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Bureau GGO / RIVM / SEC

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31. August 2011
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Application for the release into the environment of genetically modified potatoes

Dear Dr. Glandorf,

BASF Plant Science Company GmbH hereby submits the 'Application for the release into the environment of potato lines with improved resistance to *Phytophthora infestans*, 2012 - 2018' according to the Genetically Modified Organisms Decree.

None of the information contained in the application is claimed as confidential, except the information given in annex 2.

Yours sincerely,

Andreas Heise
Regulatory Compliance Manager Europe
BASF Plant Science Company GmbH

BASF Plant Science Company GmbH

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Application

for the release into the environment of potato lines with improved resistance to *Phytophthora infestans* according to the Genetically Modified Organisms Decree, 2012 - 2018

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A. GENERAL INFORMATION

A.1 Title of the Notification

Application for the release into the environment of potato lines with improved resistance to *Phytophthora infestans* according to the Genetically Modified Organisms Decree, 2012 - 2018

A.2 Give a brief description of the subject of the notification

Field trials shall be conducted with genetically modified potato lines into which resistance genes (R-genes) derived from *Solanum bulbocastanum* have been inserted. The aim of the genetic modification is to increase the resistance of potatoes to *Phytophthora infestans*, which causes late blight.

A.3 Describe the activities involved

Genetically modified potato lines as well as parental and conventional comparator varieties might be grown in parallel in the field. The cultivation will be carried out according to general agricultural practice for potatoes except that for resistance testing no fungicides will be applied. The potato plants will be exposed to *Phytophthora infestans*, either naturally or by inoculation, and the plants will be evaluated for late blight symptoms.

A.4 In which year do you intend to start up the activities?

2012

A.5 In which year do you expect to finish the activities?

2018

A.6 Will consumption and/or feed experiments be part of the activities?

No

A.7 Has there been any hybridization carried out between the primary GMP and unmodified plants? If so, please state whether such hybrids are involved in the present activities?

No

A.8 COGEM issued an advice on November 25, 2008 (CGM/081125-02) on categories of field trials. To which category of field trials does your intended field trial belong?

This application is for a category 1 permit.

A.9 Do you want to keep the information confidential? If so, please specify how the release of the information would harm your competitive position.

We only want to keep information regarding personal contact details of the applicant, contact persons, and ESO confidential. This information can be found in appendix 2.

AIM OF THE INTRODUCTION OF THE GENETICALLY MODIFIED PLANT INTO THE ENVIRONMENT

A.10 Specific aim of the activities involved in the notification

The primary aim of the release is the production of seed potatoes. Additionally demonstration trials and safety studies for the described plant lines might be carried out. In that frame plant material for further analyses (e.g. biochemistry, molecular biology) might be taken from the release site.

A.11 General aim of the activities involved in the notification

The long-term aim is to develop potatoes with improved resistance to *Phytophthora infestans*.

LEGAL PERSON NOTIFYING FOR A CONSENT

A.12 Name of the legal person

BASF Plant Science Company GmbH
Carl-Bosch-Str. 38
67056 Ludwigshafen
Germany

B. DATA FOR THE ORIGINAL PLANT SPECIES

NAME OF THE ORIGINAL PLANT SPECIES

B.1 Common Dutch name

Aardappel

Family

Solanaceae

Genus

Solanum

Species

Solanum tuberosum

Subspecies

tuberosum

Cultivar or cultivated line

P880

Properties of used cultivar	P880
Ripening	Medium
Growth habit	Stem type
Flower color	White
Flowering	Abundant
Berry formation	Frequently
Tuber shape	Oval
Tuber skin	Yellow

GEOGRAPHICAL DISTRIBUTION

B.2 In what systems (other than agricultural systems) does the original plant species occur in the Netherlands?

Potato is not found outside the agricultural system in the Netherlands. Under our climatic conditions potatoes exhibit only low potential to propagate and over winter and its capability to compete with the domestic Flora is minimal.

B.3 In what systems (other than agricultural systems) does the original plant species occur in countries surrounding the Netherlands?

Potato is not found outside the agricultural system in countries around the Netherlands.

B.4 In what kind of agricultural ecosystems is the original plant species cultivated?

Potatoes are grown in many countries worldwide. In the European Union potatoes are cultivated on around 2.1 Mio ha and of which around 152,000 ha are in the Netherlands (Eurostat, 2009). Potatoes are grown on agricultural fields and in private gardens.

B.5 In what other ecosystems (including agricultural systems) is the original plant species found?

In Europe, it is only found in agricultural ecosystems. Potato (*Solanum tuberosum* subsp. *tuberosum*) was introduced into Spain from South America around 1570 and is now cultivated as a major agricultural crop worldwide. The centre of origin is situated in Latin-America.

REPRODUCTION AND PROPAGATION

B.6 How does the plant reproduce in its natural habitat and which factors affect its reproduction?

Reproduction of potato is mainly vegetatively via tubers, though sexual reproduction is possible via botanical seeds. Reproduction is mainly affected by the cultivar, weather conditions, day-length and soil type.

B.7 How is the plant propagated in the agricultural ecosystem where it is grown and which factors affect its propagation?

Reproduction of potato is mainly vegetatively via tubers, but for some varieties sexual reproduction is possible via botanical seeds. Under field conditions selfing is most likely, with 80 - 100 % of seeds formed due to selfing. Flowering and formation of berries is dependent on cultivar and weather conditions (Askew, 1993). Climatic factors affect the survival of tubers and seedlings.

