71	226	293
2	98	82	154	348
3	60	49	234	558
3	74	51	232	394
3	70	57	327	429
3	68	50	253	399
Average	81	66	174	302
Location	*** (***)		*** (***)	
Cultivar	** (ns)		*** (ns)	

* = P<0.05 ** = P<0.01 *** = P<0.001 ns = P>0.05
( ) = level of significance for covariance and yield

Chlorogenic acid is a phenolic acid present in all potatoes, and at high concentrations contributing to, after cooking, blackening which is a serious quality problem. It is shown in table 2 that there is a significant difference (99.9% confidence) between locations. If yield is not taken into account there is a significant difference between clones (99% confidence). This difference is to the advantage of the transformed clone.

When yield is taken into account (the covariance analysis) there are no differences between clones. From the statistical analysis it is concluded that the amount of chlorogenic acid is not effected by the genetic modification.

High nitrate content is considered, especially in baby foods, as an anti-nutritional factor. In table 2 a significant difference (99.9% confidence) is shown between trial locations and between cultivars. The analysis indicates a higher content of nitrate in the transformed clone than in the untransformed one. Taken into account the covariance with yield there are no differences between clones, however. Since the amount of accessible nitrogene in the soil is strongly correlated to the amount taken up by the plants this result is to be expected. Consequently there is a statistical basis for assuming that the genetic modification does not influence the content of nitrate.

Table 3. Results of samples and statistical analysis of the glycoalkaloids solanine and chakonine. (Complete statistical data are found in Annex 8.)

Trial	Solanaine (mg/kg)		Chakonine (mg/kg)		Total glycoalkaloids (mg/kg)	
	Prevalent	EH92-527-1	Prevalent	EH92-527-1	Prevalent	EH92-527-1
1	157	78	310	270	467	348
1	161	83	306	232	467	315
1	144	145	318	289	462	434
1	147	168	286	319	433	487
2	75	77	176	239	251	317
2	84	66	211	199	295	266
2	113	129	249	273	362	402
2	68	77	177	194	244	271
3	61	56	161	187	222	243
3	46	64	143	203	189	267
3	54	97	152	247	206	344
3	61	85	169	230	230	315
Average	98	94	221	240	319	334
Location	***		***		***	
Cultivar	ns		ns		ns	

All potatoes contain various amounts of glycoalkaloids, which can be toxic to people when large amounts are consumed (see references in Part 1, B.7). The amounts reported in table 3 are given as mg per kg dry matter. Since the maximum contents recommended are given as mg per kg fresh weight (200 mg/kg), the amounts should be multiplied by 0.25 to be comparable. Moreover starch potatoes are excluded from the recommendation because they are not intended for consumption. The amount of glycoalkaloids can vary due to different reasons, e.g. cultivar differences, yield, stage of tissue development and different types of stress. The genetic modification is not supposed to influence the content of these substances, and that is verified in the analyses executed. In the table it is shown that there are differences between locations when data are analysed without taking yield differences into consideration. There are no significant differences between clones, however. It is therefore concluded that the genetic modification does influence the production of glycoalkaloids.

In summary it can be stated that that there are no increased contents of any of the toxic and anti-nutritional substances examined in the clone EH92-527-1 as compared to the recipient clone.

The intentional change in the composition of the starch which has been induced through the genetic modification is unique. So far potato clones having this starch composition were not known. Pure amylopectin starch for food purposes has previously been produced commercially by using chemical separation of common potato starch (Annex 5). There are other plants which have a genetically inherited natural high content of amylopectin, the most common ones being waxy types of maize which have been used commercially since the 1940's. In addition to those maize types there are mutants of rice, sorghum and barley, which are also referred to as "waxy". Waxy maize is also called amioka and contains about 99% amylopectin. The main use is in the food industry as thickeners and stabilizing agents. It has a much better storage stability than common cereal starches and

somewhat better than root starches. The production is about 200 000 tons per year. Waxy starches maintain a considerable part of the market for modified thickeners for all types of foods (see Annex 7).

With the present use of amylopectin starches from other crops and the earlier extensive use of amylopectin starches from potatoes in food products, there is no reason that the modified starch composition of potatoes (at least 98% amylopectin) should carry any risks for other organisms, including humans.

A potential risk for the environment could happen if the character transferred to potatoes should change the competitive value of the modified potato with other crops. Since the genetic modification does not affect the morphology, vitality, dissemination or survivability of the clone, this risk can be excluded.

8. Mechanisms of interaction between the genetically modified plant and target organisms (if applicable)

Not applicable.

9. Potentially significant interaction with other, not modified organisms

In potatoes normally existing toxic and anti-nutritional substances are glycoalkaloids and nitrates. The interaction with other organisms has been described in Part 1, B.7. In D.7, in Annex 8 and in Part 3 it is shown that the contents 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 another way than what the unmodified recipient cultivar, Prevalent, does.

The modified clone, EH92-527-1, is characterized by two changes compared to the recipient clone. Those are resistance to kanamycin and the lack of amylose. Because of the later, the content of amylopectin has been increased to >98%.

It could give reason to suspect that kanamycin resistance itself, the causal gene, nptII, or the causal protein (APH(3')II (aminoglycoside -3'-phosphotranferase II) causes a risk. A great number of studies and review analyses rebuke this.

The gene nptII which was isolated from the transposon Tn5 from *Eschirichia coli*, is coding for the enzyme (the protein) APH(3')II. APH(3')II catalyzes ATP-dependent phosphorylation in the 3' position of the aminohexose ring of certain aminoglycosides having an antibiotic effect (Sande & Mandell, 1985; Nap et al., 1992; Kärenlampi, 1996). The physical consequence is that the penetration of the membrane of the resistant cell is retarded (Sande & Mandell, 1985). APH(3')II contains 264 amino acids and is very substrate specific. Only the aminoglycosides kanamycin, neomycin and geneticin are inactivated, while later developed therapeutically more potent amikacin and netilmicin are not affected (Nap et al., 1992; Kärenlampi, 1996).

Resistance against the three antibiotics is commonly found in natural microbe populations. Such spontaneously occurring resistance can be caused by several

mechanisms, of which one is described above (Nap et al., 1992; Bergmans, 1993; Kärenlampi, 1996).

If the nptII gene would be disseminated by sexual reproduction to related potato varieties or species, or if a potato clone carrying the nptII gene would be disseminated in the ecosystem, selective advantages would appear if the antibiotics are applied as herbicides. Such use of antibiotics is, however, regarded as non-realistic (Nap et al., 1992). Another possibility for the occurrence of a selective advantage is if manure from animals which have been treated with kanamycin or neomycin is spread on the field where the potato clones are grown. Nap et al. (1992) have calculated the theoretically possible amount of antibiotics supplied to cultivated land in the Netherlands, where manure is extensively used as fertiliser and the antibiotics concerned are used in veterinary medical therapy. The maximum amount of spreading in the field would be 20 grams of antibiotics per hectare or 4×10^{-5} grams per liter soil with tillage to 5 cm. Soil colloids, mainly clay particles, bind irreversibly as much as 26 grams of kanamycin and 42 grams of neomycin per kilogram. Based on these values, it would take more than 50000 years to saturate the soil with the antibiotics to a level making it possible for the antibiotics to interfere with plant, provided that the antibiotics are used at the same rate and that the use of manure remains unchanged. In a separate study Bergmans (1993) calculates a maximum supply of 0.13 μg per ml soil. It is completely unreasonable that the presence of active antibiotics in the soil could produce a selective advantage for plants which contain the nptII gene. The above studies are in reference to conditions in Holland. In the later calculation it is assumed that none of the antibiotics break down. Despite that the conclusions are quite different on the amount of time it takes to saturate the soil with antibiotics, it is obvious that there will be no selective advantage for plants carrying resistance to the antibiotics for a very long time, if ever.

Horizontal dissemination of the nptII gene to microorganisms in the soil or in people's and animal's digestive track could also be a potential risk. Harding (1996) states that such transfer from eukaryotes to prokaryotes is theoretically possible, but the occurrence has never been shown. If it did happen the amount of risk must be calculated in comparison to the possible selective advantage for the recipient. According to Nap et al. (1992) the transfer from plants or plant cells to micro-organisms could lead to a yearly amount of maximum $8{,}7 \times 10^{-7}$ transgenic bacteria per gram soil. The natural occurrence of kanamycin resistant bacteria is estimated to 1.600 - 30.000 per gram soil. The addition by way of transformation should therefore be maximum 5.4×10^{-10}%. Bergmans (1993) stated the number of naturally occurring kanamycin resistant organisms from 1.000 to 100.000 per gram of soil.

Nap et al. (1992) calculated the addition of antibiotic resistant microorganisms by horizontal transformation in the digestive track of humans and animals in the same way. The maximum addition to naturally occurring resistant organisms would approach 2.4×10^{-15}. This addition is ascertained to be less than the amount produced by spontaneous mutations. WHO (1993) considered the possibility of such horizontal transferring as very unlikely, and states even that the gene possibly transferred would be inactive due to lack of an essential bacterial promoter. Kärenlampi (1996) states that such transfer is theoretically possible,

and that kanamycin resistance could be transferred to bacteria in the digestion track. It is also stated that there are no known mechanisms for transferring and that there has not been any reports on transfers. If it should happen the consequences should be very limited because the enzyme is normally produced by spontaneous mutant bacteria in the intestines.

Direct toxic effects of the nptII gene and APH(3')II can be disregarded. The low possible intake of genetically modified DNA in comparison with the naturally occurring amount of DNA with resistance mutations in the digestive track means that the additive possible toxic effect is negligable (Kärenlampi, 1996). The WHO report (1993) as well as Kärenlampi (1996) state that APH(3')II lacks toxicity for people and animals. Acute toxicity test made on mice which received a dose of 5.000 mg per kg body weight did not reveal any effects. This dose is equivalent to more than one million times the possible intake of potatoes or tomatoes containing the nptII gene and the APH(3')II enzyme. Kärenlampi (1996) also stated that APH(3')II does not cause allergic reactions in the human. This is also true for medicines based on aminoglycosides which are registered in Sweden (FASS, 1996).

Despite extensive research, the nptII gene has not been shown to cause any pleiotropic effects, i.e. there are no other phenotypic effects than production of APH(3')II causing specific resistance to antibiotics (Kärenlampi, 1996).

Theoretically antbitiotic resistant bacteria or its products, e.g. APH(3')II, could constitute a risk when consumed. Nap et al. (1992) states that such naturally resistant bacteria occur in food and drinking water as well as in faeces. In a sampling of human excrement from people not having been treated with antbiotics more than half of the samples contained more than 10% antibiotic resistant organisms. The addition of antibiotic resistant organisms, which might happen with the consumption of bacteria which have resistance from horizontally transferred resistance, must be considered negligable, especially considering what has been presented above regarding possible frequency of such bacteria.

Another potential risk might occur by inactivation of orally distributed antibiotics, which have been administered for therapeutic use. The WHO report (1993) as well as Kärenlampi (1996) state that the situation can only happen if the protein coded from the marker gene, APH(3')II, inactivates the antibiotic. Depending upon a low pH and the presence of certain enzymes, APH(3')II, however, breaks down very quickly in the digestive track, with a complete loss of activity in 2-5 minutes. Moreover, APH(3')II needs ATP to execute its phosphorylating activity. ATP is inactivated at a low pH.

The use of the aminoglycosides in human and veterinary therapy has decreased dramatically due to serious side-effects. Sande & Mandell (1985) describe three main types of such side-effects, namely nephrotoxicity (kidney malfunction), ototoxicity (break down of hearing sensors) and neuromuscular blockage (acute muscel paralysis). Kärenlampi (1996) states that there is nearly no kanamycin used in human therapy due to the mentioned side-effects. In Sweden there are not any registered medicines which are based on or contain kanamycin either for human or veterinary therapy (FASS, 1996; FASS Vet., 1995). The same reference

states that also the use of neomycin has decreased. In Sweden there are only two registered medicines which are allowed for human therapy, namely Nebacetin powder and Ecomytrin cream, both for external use (FASS, 1996). Nebacetin ointment was registered through 1995 for treatment of infections in the auditory canal. There are three medicines which are registered for veterinary therapy, namely Colinovina vet. mixture and Colivet vet. mixture for oral treatment of colienteritis and infectious diahrrea in pigs and cattle, and Tresaderm vet. solution (neomycin + tiabendazol) for external treatment of irritation in the auditory canal, ear scab and dermatosis in dogs and cats (FASS Vet., 1995). Both kanamycin and neomycin have been replaced by other, more potent aminoglycosides, which are not substrates for APH(3')II (Nap et al., 1992). Geneticin is no longer used for therapeutic treament in either human or veterinary medicine (Kärenlampi, 1996).

In summary the WHO report (1993) states that the presence of marker genes *per se* in food and food products can not be a safety risk, and that there are no reasons to suspect that the gene products should create problems with allergies, unless the marker genes originate from sources which are known to cause food allergies. Based upon Bergmans (1993) The Dutch Committee on Genetically Modified Organisms has submitted a recommendation to the Netherland's Environmental Department to consider the presence of kanamycin resistance as risk-free. Kärenlampi (1996) means that the nptII gene and its products must be considered to be very well investigated in all aspects. Therefore, it should be the first candidate to be included in a "positive list", that is a list of marker genes accepted by society.

There are no reasons what so ever to suppose that the changed starch composition (>98% amylopectin) should cause any danger for other non-modified organisms, including humans. There are already plants, in which this trait has occured spontaneously or by induced mutations, e.g. maize and barley. Further more "amylopectin starch" can be produced from normal potato starch by chemical treatment. No un-desired side-effects have been observed (Annexes 5 and 7).

10. Description of methods for detection and identification of the genetically modified plant

The transgenic potato clone can be detected with PCR, "Southern-blotting", polyacrylamide electrophoresis and iodine staining. In the field tubers from the modified plant is easily identified by iodine staining of a cross section of the tuber.

11. Information about previous releases of the genetically modified plant, if applicable

Deliberate release of the genetically modified potato clone:

- 1993: field trial (Annex 13)
- 1994: field trial and seed production (Annex 14)
- 1995: field trial and seed production (Annex 15)
- 1996: field trial and seed production (Annex 20)

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H. INFORMATION ON THE POTENTIAL ENVIRONMENTAL IMPACT FROM THE RELEASE OF GENETICALLY MODIFIED PLANTS

1. Likelyhood of the genetically modified plant becoming more persistant than the recipient or parental plants in agricultural habitats or more invasive in natural habitats.

The potato clone EH92-527-1 has been changed in two aspects, as compared to the recipient clone, Prevalent. Resistance to kanamycin has been introduced and the starch composition has been changed. None of the changes has brought with it any characteristic which might create improved opportunities for survival in or invasion of natural habitats. This has been studied in field trials where no differences could be shown between the recipient clone and EH92-527-1 (se Annexes 2, 10, 13, 14, 15 and 20).

2. Any selective advantages or disadvantages which could be conferred to other, plant species by transfer of genetic material from the gentically modified plant.

Introgression of genetic material to other plant species is impossible because of incompatibility barriers. Introduction to other potato varieties is possible, but there are no reasons to assume that the resulting hybrids should have any selective advantages (Conner, 1994; Eijlander & Stiekma, 1994).

3. Potential environmental impact of the interaction between the genetically modified plant and target organisms (if applicable)

Not applicable.

4. Possible environmental impact resulting from potential interactions with other, not modified organisms

Glycoalkaloids and nitrates are toxic and anti-nutritional substances normally present in potatoes. Their interaction with other organisms has been described in Part 1.B.7. In Part 1.D.7, Attachment 8 and Part 3 it has been shown that the contents of these substances have not been changed due to the modifications. Therefore it is assumed that the modified potato clone, EH92-527-1, does not affect other organisms in another way than the recipient cultivar, Prevalent. The modified clone will not differ from any other starch potato variety in its influence on the environment.

The modified clone, EH92-527-1, differs in two characteristics from the recipient clone. One is the resistance to kanamycin, and the other is the elimination of amylose causing the increase the of amylopectin content to >98%.