A majority of the cultivated tetraploid *S. tuberosum* subsp. *tuberosum* cultivars are characterized by reduced fertility. Most cultivars show reduced pollen fertility or even pollen sterility (CFIA, 1996). Flower formation does not in all cases lead to the formation

of potato berries. A frequently observed phenomenon is shedding of flowers after pollination, so that no berries and seed develop.

B.8 What is the generation time of the plant in its natural habitat and which factors affect it?

Potato is a perennial plant which in Europe is grown annually from vegetative tubers due to unfavorable climatic conditions (Eastham and Sweet, 2002).

B.9 What is the generation time of the plant in the agricultural ecosystem where it is grown and which factors affect it?

The generation time of potato as it is cultivated in Europe is one year due to climatic conditions and disease pressure.

SURVIVAL

B.10 What surviving parts or elements of the plant are formed and which factors affect them?

Potatoes survive as tubers or as seed. The survival of tubers is temperature dependent.

B.11 How long do the surviving parts of the plant persist in her natural habitat and which factors affect this persistence time?

Tubers sprout after some months of dormancy while seeds can persist in the soil for several years.

B.12 How long do the surviving parts of the plant persist in the agricultural ecosystem where the plant is cultivated in the Netherlands and which factors affect this persistence time?

Wet soils and hard frosts reduce over wintering tuber survival. Tubers are frost sensitive and cannot survive a temperature of -3 °C and lower. It is reported that potato tubers are destroyed by a frost period of 25 hours at -2 °C or a frost period of five hours at -10 °C (OECD, 1997). Under European conditions the tubers persist poorly in cold wet soils and plants rapidly become infected with a range of fungal and viral diseases (Eastham and Sweet, 2002). Even under moderate British climate conditions up to 80 % of the tubers that remained in the soil after the harvest die in mild winters (Lutman, 1977). Botanical seed over winter regardless of temperature. Their survival depends on cultivation practices and crop rotation. Normally volunteer plants are eliminated by plowing, harrowing, herbicide treatment, and competition with other crops in crop rotation.

B.13 What is the chance that the plant will survive outside the agricultural ecosystem in the Netherlands?

Negligible. Potato is not adapted for survival outside the agricultural ecosystem in the Netherlands. Volunteer tubers and plants fail to survive outside agricultural environments, due to the potato's limited competitive abilities. Feral potato could not be observed so far (Sukopp and Sukopp, 1993).

B.14 What is the chance that the plant will survive outside the agricultural ecosystem in countries surrounding the Netherlands?

Negligible, see B.13.

DISSEMINATION

B.15 What disseminating parts of the plant are formed and which factors affect them?

No real spreading parts are formed. Tubers are dispersed by activity of man in crop husbandry and transport. Dissemination is normally limited to the area of cultivation. Fruits are not often consumed as they are highly poisonous and hence seed is not dispersed by this means. Birds sometimes feed on potato berries (Hawkes, 1988), but even bird species that in principle feed on solanaceous berries (e.g. Blackcap, Spotted Flycatcher and Starling) confine themselves to indigenous plants (Goeser and Büntig, 2006). Since male and female sterility is common in cultivated potato and ripening of the berries rarely succeeds within the growing season, botanical seed is rarely formed on cultivated potatoes.

Pollen is consumed by some insects such as bumblebees. Honey bees do not forage in potato crops as its flowers lack nectar. Dissemination of pollen is executed almost exclusively by insects. Wind dissemination is considered to be marginal (OECD, 1997; Eastham and Sweet, 2002, Erasmuson et al, 2005). Pollen dissemination is limited with a maximum distance of 5 to 10 m (Bock *et al.*, 2002).

B.16 How long do the disseminating parts of the plant survive in her natural habitat and which factors affect this survival time?

Tubers sprout after some months of dormancy while seeds can persist for several years. Pollen production is limited and viability is typically limited to a few hours depending on the climatic conditions.

B.17 How long do the disseminating parts of the plant survive in the agricultural ecosystem where the plant is cultivated in the Netherlands and which factors affect this survival time?

See B.12 and B.13

B.18 What is the chance that the plant will disseminate in the Netherlands?

Negligible. Potato is not adapted for survival and reproduction outside the agricultural ecosystem in the Netherlands. Volunteer tubers and plants fail to survive outside agricultural environments, due to the potato's limited competitive abilities. Potatoes are cultivated annually on a large scale in the Netherlands without leading to an uncontrolled spread.

B.19 What is the chance that the plant will disseminate in countries surrounding the Netherlands?

Negligible. Potato is not adapted for survival and reproduction outside the agricultural

ecosystem in countries around the Netherlands. Volunteer tubers and plants fail to survive outside agricultural environments, due to the potato's limited competitive abilities. Potatoes are cultivated annually on a large scale in countries surrounding the Netherlands without leading to an uncontrolled spread.

HYBRIDISATION OR OUTCROSSING

B.20 Describe the method of pollination of the original plant species

Dissemination of pollen - if present - is executed almost exclusively by insects. As anthers of potatoes require sonication by insects to release pollen, the range of pollinating insects is restricted. Main pollinators are bumblebees (McPartlan and Dale, 1994) which tend to buzz pollinate potato flowers, releasing pollen by rapid movement of their flight muscles (Treu and Emberlin 2000). This usually leads to self-pollination of the flower (Glendinning, 1976) or to deposition of pollen from one flower only across a limited number that are subsequently visited. Potato flowers do not produce any nectar (Sanford and Hanneman, 1981) and are therefore not attractive for honey bees.

Pollen dissemination is limited with a maximum distance of 5 to 10 m (Bock et al., 2002). Wind dissemination is considered marginal (OECD, 1997; Eastham and Sweet, 2002). Under field conditions selfing is most likely, with 80 - 100 % of seeds formed due to selfing.

B.21 Is there a chance that the plant in question will hybridize or outcross with either cultivars or wild relatives in the Netherlands? If so, describe all possible accidental hybridizations.

Solanum tuberosum is compatible with other cultivated genotypes of the same species in the Netherlands. *Solanum tuberosum* is not compatible with the wild related species *Solanum dulcamara* and *Solanum nigrum* in the Netherlands. No viable seeds or plants can be formed (Eijlander and Stiekema, 1994; Raybould and Gray, 1993; McPartlan and Dale, 1994; OECD, 1997). In a study descendants of non-transgenic potato plants were analyzed that served as pollen traps for the neighboring transgenic plants. 1.3 million plants from seven trial sites and six vegetation periods were studied (Erasmuson et al., 2005). It turned out that already in a distance of 2.25 meters hardly any transgenic offspring could be detected (0 to 0.5 per 10000 progeny analyzed). A study by AVEBE in the Netherlands yielded a comparable result. In a distance of five meters no out-crossing could be detected anymore (AVEBE 2004 in: Van de Wiel and Lotz, 2005).

B.22 Is there a chance that the plant in question will hybridize or outcross with either cultivars or wild relatives in countries surrounding the Netherlands? If so, describe all possible accidental hybridizations.

See B.21. No other wild relatives with which potato can hybridize are found in countries around the Netherlands.

B.23 Has accidental hybridization or outcrossing actually been observed in the Netherlands or in countries surrounding it? If so, describe the conditions under which it occurred.

Accidental hybridization with wild relatives has not been observed. Hybridization

between different cultivated potato varieties is possible when fertile cultivars are grown in fields located very closely to each other. However, this is of no importance for potato production as potato is a vegetatively propagated crop. Measures taken according to conventional agricultural practice prevent possibly emerging seedling plants to establish.

INTERACTIONS WITH OTHER ORGANISMS

B.24 Describe the known interactions between the plant in question and other organisms in the ecosystem where it is cultivated

Potato like every other plant is known to interact with other organisms in the environment including microorganisms, viruses, insects, birds, and mammals. Potato is susceptible to a range of pests and diseases.

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 European potato cultivation, as are nematodes (*Globodera* spp, *Ditylencus* spp, *Paraditylencus* spp, *Tricodorus* spp and *Paratricodorus* spp).

Just like for other plants, there are many microorganisms, viruses and viroids interacting with the potato plant. Well known pathogenic fungi are for example potato late blight (*Phytophthora infestans*), black scurf (*Rhizoctonia solanii*), potato wart disease (*Synchythrium endobioticum*), early blight (*Alternaria solani*), powdery scab (*Spongospora subterranea*), 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).

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.

There are many viruses that attack the potato plant. Economically most important are Potato leaf roll virus (PLRV), Potato virus Y (PVY), Potato virus A (PVA), Potato virus X (PVX), Potato virus S (PVS), Potato virus M (PVM), Tobacco rattle virus (TRV) and Potato mop-top virus (PMTV). Among viroids the Potato spindle tuber viroid (PSTVd) is the most important one.

Potatoes are a significant part of the diet in large parts of the world. The only part of the plant that is consumed is the tubers. The main toxic or anti-nutritional substances that occur in potatoes are glycoalkaloids, which are toxic to mammals in high concentrations, trypsin inhibitor and nitrates (OECD, 2002). Glycoalkaloids being particularly associated with green tissues are found in harmful amounts mainly in the above ground parts of the plant such as stems, leaves and fruits. In the tubers of cultivated table potato varieties the content is usually low, in a range between 100 mg to 200 mg per kilogram fresh weight. The highest content can be found in inflorescence and in sprouts, in tubers generally in skin and upper layers of flesh.

Nitrates are found in the entire plant and are considered anti-nutritional, especially for babies. Therefore plant breeders aim at maintaining very low contents in new potato cultivars.

Potatoes are also commonly used as feed throughout the world. Wild animals 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.

IDENTIFYING CHARACTERISTICS

B.25 Describe the identifying characteristics that distinguish the original plant species from its relatives

Solanum tuberosum subsp. *tuberosum* can be distinguished from other solanaceous species via morphologic criteria as well as via molecular tools such as Restriction Fragment Length Polymorphism (RFLP) or Amplified Fragment Length Polymorphism (AFLP).

The most distinguishable feature of *S. tuberosum* as compared to the wild relatives *S. dulcamara* and *S. nigrum* is the formation of tubers.