In a report to the Netherland's Environmental Department, Bergmans (1993) assumes that the environmental consequences of resistance to kanamycin (or the

carrier gene nptII) can be disregarded. In Holland as a result of the use of kanamycin in veterinary medicine, antibiotics are supplied to the soil mainly through the use of manure. The concentration of kanamycin is less than 0.13 µg per ml soil, which is considered to be far too little to give plants containing the nptII gene any selective advantages. Of course this does not only apply to the transgenic clones, but also to other plants to which the gene has been transferred by pollen.No differences in competetiveness between plants resistant or susceptible to antibiotics have ever been observed (Bergmans, 1993). It can not be assumed that kanamycin resistance is added to the soil microflora from transgenic plants in measurable amounts. Soils in the Netherlands normally contain 1000 - 100 000 naturally kanamycin resistant microorganisms per gram. The harms associated with transfer of the nptII gene to the microflora of the intestines of humans or animals by way of food or feed are also regarded as negligable. This is because the content of kanamycin resistant microorganisms in the intestines is normally high without causing any harmful effects. Further information is found in D.9.

In regards to the changing of the starch composition, i.e. the increase of the amylo-pectin content from about 75% to >98%, there are no reasons to even suspect any environmental consequences. Potatoes are consumed not only by humans but also by animals, and by microorganisms in the process of biological decomposition. The only change in connection with the digestion of the modified starch is a somewhat faster break down to sugars.

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Part 2

SPECIAL CONDITIONS IN OTHER AREAS OF THE EU WHICH MIGHT CAUSE OTHER EFFECTS TO THE ENVIRONMENT OR MIGHT AFFECT THE HEALTH OF HUMANS AND ANIMALS

The transgenic clone/variety is compatible with other cultivated potato varieties and with true seedling plants which have been produced by hybridization of potato varieties along with vegetative progenies of such hybrids, all of which belonging to the species *Solanum tuberosum*. In northern Europe potatoes are only found in cultivated fields, while in southern Europe they can also be found outside such areas (”wild”).

Solanum tuberosum is not compatible with its related wild species, *S. nigrum* and *S. dulcamara* (Eijlander & Stiekma, 1994; Conner, 1994).

Concerning possible effects to the environment, these are limited to *Solanum tuberosum*, while other sexually compatible relatives are not found in Europe. The transgenic characteristics in EH92-527-1 cannot be considered to influence the environment in Europe, regardless if the trait is maintained in the transgenic clone itself or if it is transferred to other potatoes. The characteristic transferred is not considered to cause any selective advantages as compared to other clones *of Solanum tuberosum*.

Potatoes are found throughout Europe as a cultivated crop. This means that the European population has been exposed to potatoes as a food during a long period of time. In Part 3 a general risk assessment concerning EH92-527-1 is presented. Considering the fact that the exposure of humans to potatoes and potato products are similar in large parts of the EU the assessment is relevant also for this chapter.

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Part 3.

PRELIMINARY ASSESSMENT OF ANY HARMFUL EFFECTS WHICH MIGHT BE CAUSED BY THE MODIFIED PLANT

A. THE POSSIBILITIES THAT THE TRANSFORMED POTATO CLONE WILL DEVELOP INTO AN OBNOXIOUS WEED

In northern Europe potatoes are only found in cultivated areas, while in southern Europe they can also be found in the wild, i.e. outside cultivated areas. In no case potatoes are considered to create any ecologically competitive factor of importance.

A condition for the clone EH92-527-1 to become an obnoxious weed would be that the clone has received radically changed characteristics in comparison with other potatoes as a result of the genetic modification. Examples of characteristics needed to classify the clone as an obnoxious weed would be high competitive ability with other plants, improved survivability when subject to mechanical or chemical weed control and improved ability to establish itself outside cultivated areas.

Establishing and development of plants from seed of the clone EH92-527-1 has been studied in comparative yield trials 1995 and in selection trials 1993, 1994 and 1995. In all of these trials it has been shown that establishment and development of potato plants are comparative to the recipient clone, above as well as below ground. There were no cases where it could be shown that there was a higher competitive ability with other plants or a better ability for establishment. (see Annexes 2, 10, 13, 14, 15).

The ability to resist mechanical weed control has been indirectly tested in trials 1993, 1994 and 1995. After harvest the fields were superficially cultivated according to the conditions stated in permissions for deliberate release. The fields were then fallow in the following summers, and they were officially inspected by the Swedish Board of Agriculture. There were no observations of potato plants which might have been left in the field, which indicates that survivability had not been changed. In some trials the herbicides Sencor or Sencor+Boxer were applied for weed control. There were not any differences observed between the transgenic clone and the parent with respect to herbicide tolerance.

The modification has caused two changes compared to the recipient clone. These are a change in starch composition and resistance to an antibiotic, kanamycin. The change in starch composition is only a change of quality of the starch produced. The quantity of starch produced has not been changed to a significant extent. The amount of energy in the two starch components is similar. There is a possibility that there is a somewhat faster availability of amylopectin because this molecule has a larger number of α-D-(1$\rightarrow$6) bonds, which are more easily broken down. The availability of the energy stored in the tubers is not a limiting factor and lacks importance for the establishment and development of the potato plants.

Considering the small amounts of kanamycin present in the environment, resistance to kanamycin does not involve any advantages to the plant. This has been demonstrated by Bergmans (1993) in a report to the Netherlands Environmental Department stating that consequences for the environment by kanamycin resistance (or the causal gene, nptII) can be excluded. In the Netherlands as a result of the use of kanamycin in veterinary therapy, the antibiotics are spread to the soil through use of manure. The concentration, however, is less than 0.13 μg/ml, which is far to low a concentration to give plants containing the nptII gene a selective advantage. Of course this does not only apply to the transgenic plants, but also to other potatoes having received the gene through pollen. However, no differences in competetiveness between plants that are susceptible or resistant to antibiotics have ever been observed (see also Part 3, D and Part 3, Resistance to kanamycin).

In summary, it can be ascertained that the risk for EH92-527-1 to develop into an obnoxious weed is negligable. If this should happen, research has shown that the clone can be eliminated by mechanical cultivation as well as by treatment with chemical herbicides.

B. THE POSSIBILITIES THAT THE GENES INTRODUCED INTO THE PLANT CAN BE TRANSFERRED TO ITS WILD RELATIVES

The transgenic clone/cultivar is compatible with other cultivated potato varieties and with true seedlings and their vegetative offspring created by hybridization of potato varieties, all belonging to the same species, *Solanum tuberosum*. *Solanum tuberosum* is not compatible to any of its relatives present in Europe, *S. nigrum* and *S. dulcamara* (Eijlander & Stiekma, 1994; Conner, 1994).

With the facts stated above, there cannot be seen any risk in transfer of genes to the wild relatives.

C. THE POSSIBILITIES THAT THE GENETICALLY MODIFIED PLANT WILL BE TOXIC OR IN ANY WAY CAUSE HARM

The transformation of recombinant DNA into the clone EH92-527-1 had been made with *Agrobacterium tumefaciens*. The kanamycin resistance gene, nptII, originating from Tn5 has been utilized as a marker gene. In the transformation an inverse gene sequence has been transferred into the potato. The inverse sequence (antisense) eliminates the expression of the normal sense sequence being necessary for the synthesis of amylose.

When it comes to determining the risk that EH92-527-1 could be harmful to humans and animals, the evaluation should be divided into three parts:

- Substantial equivalence between EH92-527-1 and Prevalent considering important characteristics which have not been directly changed as a result of gene technology.

- Substantial equivalence between the transferred change of starch composition and the experience of this starch composition from other crops and from the use of such starch produced from potatoes by a chemical process.

- Evaluation of possible dangers caused by the introduction of the kanamycin resistance gene.

As a basis for the evaluation a summary of the concept of Substantial equivalence is included as well as a molecular characterization of the transferred DNA.

Substantial equivalence

The concept Substantial equivalence is given by OECD as the most practical way to evaluate the safety aspects for new food products which are produced by modern biotechnology.

The method of evaluation is to compare molecular characteristics, agronomic traits, important nutritional substances and possibly toxic substances present in the modified clone with the counterparts in the parent clone. The purpose is to show that the two clones are equal except for the traits which have been deliberately changed. The modified traits must be shown to be equivalent to other materials, which have been proven not to be harmful to humans and animals.

In those cases where Substantial equivalence cannot be shown completely, e.g. concerning transferred traits, further trials have to be carried out in order to confirm that the products is not harmful. The type of trials or examinations to be executed isdetermined case by case.