The general description of the morphology of *S. tuberosum* subsp. *tuberosum* is as follows: Herbaceous perennial with weak stems that grow to a maximum of three feet, long pinnate leaves, and ovate leaflets with smaller ones disposed along the midrib. The flowers are white, purple, pinkish, or bluish, in clusters, usually with a five-part corolla and exerted stamens with very short filaments. The fruits are yellowish or green, globose, and less than one inch in diameter. Some lack seeds, but others may contain several hundred. The fruits are inedible by humans due to the presence of toxins. Tubers are borne at the end of underground stolons. They are round to long oval. The flesh is generally white or cream to yellow, the skin color light brownish to red. Tubers can contain high levels of solanine, a toxic alkaloid (OECD, 1997). *S. dulcamara* is a perennial subshrub with violet flowers smaller that potato flowers. *S. nigrum* is an annual smaller than potato and has white flowers with a diameter of 1 cm.

C. GENERAL INFORMATION ABOUT THE GENETIC MODIFICATION

EARLIER OR OTHER MODIFICATIONS

C.1 Is the original plant species already genetic modified?

No, the recipient plants have not undergone any genetic modification prior to the one described in this notification.

GENERAL DATA ON THE GENETIC MODIFICATION

C.2 What type of genetic modification has been used?

Transformation with recombinant DNA to potato was performed using *Agrobacterium tumefaciens*, strain AGL0, AGL1 or LBA4404. 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 mobilizing functions are found on a modified Ti-plasmid (Hoekema et al., 1983). Transformation was carried out on cut leaf or stem tissue after which *A. tumefaciens* was killed with Claforan (Visser, 1991). Shoots were regenerated under imazamox selection (Andersson et al., 2003).

C.3 What is the intended result of the genetic modification?

The intended result of the modification is that the resistance genes Rpi-blb1 and Rpi-blb2 from *Solanum bulbocastanum* will be transferred into the potato thus conferring improved resistance to *Phytophthora infestans*.

C.4 Has the genetic modification been carried out in the Netherlands? If so, please give the relevant consent number.

No, the genetic modifications have been carried out in Sweden.

THE DNA THAT HAS BEEN USED FOR MODIFYING THE PLANT

C.5 Describe the composition of the whole DNA construct that has been used for the modification process. Specify the origin of and the function assigned to all the components of the construct.

The binary vector VCPMA16 is derived from plasmid pSUN (WO 02/00900) which is based on pPZP200 (Hajdukiewicz *et al.*, 1994) and can be propagated in *E. coli* as well as *A. tumefaciens*. The backbone contains a ColE fragment (o-ColE1) from pBR322 containing origin of replication in *E. coli* as well as a *bom* site for mobilization from *E. coli* to *A. tumefaciens*. A fragment derived from plasmid pVS1 contains broad host range replication functions (o-VS1-repA) including origin of replication and the *repA* gene, as well as a *sta* gene (c-sta) encoding stabilizing functions. Additionally, the backbone contains a gene coding for spectinomycin resistance (c-aadA) allowing for bacterial

selection but which is not intended to be transferred to the plant.

The T-DNA in VCPMA16 is delimited by pTiT37 right and left T-DNA border regions (b-RB and b-LB) originating from *A. tumefaciens*. T-DNAs in the plasmid VCPMA16 contain an acetohydroxyacid synthase gene for selection of transformed plant tissue and genes for improving resistance to *Phytophthora infestans*.

The acetohydroxyacid synthase gene (*ahas*, EC 2.2.1.6, 2013 base pairs (bp); mutation S653N) originates from *Arabidopsis thaliana* and has a point mutation corresponding to S653N in the expressed AHAS protein (Chang and Duggleby, 1998). The *ahas* gene is flanked by the nopaline synthase gene promoter (288 bp) and the *ocs* polyadenylation sequence (253 bp) from *Agrobacterium tumefaciens* and serves as selectable marker gene conferring tolerance to imazamox (Andersson *et al.*, 2003).

The genes for improving resistance to *P. infestans* are R-genes from *Solanum bulbocastanum*. Plasmid VCPMA16 contains a genomic fragment from *S. bulbocastanum* containing the *Rpi-blb2* gene with endogenous promoter and terminator regions. Both constructs also contain genomic fragments from *S. bulbocastanum* containing the *Rpi-blb1* gene with endogenous promoter and terminator regions.

R-genes can be divided into different classes, where the majority of genes belong to the nucleotide binding site (NBS)-leucine rich repeat (LRR) class (Young, 2000). The *Rpi-blb1* (van der Vossen *et al.*, 2003) and *Rpi-blb2* (van der Vossen *et al.*, 2005) both belong to the NBS-LRR class of R-genes.

There are no components of the vectors known to code for harmful substances.

Table 1. List of genetic elements in T-DNAs of VCPMA16.

Abbreviation	**Name and function**	***Size (bp)***	**Origin**
VCPMA16			
T-DNA		≈ 16700	
p-blb2	Promoter region of gene *Rpi-blb2* (including intron)	1530	*Solanum bulbocastanum*
c-blb2	Coding region of gene *Rpi-blb2* (including intron)	3890	*S. bulbocastanum*
t-blb2	Terminator region of gene *Rpi-blb2*	2530	*S. bulbocastanum*
p-blb1	Promoter region of gene *Rpi-blb1*	1173	*S. bulbocastanum*
c-blb1	Coding region of gene *Rpi-blb1* (including intron)	3592	*S. bulbocastanum*
t-blb1	Terminator region of gene *Rpi-blb1*	406	*S. bulbocastanum*
p-nos	Promoter of nopaline synthase gene	288	*Agrobacterium tumefaciens*
c-At*ahas*	Coding region of acetohydroxyacid synthase gene containing mutation S653N	2013	*Arabidopsis thaliana*
t-nos	Terminator of nopaline synthase gene	253	*A. tumefaciens*

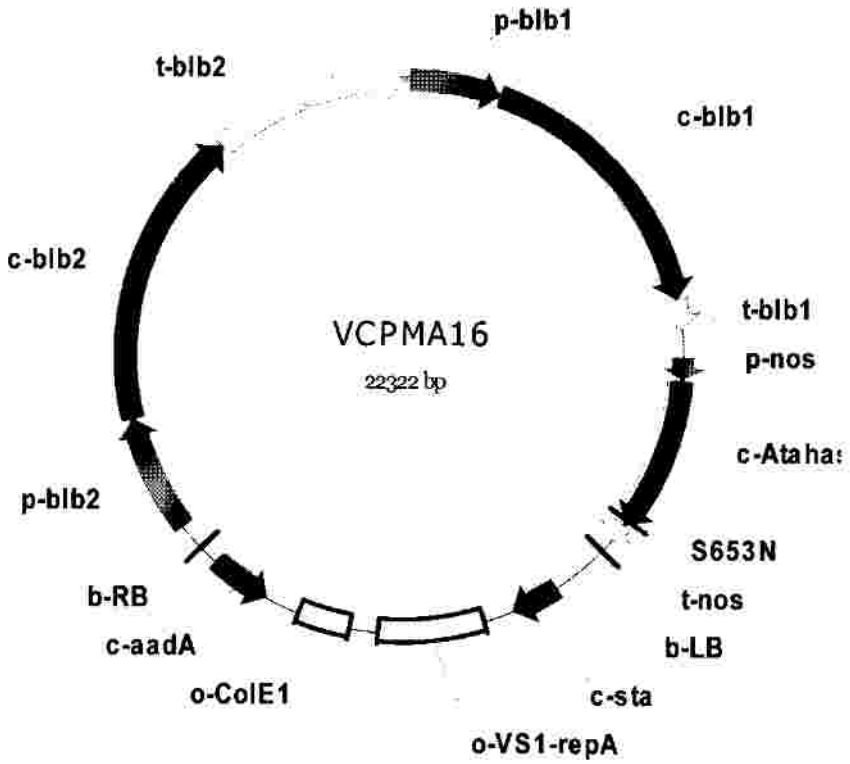

Figure 1. Map of plasmid VCPMA16

C.6 Does the construct code for one or more gene products that are functionally homologous to the gene products occurring naturally in the original plant species?

Potato already contains many other resistance genes of the NBS-LRR class (Wouters et al., 2004). Some NBS-LRR genes are introgressed from *Solanum demissum* and confer resistance to some races of *Phytophthora infestans* (Ballvora et al., 2002). The exact amount of NBS-LRR genes in potato is not known. For plants where the genomic DNA sequence has been analyzed in more detail NBS-LRR genes are among the most abundant (Arabidopsis has roughly 200, rice over 500 NBS-LRR genes).

Potato also expresses a functional homolog of the wild-type *Arabidopsis thaliana* AHAS protein.

C.7 Does the construct contain sequences that code for toxins or allergens?

No. For more details see D.19.

C.8 Does the construct contain sequences whose products are unknown?

No

D. INFORMATION ABOUT THE GENETICALLY MODIFIED PLANT (GMP)

HISTORY

D.1 Have there been activities carried out already with the genetically modified plant in question or with plants containing a similar genetic modification? If so, please describe these activities, together with their results.

Transgenic potato containing the *Rpi-blb2* gene were tested in a field trial in Sweden starting in 2005 (consent B/SE/05/450).

Field trials with transgenic potato lines containing both resistance genes (*Rpi-blb1* and *Rpi-blb2*) based on construct VCPMA16 have been conducted since 2006 in the Netherlands (B/NL/05/03 and B/NL/07/07), in Sweden (B/SE/05/8615), and in Germany (B/DE/05/174, B/DE/06/183, B/DE/07/191). Since 2007, the respective potato lines have also been planted in field trials in the Czech Republic (B/CZ/07/01) and in the UK (B/GB/06/R42/01).

For some of the consents (B/SE/05/450, B/SE/05/8615, B/NL/05/03, B/DE/05/174, B/CZ/07/01) the final report is available on the website from the Joint Research Center at the European Commission (http://gmoinfo.jrc.ec.europa.eu/gmp_browse.aspx).

Field trials with potatoes modified for starch composition carrying the ahas gene as selection marker gene have taken place at several locations in Sweden (since 2002), Germany (since 2004), the Netherlands (since 2004), the Czech Republic (since 2005) and in the UK (since 2007).

Except the better resistance against *Phytophthora infestans* there were no deviations of the GM potato lines in comparison to the non-GM plots.

During these trials no unforeseen effects as compared to conventional potato varieties have been observed. Regarding survivability of tubers and berries and dissemination into the environment no differences to the comparator lines have been observed. The agronomic characteristics observed during these trials are very similar to their comparator lines.

D.2 Has there been any hybridization between the primary GMP and other genetically modified plants? If so, please state whether such hybrids are involved in the present activities.

No. The lines have been propagated vegetatively by tuber production. The material that will be planted is seed potatoes (i.e. tubers used as starting material).

CHARACTERISTICS

D.3 Describe the new or modified characteristics of the genetically modified plant.