Substantial equivalence between EH92-527-1 and the mother variety Prevalent considering important characteristics which have not been changed as a result of gene technology

Agronomic traits

The agronomic traits of the modified clone EH92-527-1 and the recipient clone Prevalent have been compared in the following reports of field trials:

- Sortansökan, Anmälan till officiell värdeprovning av EH92-527-1, 1995 (”Cultivar application, Registration of EH92-527-1 for official trials, 1995”). Annex 2.
- Känno, Jüri. Observationer i praktiska odlingar av konventionell Prevalent och transgen Prevalent EH92-527-1 1994-1995, 1996 (”Observations in practical cultivation of the cultivar Prevalent and the transgenic Prevalent EH92-527-1 1994, 1995, 1996”. Annex 10.
- Erjefält, Lennart. Rapport rörande fältprövning av transgen potatis 1993 (”Report about field trials with transgenic potatoes 1993”). Annex 13.
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- Erjefält, Lennart. Rapport rörande fältförsök och utsädesodling med transgen potatis 1995 (”Report about field trials and seed production with transgenic potatoes 1995”). Annex 15.

- Erjefält, Lennart. Rapport rörande fältförsök och utsädesodling med transgen potatis 1996 (”Report about field trials and seed production with transgenic potatoes 1996”). Annex 20.

Results of comparative trials

Comparisons with the conventional parental cultivar Prevalent have been made ever since field trials with EH92-527-1 began in 1993. In these comparisons no differences in agronomic traits have been observed. In the field trials 1995 and 1996 some yield differences were recorded. This could be explained by differences in seed quality.

Chemical characterization along with statistical analysis of the data obtained

In a preliminary trial with Prevalent it was investigated what level of variation in different characters could be normally expected. In samples of the recipient cultivar Prevalent from 13 locations carbohydrates was measured at 83.5 g with a standard error (SE) of 0.26. The standard deviation was 0.92. This means that 7 observations of each Prevalent and EH92-527-1 are needed to determine the true difference in 5 out of 6 cases at a 5% level of significance if a difference of 3 x SED (standard error of the difference of the mean) is accepted. This confidence level is usually used for biological traits.

In order to increase the confidence level even more, the number of observations was increased to 12 per clone. The true difference that can be detected is reduced to 0,77, i.e. 2 x SED. From this calculation the transgenic clone EH92-527-1 and its recipient cultivar Prevalent were grown in three randomized blocks in 1996 with four replications at each of the following locations: Svalöv, Kristianstad and Fjälkinge.

Samples were taken from all plots and analyzed for contents of nutritional, toxic and antinutritional substances. All results from the analyses were registered as parts per dry matter. Simple analyses of variance with location and cultivar as variables were calculated. In general both replications and cultivar x location interactions were not significant. Their variance was therefore carried over as the variance of the remainder in order to aquire the maximum degrees of freedom. With the test of homogeneity it was indicated that the values for digestible fibres, glucose, energy and cadmium were not normally distributed. With a closer examination it was found that one of the valuesdeviated from the normal distribution, and therefore it was discarded from the analysis of variance.

For traits which showed a significant difference for cultivars, an analysis of covariance was also calculated with yield as covariant. The statistical program STATGRAPHICS, Manugistics, Inc. has been used both for planning of the trial and the analysis of variance.

The results of the analyses are shown in the following tables. The average is shown for all plots of each clone along with location and cultivar differences. When appropriate the level of significance after considering the covariance of yield is shown within brackets ( ).

Table 4. The statistical analysis of the data for dry matter and yield. (The complete statistical data are found in Annex 8.)

	Dry matter content (g/100 g)		Yield (kg dry matter)	
	Prevalent	EH92-527-1	Prevalent	EH92-527-1
Mean	26.0	24.8	21.5	16.0
Local	**		***	
Cultivar	***		***	

* = P<0.05 ** = P<0.01 *** = P<0.001 ns = P>0.05

In the trials there was a significant difference regarding yield between Prevalent and EH92-527-1. It has been mentioned earlier that a difference in the seed quality was observed, and that this difference most probably affected yield. It cannot be disregarded, however, that the change in starch composition might also affected the yielding ability of the trangenic clone. There is also a significant difference in dry matter content between the clones. The greater part of that difference is most probably due to a difference in starch content. It cannot be disregarded that this difference is also a consequence of the modification of the starch synthesis, which eliminates the ability of the transgenic clone to produce amylose. The statistical analysis does not reveal a true difference between clones regarding starch content, but the result is very close to a significant difference. It cannot be disregarded that a larger experimental material would explain the difference in dry matter content as being a consequence of a difference in starch content.

It's concluded that there is reason to consider the differences in dry matter content and yield in this statistical analysis.

Other analyses are shown in tables 5 - 8.

Table 5. The statistical analysis of the data for protein, fat (lipids), ash and carbohydrates. (The complete statistical data are found in Annex 8.)

Mean:	Protein g/100g DM	Fat g/100g DM	Ash g/100g DM	Carbohydrates g/100g DM
Prevalent	7.33	0.12	3.72	83.91
EH92-527-1	7.47	0.13	3.80	84.17
Local	***	ns	*	***
Cultivar	ns	ns	ns	ns

* = P<0.05 ** = P<0.01 *** = P<0.001 ns = P>0.05

None of the analyses could show any differences in protein, fat (lipids), ash or carbohydrates between the clones.

Table 6. The statistical analysis of the data for plant fiber, digestible fiber, starch and energy. (The complete statistical data are found in Annex 8.)

Mean:	Plant fiber	Digestible fibre	Starch	Energy
	g/100 g DM	g/100 g DM	g/100 g DM	kJ/100 g DM
Prevalent	1.64	4.92	74.6	1555
EH92-527-1	1.63	4.44	75.2	1562
Local	**	** (*)	*	** (**)
Cultivar	ns	** (ns)	ns	* (ns)

* = P<0.05 ** = P<0.01 *** = P<0.001 ns = P>0.05

( ) = level of significance of covariance with yield

There is no significant difference in plant fibre and starch between the two clones. Digestible fibres and energy were not normally distributed. After 1/x transformation, the digestible fibres had a normal distribution, while energy was still one-sided because of one deviating value. This value was disregarded. According to the simpleanalysis of variance the modified clone had a significantly lower content of digestible fibers than the recipient cultivar.When the difference in yield was taken into consideration the difference in digestible fibres disappeared. It is probable that the modification of the starch synthesis also affects parts of the constituents included in the analysis of digestible fibres, e.g. retrograded amylose. Also the significant differences in energy disappear when differences in yield are taken into consideration.

Table 7. The statistical analysis of the data for fructose, glucose and saccharose.(The complete statistical data are found in Annex 8.)

Mean:	Fructose	Glucose	Saccharose
	g/100 g DM	g/100 g DM	g/100 g DM
Prevalent	492	855	1425
EH92-527-1	756	1000	2182
Local	*** (***)	***	* (*)
Cultivar	*** (*)	ns	*** (***)

* = P<0.05 ** = P<0.01 *** = P<0.001 ns = P>0.05

( ) = level of significance of covariance with yield

Fructose, glucose and saccharose contents are higher in the modified clone, but only regarding fructose and saccharose significantly higher. Also when the level of yield is taken into consideration the differences remain, but with a lower significance. All mono- and disacharides are intermediates in the starch synthesis and are therefore affected as a consequence of the inhibition of the amylose production.

Table 8. The statistical analysis of the data for chlorogenic acid, glycoalkaloids, nitrate and vitamin C. (The complete statistical data are found in Annex 8.

Mean:	Chlorogenic acid	Glycoalkaloids	Nitrate	Vitamin C
	µmol/100 g DM	mg/kg DM	mg/kg DM	mg/100 g DM
Prevalent	81	319	174	63
EH92-527-1	66	334	302	88
Local	*** (***)	***	*** (***)	*** (***)
Cultivar	** (ns)	ns	*** (ns)	*** (***)

* = P<0.05 ** = P<0.01 *** = P<0.001 ns = P>0.05

( ) = level of significance of covariance with yield

Comments about chlorogenic acid, glycoalkaloids and nitrates are found in Part 1.D7.

There is a significant difference between the clones for Vitamin C, also when yield differences are accounted for. The content of Vitamin C is higher in the transgenic clone. A possible explanation could be that the higher amounts of mono- and disaccharides is inducive to the production of Vitamin C. The differences are therefore associated with the deliberate modification of the starch synthesis.

Regarding analyses of minerals (see analysis data in Attachment 8) there is a strong association with the level of yield.

In order to further elucidate that the analysis values obtained for EH92-527-1 do not deviate from normal variation in potatoes, the following comparison between the values obtained and normal reference values has been compiled.

Table 9. Comparison between the obtained analysis values for clone EH92-527-1 and values collected from the literature.

Analysis	Starch potatoes			Table potatoes		
	Mean values		Own study (6) Parent clone	SLV* 1996	SLV** 1988	Danish data***
	EH92-527-1	Parent clone				
DM content (1)	24.8	26.0	22.7-25.8	-	16.0-24.0	18.2-25.2
Protein (1)	1.9	1.9	1.3-2.4	1.8	0.9-2.6	1.4-2.5
Fat (1)	0.03	0.03	0.0-0.06	0.1	-	0.1-0.5
Ash (1)	0.9	1.0	0.9-1.2	1.0	0.9-1.1	0.7-1.1
Carbohydrates (1)	20.9	21.8	19.1-21.5	16.1	-	12.8-26.4
Plant fiber (1)	0.4	0.4	0.4-0.5	-	-	-
Digestible fiber (1)	1.1	1.3	1.2 -1.4	1.4	-	1.5
Starch (1)	18.6	19.4	17.2-19.5	-	-	17.0
Energy (2)	387	405	-	306	-	355
Fructose (3)	186	128	30-208	-	-	70
Glucose (3)	246	222	84-378	-	-	180
Saccharose (3)	541	371	173-597	-	-	780
Chlorogenic acid (4)	16.4	21.1	16.3-45.7	-	-	-
Glycoalkaloids (5)	83	83	72-219	-	-	-
Nitrate (5)	75	45	19-209	-	-	-
Vitamin C (3)	21.8	16.3	12.1-18.1	11	4-23	27
Na (3)	1.6	1.7	0.2-1.2	2	1-4	3,9-10.9
K (3)	474	478	405-558	488	380-640	242-480
Ca (3)	12.2	10.3	4.2-13.2	4	3-19	5-7
Mg (3)	20.8	24.1	19.2-26.0	24	14-28	15-28
P (3)	47.0	49.1	37.2-81.2	31	27-66	28-56
Fe (3)	0.8	0.8	0.6-1.6	0.48	0.41-1.58	0.46-3.00
Zn (3)	0.2	0.2	0.20-0.36	0.4	0.15-0.87	0.22-0.49
Cu (3)	0.06	0.06	0.04-0.17	-	0.05-0.16	0.033-0.194
Mn (3)	0.10	0.13	0.13-0.23	-	0.13-0.44	0.13-0.44
Cd (3)	0.001	0.002	-	-	-	-

(1) = g per 100 g fresh weight

(2) = kJ per 100 g fresh weight

(3) = mg per 100 g fresh weight

(4) = μmol per 100 g dry matter

(5) = mg per kg fresh weight

(6) Analyses from 20 lots of Prevalent grown in southern Sweden 1995

* Livsmedelsverket, 1996

** Statens Livsmedelsverk, 1988

*** Levnedsmiddelstyrelsen, 1996

In summary it can be concluded that the clone EH92-527-1 does not differ in any way from analysis values which are normal in potatoes.

The statistical analysis does not reveal any differences between the parent clone and the modified clone which did not have a natural explanation that the cause was due to the inhibition of amylose production in EH92-527-1.

Modification of the starch composition

The reason for the genetic modification was to change the composition of the starch from a normal starch consisting of a mixture of amylose and amylopectin into a starch consisting of at least 98% amylopectin. This modification of the starch composition was successful. The tubers of EH92-527-1 show a starch composition which agrees with the objectives. Analyses of the starch composition are shown in Annex 6.

In Annex 5, a review has been made about the occurrance of starches with high amylopectin content in other plants than potatoes. The review shows a number of plants which produce this type of starch. In Attachment 7, there is a review on different uses of high amylopectin starches in food products. This review shows that starch with high amylopectin content has been used in a great number of food products for quite some time. The review also shows that pure amylopectin starch, produced from potatoes by a chemical process, has been used in food products from the 1950's to the 1980's.

With the knowledge that today there is an extensive use of amylopectin starch in food products it is regarded as ascertained that the deliberate change caused by the genetic modification of EH92-527-1 is Substantially equivalent with a number of food starches present on the market, which have been produced from other crops. The equivalence is confined to the chemical composition of the starch, while differences between potato starch and starches from other crops remain, e.g. granule size, content of phosphorus, presence of proteins etc.

Kanamycin resistance

In the potato clone EH92-527-1 resistance to the aminoglycosides kanamycin, neomycin and geneticin was transferred with the gene nptII. This gene and its gene products has shown in many studies and reviews to be unharmful for the environment as well as humans and animals.

The gene nptII, which was isolated from the transposon Tn5 from *Eschirichia coli*, codes for the enzyme APH(3')II, which specifically catalyzes ATP dependent phosphorylation (and inactivation) of the aminoglycosides kanamycin, neomycin and geneticin (Sande & Mandell, 1985; Nap et al., 1992; Kärenlampi, 1996).

Resistance to those antibiotics commonly occurs in natural microbe populations (Nap et al., 1992; Bergmans, 1993; Kärenlampi, 1996).

If the gene nptII is disseminated to other potato cultivars or related species through sexual reproduction, a selective advantage should only be found if the specific antibiotics are applied as herbicides or with manure to the soil. Nap et al. (1992) and Bergmans (1993) have shown that this is nearly impossible.

Under certain assumptions horizontal transfer of the nptII gene from plants to micro-organisms in the soil is estimaded to supply a yearly maximum amount of 8.7×10^{-7} transgenic bacteria per gram soil (Nap et al., 1992). As the natural occurrance of resistant organisms is 1 600 - 100 000 per gram soil (Nap et al., 1992; Bergmans, 1993), the addition of 8.7×10^{-7} transgenic bacteria per gram would cause an increase of resistant organisms not exceeding 5.4×10^{-10} %. In the same way, Nap et al. (1992) have calculated the addition of resistant bacteria in the digestive track in humans and animals through transformation to 2.4×10^{-15}%, which is estimated to be less than the natural supplement through spontaneous mutations. The WHO Report (1993) as well as

Kärenlampi (1996) and Harding (1996) estimate that the possibilities for spontaneous transformation are very unlikely and that the consequences of such transformations would be very small.

Toxic effects of the nptII gene as well as the enzyme APH(3')II can be disregarded (WHO, 1993; Kärenlampi, 1996). No pleiotropic effects of the gene or the enzyme have been shown (Kärenlampi, 1996).

Taking into account of the high frequency of naturally occurring bacteria which are resistant to antibiotics, the risks connected with consumption of food containing transgenic resistant organisms are estimated to be negligable (Nap et al., 1992). Also, there is no risk for inactivation of antibiotics used for therapeutical purposes (WHO, 1993; Kärenlampi, 1996).

The declining use of antibiotics containing kanamycin or neomycin also reduces the risks for possible interactions (Kärenlampi, 1996; FASS 1996; FASS Vet. 1995).

The Nordic Council of Ministers (Nordiska Ministerrådet) has suggested that the gene nptII should be included in a so called "positive list" of marker genes which are considered to be so well investigated that their use (as markers) in commercial products actually could be recommended from a health safety perspective (Kärenlampi, 1996).

Further descriptions are found in Part 1, D.9.

In a Swedish investigation on the occurrence of bacteria carrying resistance to kanamycin it was shown that a considerable share of bacteria present in soil from four different locations in Scania (southern Sweden) carry such resistance (annex 22). Based upon the results of this study as well as Dutch investigations it is assumed that kanamycin resistant bacteria are universally distributed in the EU. The abundant occurrence of kanamycin resistant bacteria indicated in Sweden, in spite of an extremely limited use of the antibiotics in the country, supports the assumption that kanamycin resistance is common among soil bacteria.