The inserted resistance genes, *Rpi-blb1 and Rpi-blb2*, confer improved resistance against *P. infestans*.

The introduced selectable marker gene *ahas* confers tolerance to imazamox during the selection of the transformed plant tissue.

INSERTION

D.4 Specify the sequences that have been introduced

The T-DNA as described in section C.5 (table 1)has been introduced.

D.5 Is the insert fully or partially present in the genetically modified plant? Please state how this was determined.

According to real-time polymerase chain reaction (PCR) analysis (Ingham et al., 2001) all genes of the insert (Rpi-blb1, Rpi-blb2 and ahas gene) are confirmed to be present in all transgenic lines intended for field trial.

D.6 How many copies of the insert are present in the plant?

According to real-time PCR analysis (Ingham *et al.*, 2001) with primers and probe directed against the *ahas* gene all transgenic lines intended for field trials contain 1 or 2 copies.

D.7 Is the insert located in the nucleus or located extra nuclear?

The transgenic plants are produced by Agrobacterium-mediated transformation thus resulting in nuclear localization of the insert (Zambryski et al., 1980).

D.8 Is the insertion stably present?

Agrobacterium-mediated transformation generally results in stable insertions. The transgenic lines were vegetatively propagated in at least two cycles via cuttings or tubers, respectively. The genetic stability of transgenic potato lines was analyzed by PCR-based detection of the T-DNA and the inserts have been found to be stably present.

D.9 Is the absence of the vector backbone in the genetically modified plant confirmed? If yes, supply the method and the results.

When analyzed by real-time PCR (Ingham *et al.*, 2001) no vector sequences outside the T-DNA have been detected in any of the transgenic lines intended for field trial.

In the vector VCPMA16 the backbone is derived from pPZP200. This backbone contains the aadA gene encoding resistance to spectinomycin. The spectinomycin resistance gene is homologous in sequence to accession numbers AAX97761 (protein) and AY995143 (nucleotide, first CDS).

Presence of backbone for lines transformed with VCPMA16 is analysed by real-time PCR with two different primer-probe sets within the backbone, one close to left border (LB) and one close to right border (RB). Figure 2 shows the location of the RB-aadA and LB primer-probe sets on the backbone of the base vector that was used for preparing construct VCPMA16. The primer and probe set at the right border is directed to a sequence within the aadA gene (spectinomycin resistance). A negative analysis result with this primer-probe set will thus show the absence of the aadA gene.

The analyses with the RB-aadA primer-probe set also included primers and probe for an endogenous control as reference. If the difference in Ct values between the RB-aadA sequence and the endogenous control (dCt) is higher than 4, the sample is judged as negative (a difference of more than 4 in the analysed sample is equivalent to less than 0.125 (0.53) copies of the target; such values likely arise due to very small levels of contamination during sample preparation or assay set-up or when disproportionately low Ct values have been obtained without any true amplification). DNA extracts from potato plants known to contain backbone sequences were used as positive controls to confirm the assay. All the lines presented in table 2 were shown not to contain the right border aadA-sequence.

Similarly, the presented VCPMA16 lines were shown to be negative also when analysed with the LB primer-probe set (data not shown).

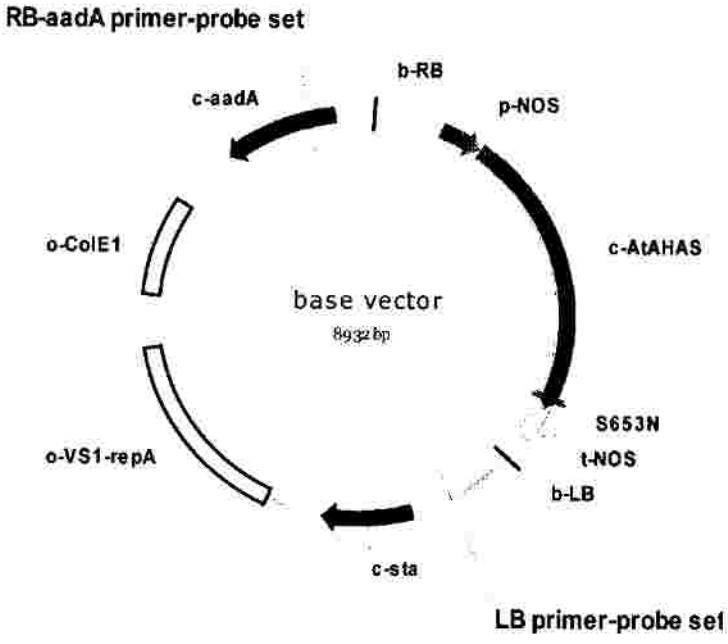

Figure 2. Location of primer-probe sets for detection of backbone sequences shown in

the base vector used.

Table 2. Data for lines transformed with construct VCPMA16

Line no	Parental variety	Backbone analysis			
		Ct RB-aadA	Ct end ctrl	dCt	Result
TS-PH05-017-0005	P880	35,17	27,09	8,08	negative
TS-PH05-026-0025	P880	40,00*	25,66	14,34	negative
TS-PH05-026-0048	P880	40,00*	30,02	9,98	negative
TS-PH05-028-0011	P880	40,00*	25,39	14,61	negative
TS-PH05-037-0118	P880	40,00*	29,55	10,45	negative
TS-PH05-037-0281	P880	40,00*	28,10	11,90	negative

The plasmid map (fig. 2) shows the location of the RB-aadA and LB primer-probe sets on the backbone of the base vector that was used for preparing construct VCPMA16.