D. THE RISK THAT THE PLANT CAN BE MORE SUSCEPTIBLE TO PREDATORS AND DISEASES.

The establishment and development of plants from seed of the clone EH92-527-1 has been studied in comparative trials in 1995, and in observation trials in 1993, 1994, 1995 and 1996. In all trials it has been shown that the establishment and development of plants are equal to the recipient clone both above and below ground. During the growing season, both the modified potato clone and the recipient clone were exposed to attack by insects and diseases, mainly potato late blight. In order to avoid such damages, treatments were applied according to the requirements of the recipient clone. There were no observations indicating that the transgenic clone is more (or less) susceptible to any pest or disease than the recipient variety. (Annexes 2, 10, 13, 14, 15 and 20.)

E. THE POSSIBILITIES FOR NEGATIVE ECOLOGICAL AND OTHER EFFECTS WHICH ARE NOT RELATED TO WEED CHARACTERS

Such effects have not been observed during any of the trials. It is not expected that such effects should occur as a consequence of the modifications present in EH92-527-1 (Annexes 2, 10, 13, 14, 15, 20).

This application includes the use of potato pulp as animal feed. Potato pulp is a by-product from the production of starch from potatoes. The starch granules are located inside the potato cells. In the production process the cells are broken and starch can be separated. The remainder (the pulp) consists of cell residuals and small amounts of starch. The same rest product is of course obtained when starch is produced from the clone EH92-527-1. Potato pulp is mainly used for cattle feed in the autumn. It is supplied as a nutritional supplement during the grazing period as well as in the stable. Following the industrial process of starch production the pulp is stored in a silo located at the factory. The capacity of the silo corresponds to about one day's production. Farmers using potato pulp for feed collect the pulp from the factory either as a return freight when delivering potatoes or in separate transports. The pulp is unloaded on a hard surface on the farm to be stored before it's used as feed. As the pulp contains easily accessible carbohydrates a fermentation process is rapidly initiated in the pulp. The pulp may also be used as feed for grazing cattle immediately after delivery. In that case it's unloaded directly on the feeding spot.

Previously it has been shown that essentially no differences exist between non-transformed potatoes and EH92-527-1, besides those differences related to the starch composition. This is assumed to be valid also concerning the pulp. The starch present in the pulp from EH92-527-1 differs from common starch only by the lack of the amylose component, thus creating no additional risk.

Also potato DNA is present in the potato cells. Therefore such DNA is expected to be present also in the pulp. A study was carried out in order to investigate if whole, vital cells or the introduced gene construction were to be found in the pulp from EH92-527-1 (annex 1). Normal potato pulp was analysed as a control. No whole cells could be

detected. The occurrence of DNA fragments containing the kanamycin resistance gene was indicated in fresh pulp, and in pulp that had been cold-stored, from EH92-527-1. Also in pulp from non-transformed potatoes the kanamycin resistance gene was found after some days of storage. The increase of the gene in pulp from EH92-527-1 during storage, as well as the presence of the gene in stored potato pulp from non-transformed potatoes, is assumed to originate from kanamycin resistant bacteria present in the environment, which invade the pulp and multiply during storage. With respect to the risk of kanamycin resistance being transferred to bacteria in the digestive track of humans or animals, it has been shown that the probability is very low. In the study on kanamycin resistant bacteria in soil it was indicated that the occurrence is very abundant in Sweden (annex 22). Consequently the occurrence of the kanamycin resistance gene in transgenic potatoes, and the very limited probability of dispersal of that gene to the bacteria population, justifies the assumption that the risk for increase of kanamycin resistant bacteria can be regarded as negligable. Therefore it's concluded that there are no risks connected with the use of pulp from EH92-527-1 as feed.

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Part 4.

PREVIOUS RELEASES

Field trials have been carried out with the modified potato clone and other modifications of the same type in

- 1993:field trial (annex 13)
- 1994:field trial and seed production (annex 14)
- 1995:field trial and seed production (annex 15)
- 1996:field trial and seed production (annex 20)

Part 5.

OTHER INFORMATION

Part 6.

Form no. E 12.6.

SUMMARY OF NOTIFICATION ON PRODUCTS CONTAINING GENETICALLY MODIFIED ORGANISMS (GMO)

(article 12, directive 90/220/EEG)

INTRODUCTION

This document is to be used as a form for summary of the notification delivered to The Commission when a product containing genetically modified organisms is to be released on the market (part C, article 12.3 in directive 90/220/EEG). This does not involve that the regulations of directive 90/220/EEG are set aside.

The completed summary of notifications regarding products containing genetically modified organisms will contain a summary of the information under the corresponding paragraphs in the complete act. It is therefore obvious that the risk assessment according to article 12 in directive 90/220/EEG can not be made based upon the summary.

A. GENERAL INFORMATION

1. *Information on the notification*

   a) Member State where notification was made: Sweden

   b) Notification number: C/SE/96/3501

   c) Denomination of product (trade or other name): EH92-527-1

   d) Date of registration: 1996-08-05

2. *Notifier/producer/importer*

   a) Name of notifier: Amylogene HB

   b) Address of notifier: c/o Svalöf Weibull AB, S-268 81 Svalöv, Sweden

   c) The notifier is

   a national producer ☒

   an importer ☐

   d) In case of import

      i) Name of producer

      ii) Address of producer

3. *Description of genetically modified organisms in the product*

   Name and species of each type of genetically modified organisms contained in the product

   Potatoes, *Solanum tuberosum*, clone EH92-527-1

4. *General description of the product*

   a) Type of product

   Potatoes intended for starch production

   b) Structure of the product

   Potatoes with modified starch composition, the starch containing at least 98% amylopectin

c) Specific characteristics of the product

The starch of the potatoes has been modified to contain at least 98% amylopectin

d) User category

The potato starch industry

e) Specified conditions for use and handling

The potato clone is intended to 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. Cultivation of seed potatoes will be for 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 will be applied as for other seed potatoes. Mainly seed potato production will be carried out in Sweden, but may also be considered in other parts of the EEC where starch potatoes are grown.

The starch potatoes produced will be used for the production of potato starch. With respect to the starch potatoes the same growing conditions will be applied as for other starch potatoes, except that the potatoes will be handled strictly separated from other starch potatoes. This means that starch will be extracted from the product and used according to A.4., and also that 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 waste water. In 1998 the potato pulp will be spread on cultivated land. Subsequent to 1998 the intention is to use the pulp as cattle feed when the appropriate permit is granted. The cultivation area is identical with the cultivation area of starch potatoes in the EEC.

f) Geographical territory for which the product is intended

Areas in Europe where starch potatoes and potato seed is cultivated

g) Environments suitable for the product

Arable land

h) Calculated yearly production in and/or import to the Community

In the next five years a yearly production of 50.000 to 75.000 tons of potatoes is estimated to be reached.

5. *Has the combination of genetically modified organisms in the product been notified according to part B of directive 90/200/EEG?*

Yes ☒ No ☐

i) If *yes*, state country and number of notification:

Sweden: SJV Dnr 22 28/95, 22 530/96 22 1782/97 and before Sweden joined the European Union 22 3414/92, 22 4363/93 and 22 4664/93

ii) If *no* state data of risk analyses based upon the categories prescribed in part B of directive 90/220/EEG

6. *Is the product simultaneously notified in any other member state?*

Yes ☐ No ☒

If *yes* state which one

7. *Has another product with the same combination of genetically modified organisms been placed on the market within the EU by another notifier?*

Yes ☐ No ☐ Not known☒

If *yes*, state notifier

8. *Information on release of the same genetically modified organism or the same combination of genetically modified organisms having been notified or carried out by the notifier within or outside the Community*

Deliberate release in Sweden:1993: SJV Dnr 22 4314/92
1994: SJV Dnr 22 4363/93 and 22 4664/93
1995: SJV Dnr 22 28/95
1996: SJV Dnr 22 530/96
1997: SJV Dnr 22 1782/97

9. *State instructions and/or recommendations with respect to storage and handling*

Seed potatoes shall be handled and stored with the same demands regarding separation of from other potatoes as for other seed potatoes.
Starch potatoes shall be handled and stored with the application of specific and careful precautionary measures to ensure separation from other potatoes.

10. *Proposed package*

Seed potatoes shall be packed in sacks or be handled in bulk.
Starch potatoes are handled in bulk.

11. *Proposed labelling*

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.

12. *Precautions to prevent non-deliberate release or abuse*

Seed potatoes which by mistake have been mixed with other seed 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.

13 .*Measures with respect to removal and/or handling of by-products and waste*

Potatoes which can not be used for starch production or seed will be destructed in a 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. INFORMATION ON THE NATURE OF GENETICALLY MODIFIED ORGANISMS INCLUDED IN THE PRODUCT.

INFORMATION ON THE RECIPIENT AND/OR MATERNAL ORGANISM(S) FROM WHICH GENETICALLY MODIFIED ORGANISMS ORIGINATE

14. *Scientific and other denominations*

Solanum tuberosum, potatoes, cv Prevalent

15. *Phenotypic and genotypic characteristics*

Starch potato variety 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.

16. *The geographical distribution of the organism, and its natural habitat*

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

17. *Genetical stability of the organism and factors which could influence the stability*

Potato varieties are vegetatively multiplied (clones), and are therefore genetically stable.

18. *Possibilities for genetic transfer to and genetic interchange with other organisms.*

Hybridisation with wild relatives present in Europe is not possible, due to efficient incompatibility barriers.

19. *Information on reproduction and factors which could have an influence*

Reproduction can be executed vegetatively by tubers as well as sexually by botanical seed. Due to frost sensitivity the survivability of the tubers is influenced the winter temperature. The generation period is one year.

20. *Information on survivability and factors influencing survivability*

Potatoes survive as tubers or as botanical seed. Survivability is influenced by temperature, by standard procedures in the subsequent crop(s) like herbicide application and soil treatment, and by competition from the subsequent crop(s). Potatoes does not compete successfully in the natural ecosystem outside cultivated fields.

21 *.Mechanisms of dissemination and factors affecting dissemination*

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

22. *Interactions with the environment*

Potatoes appear only on cultivated land. Dissemination to the surrounding environment may occur, but due to frost sensitivity (northern Europe) and inferior adaptation to the conditions outside the cultivated land such plant do not execute any interaction with the wild flora.

23a. *Methods of detection*

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

23b. *Methods of identification*

See 23a.

24. *Classification according to according to directives regarding protection of human health and the environment.*

Potatoes are not classified.

25a. *Pathogenic traits*

None

25b. *Other harmful traits in the organism (alive or dead) including extracellular products*

High contents of glucoalkaloids may appear in potatoes which have been exposed to stress, e.g. light, oxygen deficiency and mechanical damaging.

26. *Extrachromomal genetical elements and a description of those.*

There are no such elements in potatoes or in the actual variety, which are known to us.

27. *Information on previous genetical modifications*

The clone has not previously been genetically modified.

INFORMATION ON THE GENETIC MODIFICATION

28 *.Methods used to accomplish the genetic modification*

Transformation of potato with 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 (Hoekema et al., 1983). Transformation has been made using 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 eradicated, since subsequent cultivation without any selection agent does not generate any bacteria growth.

29. *Properties of the vector*

a) Specification of the vector and its origin

Agrobacterium tumefaciens strain LBA4404 with Ti-plasmid pAL4404 was used for transformation of potatoes (Ooms et al., 1981). In pAL4404 T-DNA and oncogenic traits have been deleted. The binary vector (pHoxwG) which was used as a carrier of traits transformed to plant tissue originates from pBIN 19 (Bevan, 1984). 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 (Zambryski et al., 1980). 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. T-DNA in pHoxwG is described below.

b) Description of the vector construct

The binary transformation vector pHoxwG is derived from pBIN19, by inserting a gene construct into the multiple cloning site. This gene construct consists of the gbss promoter (987 base pairs), which was isolated from potato, and a gbss-segment isolated from potato (1.945 base pairs) that has been cloned in a reverse orientation in relation to the gbss promoter. This recombinant gene is then terminated by the polyadenylation sequence from the nopaline synthase gene (252 base pairs). The gene construct is situated on a T-DNA that is limited by right and left border sequence from pTiT37. Within the border limits, in pBIN19 and pHoxwG, there is also an *nptII* gene that is driven by a nopaline synthase promoter and terminated by a polyadenylation sequence from the nopaline synthase gene. Outside the T-DNA border sequence is a gene conferring kanamycin resistance. That gene makes selection for resistance possible in bacteria, but the gene is not intended to be transferred to the plant. T-DNA in pHoxwG is further described below.

c) Gene map and/or restriction map of the vector

See attachment A of this summary and appendix 3 in the complete notification.

d) Sequence data

See attachment B.

e) Information on to what extent the vector contains sequences, the products or functions of which are not known

The complete vector is known.

f) The capacity of the vector regarding genetic transfer

Agrobacterium tumefaciens containing the vector is capable of transferring a fragment of the vector, T-DNA, into plant cells. The fragment is integrated in the plant cell chromosomes.

g) Mobilising frequency of the vector

A fragment of the vector is chomosomally integrated in the plant genome. That DNA is inherited in the same way as other DNA in the genome.

h) Part of the vector remaining in the genetically modified organism

No other parts than the intended ones have been detected in this genetically modified organism. ”Intended parts” is DNA sequence inside the T-DNA border sequences of the transformation vector pHoxwG.

30. *Information on the transferred genetical material*

a) Methods used for the construction of the genetical material

Restriction enzyme cutting, ligation and applicable DNA purification methods have been used.

b) Restriction sites

See attachment C.

c) Sequence of the inserted gene material

See attachment D.

d) Origin and function of each part of the inserted gene material in a genetically modified organism

T-DNA in pBIN19 has a size of about 3.213 base pairs and is limited by border sequences from pTiT37. Inside these borders there is a kanamycin resistance gene which is expressed in plant tissue, and a multiple cloning sequence from M13mp19 (see annex 3 in the complete notification). The kanamycin resistance gene is a *nptII* gene originating from Tn5 (Rothstein et al., 1981). For expression in plant tissue the gene is regulated by a nopaline synthase promoter and carries in the other end a polyadenylation sequence from the nopaline synthase gene originating from *A. tumefaciens*. T-DNA border sequences are used to insert foreign DNA in plant chromosomes. The *nptII* gene with nopaline synthase DNA-segment is used for selection of transformed plant tissue. The multiple cloning sequence contains a gene that modifies the starch composition inserted. The gene consists of the gbss promoter (987 base pairs) which was isolated from potato and the polyadenylation sequence from the nopaline synthase gene (252 base pairs). The promoter gives a strong expression in tubers, pollen and root tips. This has been verified by the expression of a marker gene (uidA) in transgenic potatoes. Between those two DNA segments a segment of the gbss gene has been cloned in a reverse order in relation to the promoter. The DNA segment present in the plasmid pHoxwG amounts to 1.945 base pairs of the potato gbss gene, and intends to reduce the amount of gbss the potato tuber, and consequently reduce the amount of amylose.

gbss = ”granule bound starch synthase” EC 2.4.1.11.

e) Information on the limitation of the inserted gene material with respect to the intended function

No other function has been observed.

f) The position of the inserted gene material in the genetically modified organism

Chromosomal integration has been verified by Southern blotting (see attachment 4 of the complete notification). The recombinant gbss gene has reduced the amount of gbss which is derived from an endogenous nuclear gbss gene. The modified characteristic has been stable over a number of tuber generations.

INFORMATION ON THE ORGANISM(S) FROM WHICH THE INSERTED GENETIC MATERIAL ORIGINATE(S) (DONOR ORGANISM(S)

31. *Scientific and other denominations*

The inserted genetic material originates from *Agrobacterium tumefaciens*, *Solanum tuberosum* and the transposon Tn5 present in e.g. *Eschirichia coli*.

32a. *Pathogenic traits in the donor organism(s)*

Strains of *Agrobacterium tumefaciens* are plant pathogens. *Solanum tuberosum* is not a pathogen. Tn5 is not an organism.

32b. *Other harmful traits in the organism(s), alive or dead, including extracellular products*

Not known

33. *In case the donor organism(s) contain(s) pathogenic or harmful traits, state if the inserted sequences are of any importance*

The DNA-fragment originating from Agrobacterium tumefaciens does not involve any consequences with respect to pathogenic or harmful traits.

34. *Classification according to Community regulations regarding human health and the environment*

35. *Potential with respect to natural exchange of genetic material between donor and recipient organisms*

There is a potential for such exchange between Agrobacterium tumefaciens and Solanum tuberosum and between S. tuberosum genotypes.