The RB-aadA primer probe set is located about 300 base pairs from the RB and lies completely within the aadA gene. The amplicon size is 137 bp.

The LB primer-probe set is located about 400 base pairs from the LB and has an amplicon size of 103 bp.

The endogenous control primer-probe set is located on the potato genome within an endogenous gene of low copy number. The amplicon size is 94 bp.

In real-time PCR the amplification can be followed for each cycle. The maximum number of PCR-cycles used in our analysis is 40. In the analyses the fluorogenic 5′ nuclease (TaqMan) assay has been applied, where the probe emits fluorescence and the fluorescence detected by the instrument is proportional to the amplification product. This change in fluorescence is shown in a graph on the instrument (ABI Prism 7900HT). To transfer these results into figures a threshold line is set and the Ct determined as "the fractional cycle number at which the amount of amplified target reaches a fixed threshold" ("User Bulletin #2:ABI PRISM 7700 sequence Detection System", Applied Biosystems).

In the analysis of presence/absence of the aadA gene, all samples are analysed in duplex reactions that in addition to the RB-aadA primer-probe set also contain primers and probe for the endogenous control. The endogenous control is used to confirm that the quality and amount of DNA as well as reaction conditions are satisfactory. Comparing the Ct value of the RB-aadA primer-probe set to the Ct value of the endogenous control gives a dCt value (dCT = CT,X – CT,R, the difference in threshold cycles for target and reference). This value is used for the copy number calculation but also for the RB-aadA assay and can be considered to distinguish true insertions from signals arisen due to e.g. potential contaminations or similar reasons. All plates analysed contain both positive (potato samples known to contain backbone sequences) as well as negative controls. As negative controls non-transformed potato as well as NTC (non-template controls) are used.

EXPRESSION

D.10 In which tissues or in which developmental stages of the plant are the new or modified characteristics expressed?

The *Rpi-blb1* and *Rpi-blb2* genes are expressed by their respective native promoters. Other resistance genes of the NBS-LRR class have been shown to have very weak constitutive expression (Michelmore *et al.*, 2001). In the transgenic lines a low level of expression has been demonstrated by real-time PCR analysis in leaves. The *ahas* gene is controlled by the nopaline synthase promoter which is known to result in low expression levels in all parts of the plant. Expression of the *ahas* gene in the transgenic material has been demonstrated in tissue culture by survival on media containing imazamox.

D.11 What is the level of expression in these tissues and during these developmental stages? Please also specify the method used to determine this.

See D.10.

DIFFERENCES BETWEEN THE ORIGINAL PLANT SPECIES ON THE ONE HAND AND THE GMP (AND IF APPLICABLE ANY HYBRID OF IT) ON THE OTHER HAND

D.12 Way of reproduction or propagation and/or duration of reproduction or propagation?

No differences regarding mode or frequency of reproduction or propagation are expected. Neither resistance genes nor ahas gene are expected to affect tuber formation, seed setting, flower development or pollen formation. Comparative observations regarding flowering frequencies, fruit setting and tuber formation between the modified lines and the recipient variety or other comparator varieties have not revealed any differences.

Other potato plants expressing the AHAS protein have been studied in several field trials and no changes in the properties mentioned have been observed (see details in answer D1).

D.13 Parts of the plant that survive and/or the duration of survival?

Survival of potato tubers is depending on temperature. The introduction of additional R-genes or ahas gene is not expected to alter the frost sensitivity. Potatoes expressing the AHAS protein have been evaluated for frost tolerance in field, but no alterations compared to the parental varieties were observed (see details in answer D1).

D.14 Way of dissemination and/or duration of dissemination?

Field trials have shown that there are no differences between the recipient clone or other standard comparator lines and the modified lines in pollen production, fruit setting and tuber formation. Thus, neither resistance genes nor the ahas gene are expected to affect pollen or seed dispersal. There is thus no reason to assume that the modified potato clones differ from the parental varieties in this respect.

D.15 Pollination?

Neither R-genes nor the ahas gene have any connection to pollen formation or flower morphology and can thus no differences can be expected regarding pollination.

In previous field trials no differences in flower morphology between the recipient clone and the modified lines have been observed that would indicate a change in the ability to produce and release pollen.

D.16 Outcrossing?

Outcrossing to related European wild species is not possible for potato and there is no reason to assume that the inserted R-genes or the ahas gene could influence this property. The possibility of hybridization with other potato varieties is expected to be the same as for the respective parental varieties.

D.17 Biological containment?

No changes expected. Neither the traits, nor the field performance recorded in previous releases suggests any change.

D.18 Competitive characteristics?

No competitive advantage of modified lines compared to the recipient clone is expected under field conditions. Increased survival ability in non-fungicide treated fields infected with *P. infestans* can be foreseen as an intended effect of the trait. This is an advantage applicable in the agricultural environment and only in those cases where no other plant protection measures against *P. infestans* are applied. Potato plants are never seen established outside the agricultural environment and resistance to *P. infestans* is not a key determinant for potential invasiveness of potatoes.

The *ahas* gene confers tolerance to the herbicide imazamox during the selection process for transformed plants, and thus is used as selectable marker (Andersson *et al.*, 2003). No field tolerance to imazamox is intended. The herbicide is not used on potatoes in general and is not registered for use in the Netherlands at all. No other effects on the survival ability, e.g. altered frost sensitivity, are expected due to the introduced genes.