INFORMATION ON GENETICALLY MODIFIED ORGANISMS CONTAINED IN THE PRODUCT

36. *Description of genotypic or phenotypic characters and especially new traits which might be expressed, or which are no longer expressed*

By means of a recombinant *nptII*-gene the plant expresses resistance to kanamycin.
By means of a recombinant gbss-gene the production of amylose in tubers is reduced. This has been verified by analysis of starch composition in tubers (see attachment 6 in the complete notification).

37. G*enetic stability of the genetically modified organism(s)*

The genetically modified organism has been found to be stable with respect to the inserted trait during three growing seasons.

38. *Expression rate and expression level of the new genetic material*

The indended characteristics are expressed in the modified plant.

39. *Activity of the expressed proteins*

The intended characteristics are expressed in the modified plant.

40a) *Method for detection of genetically modified organisms in the environment*

The transgenic clone is detected by means of PCR, ”Southern blotting”, polyacrylamid electrophoresis and iodine staining of the starch. In the field tubers from the modified plant can easily be detected by iodine staining of a cut tuber surface.

40b) *Identification methods*

see 40a.

41. *Health aspects*

a) toxic or allergy inducing effects of non-vital genetically modified organisms and/or their metabolic products

Not known

b) risks connected with the product

No risks are foreseen.

c) comparison between the genically modified organism and the donor, recipient or maternal organism with respect to pathogenicity

None of the organisms is pathogenic

d) colonisation ability

Not relevant

e) if the organism is pathogenic to humans carrying a functional immunological defence, supply information according to §attachment 2, part C

Not relevant

INTERACTION BETWEEN GENETICALLY MODIFIED ORGANISMS AND THE ENVIRONMENT

42. *The survival, propagation and dissemination of the genetically modified organisms in the environment*

Not different from the recipient organism (se paragraphs 19-21)

43. *Interaction between the genetically modified organisms and the environment*

Not different from the recipient organism (se paragraph 22)

44. *Environmental consequences*

As the genetically modified organism does not differ from the recipient organism with respect to survival, multiplication, dissemination or interaction with the environment (see paragraphs 42 and 43), dissemination does not involve any new or different environmental consequences.

C. EXPECTED BEHAVIOUR OF THE PRODUCT

1. INFLUENCE OF THE PRODUCT ON THE ENVIRONMENT

Because of information supplied in the notification the genetically modified potato clone is not assumed to bring about any influence on the environment.

2. INFLUENCE OF THE PRODUCT ON THE HEALTH OF HUMANS AND ANIMALS

Because of information supplied in the notification the genetically modified potato clone is not assumed to bring about any influence on the health of humans and animals. Since the pulp - a by-product - is intended to be used as cattle feed possible risks connected with that use have been studied separately. Based upon the results of those studies it is assumed that the risk caused by transfer of the kanamycin resistance gene is very small. The pulp contains no or extremely few whole, vital cells. As the pulp contains starch a fermentation process in which residual DNA is broken down starts immediately after the separation process in the factory. If a transfer should happen anyway, the consequences are negligible as resistance to kanamycin is very common also in natural bacteria populations.

D. INFORMATION ON PREVIOUS RELEASES

1. INFORMATION ON PREVIOUS RELEASES WHICH HAVE BEEN NOTIFIED ACCORDING TO THE DIRECTIVE, PART B

1. *Notification numbers:* SJV Dnr 22 4314/92, 22 4363/93, 22 4664/93, 22 28/95, 22 530/96, 22 1782/97

2. *Release localities:* Teckomatorp, Häljarp, Fjälkinge, Händene (Skara), Habo, Flaskebo, Tegsnäset, Norra Sunderbyn.

3. *Purpose of releases:* Control of identity, stability and agronomic characteristics. Seed production.

4. *Release duration:* 4-5 months each year, 1993-1996.

5. *Duration of supervision after release:* 1 year fallow after each release + 3 years without potato cultivation.

6. *Purpose of supervision after release:* To verify absence of potato plants emerging from groundkeepers or botanical seed, and in case such plants are found to remove and destroy them.

7. *Conclusion of supervision after release:* No potato plants have ever been found in the fallow following cultivation of modified potatoes or in the subsequent 3 years without potatoes. It is therefore concluded that the modified clone does not differ from the recipient clone with respect to survivability.

8. *Results of release with respect to risks for human health and the environment (supplied to the relevant authority according to article 8, directive 90/220/EEG):*

With reference to reports supplied to Statens Jordbruksverk (SJV; Swedish Board of Agriculture) regarding trials executed in 1993, 1994 and 1995 (SJV Dnr 22 4314/92, 22 4363/93, 22 4664 and 22 28/95) release of the potato clone EH92-527-1 can not be assumed to involve any risks with respect to human health and the environment.

II. INFORMATION ON PREVIOUS RELEASES IN OR OUTSIDE THE COMMUNITY

1. *Country of release:*

Sweden: Deliberate release: SJV Dnr 22 4314/92, 22 4363/93, 22 4664/93, 22 28/95, 22 530/96 and 22 1782/97.

2. *Authority:*

Swedish Board of Agriculture

3. *Release sites:*

Teckomatorp, Häljarp, Fjälkinge, Händene, Habo, Flaskebo, Tegsnäset, Norra Sunderbyn

4. *Purpose of releases:*

Control of identity, stability and agronomic characteristics. Seed production.

5. *Duration of supervision after release:*

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

6. *Purpose of supervision after release:*

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

7. *Conclusion of supervision after release:*

No potato plants have ever been found in the fallow following cultivation of modified potatoes or in the subsequent 3 years without potatoes. It is therefore concluded that the modified clone does not differ from the recipient clone with respect to survivability.

8. *Results of release with respect to risks for human health and the environment:*

With reference to reports supplied to Statens Jordbruksverk regarding trials executed in 1993, 1994 and 1995 (SJV Dnr 22 4314/92, 22 4363/93, 22 4664 and 22 28/95) release of the potato clone EH92-527-1 can not be assumed to involve any risks with respect to human health and the environment.

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NOTIFICATION FOR PLACING THE POTATO CLONE EH92-527-1, BEING GENETICALLY MODIFIED FOR INCREASED CONTENT OF AMYLOPECTIN, ON THE MARKET.

Attachments to Part 6, Summary of notification on products containing genetically modified organisms (GMO)

A. ***Plasmid map of pHoxwG (size approx. 15.2 Kb)***

B. ***Sequence data of the vector***

C. ***Restriction sites (pHoxwG T-DNA Cut Site Map and Enzyme Cutters)***

D. ***Sequence data of inserted genetic material***

Annexes

1. **Studies on pulp from EH92-527-1**

2. **The Swedish Seed Testing and Certification Institute: Statement on variety distinctness**

3. **Description of the T-DNA in vector pHoxwG**

4. **Southern blotting for determination of the number of gene copies incorporated in EH92-527-1**

5. **A comprehensive description of starch, the amount of amylopectin found in starch from various crops, and previous experience in the field of separating potato starch into amylose and amylopectine**

6. **Helmersson Karin. Characterising starch from genetically modified potatoes**

7. Helmersson Karin. The use of amylose and amylopectin in food applications.A literature review

8. **Analyses of the chemical composition of tuber samples of Prevalent and EH-92-527-1.** Doc a, Doc b

9. Flow chart, amylopectin potatoes (100% amp); Starch production 1995-2000

10. Observations in practical cultivation of conventional Prevalent and transgenic Prevalent EH92-527-1, 1994-1995, and in laboratory freezing tests 1996/1997

11. Analyses of the presence of GBSS in starch

12. **Characterization of starch in different parts of the transgenic clones EH92-527-1 and EH93-1069-1.** Doc a, Doc b, Doc c

13. Report about field trial with transgenic potatoes 1993

14. Report about field trials and seed production with transgenic potatoes 1994

15. Report about field trials and practical cultivation with transgenic potatoes 1995

16. Design of labelling of potato seed (example)

17. **Lyckeby Starch. From potatoes to starch**

18. Investigation of DNA integrated into the clone EH92-527-1 using PCR

19. Investigation of chloroplast DNA in the clone EH92-527-1 regarding non-intended integration of DNA from the transformation vector pHoxwG

20. Report about field trials and practical cultivation with transgenic potatoes 1996

21. Southern blot analysis of total DNA from the potato clone EH92-527-1 for the presence of unintended parts of the transformation vector

22. Investigation of kanamycin resistance among bacteria from soil