D.19 Toxic or allergenic effects?

No change in toxic or allergenic effects is anticipated.

Potato already contains other resistance genes of the NBS-LRR class with similar protein structures, including R-genes derived from the wild potato species *Solanum demissum* (Wastie, 1991). No NBS-LRR gene has been identified to confer toxic or allergenic properties. Other R-genes of the NBS-LRR class have been shown to have very weak constitutive expression (Michelmore *et al.*, 2001). In the transgenic lines a low level of expression has been demonstrated by real-time PCR analysis in leaves.

AHAS (acetohydroxyacid synthase, EC 2.2.1.6) is found in bacteria, other microorganisms, and plants. It catalyses the first step in the biosynthesis of the essential branched chain amino acids isoleucine and valine. A single amino acid substitution in the AHAS enzyme alters the binding site for imidazolinone herbicides and thus confers a tolerant phenotype. Induced as well as acquired mutations are known to confer

tolerance to a particular group of herbicides in crop plants (Chang and Duggleby, 1998). The safety of plants tolerant to imidazolinone has been assessed by the Canadian Food Inspection Agency for imidazolinone tolerant maize, rice, canola and wheat. Imidazolinone herbicide tolerant maize or CLEARFIELD maize has been cultivated in the US since 1992, CLEARFIELD canola since 1996 and CLEARFIELD wheat since 2001.

All potato contains varying amounts of glycoalkaloids, which in large doses can be toxic to humans and animals. The introduced R-genes as well as *ahas* gene are not connected to the glycoalkaloid biosynthesis pathways. The modified potato clones can thus not be assumed to differ from the parental lines in this respect.

D.20 Other harmful effects?

No changes regarding agronomic characteristics have been introduced; the genetically modified potato lines exhibit the same properties towards the environment during cultivation as its conventional starch potato comparators. No effects are foreseen.

D.21 Symbiotic characteristics?

There are no known specific symbionts interacting with potato and thus no changes are expected. No changes in mycorrhizal interactions are foreseen as the used R-genes function only in living plant cells, and can only affect potato-penetrating organisms.

D.22 Resistances and tolerances?

An improved resistance to *Phytophthora infestans* is foreseen as an intended effect of the trait. A reduction in the ability of *Phytophthora infestans* to infect the genetically modified potatoes is expected due to the inserted resistance genes. The resistance genes encode receptors that will recognize specific elicitors injected by the pathogen. This recognition will through a signaling network trigger both local and systemic defense responses. The local response aims at trapping the pathogen in the cells first penetrated by localized cell death thus stopping further penetration and spreading. The systemic response induces expression of defense related genes also in remote parts of the plant (Heil and Bostock, 2002).

The *ahas* gene confers tolerance to the herbicide imazamox during the selection process for transformed plants, and thus is used as an alternative selectable marker (Andersson *et al.*, 2003). No field tolerance to imazamox is intended. The herbicide is not used on potatoes in general and is not registered for use in the Netherlands at all. Therefore no competitive advantage is expected under field conditions. See also D.18.

D.23 Interactions with target organisms?

Not applicable. There is no target organism in the usual sense. The mode of action of the introduced *blb*-genes is a hypersensitive response upon infection by the fungus leading to plant cell necrosis. *Phytophthora infestans* is not a direct target of the introduced genes or of their gene products. The resistance mechanism already existing in the plant is being complemented toward the pathogen *P. infestans*.

D.24 Interactions with non-target organisms?

Resistance genes of the NBS-LRR class are usually very specific, limited to species or race, for initiation of a resistance response (Hammond-Kosack and Parker, 2003). Thus, no effects on other organisms than *P. infestans* are expected.

D.25 Interactions with the abiotic environment?

None of the introduced genes are related to frost or drought tolerance, salt tolerance or changes in heavy metal accumulation, thus no changes in the interaction with the abiotic environment are foreseen.

D.26 Does the genetically modified plant differ from the original plant species in other respects than those mentioned above?

No

D.27 Describe the techniques that can be used to distinguish the genetically modified plant from the original plant species

Assays based on real-time PCR has been developed for the Rpi-blb1, the Rpi-blb2 as well as the ahas gene and can all be used to distinguish the transgenic lines from their parental varieties.

E. DATA ON THE INTRODUCTION OF THE PLANT INTO THE ENVIRONMENT

LOCATIONS

E.1 How many locations are mentioned in the notification?

One location is mentioned in the notification.

E.2 In what municipalities are the experimental fields located?

The experimental fields will be located in the community of Steenbergen (province Noord-Brabant).

CATEGORY 1 FIELD TRIAL WITHOUT ISOLATION DISTANCE

E.3.b. Maps of locations

The maps showing the land parcel are given in appendix 3.

E.4 How many locations will be effectively used for the environmental introduction in any given year?

One location will be used for the release every year from 2012 to 2018.

E.5 Have the locations mentioned in E.1 above already been used earlier for the introduction of GMPs into the environment?

No, to our knowledge the location has not been used for the introduction of GMPs into the environment.

If yes, please specify the GMPs involved

Not applicable

Are these locations still subject to monitoring obligations?

Not applicable

If yes, please specify the corresponding consent number

Not applicable

E.6 Area of each location

The maximum area for the release per site and year will be 1 ha.

E.7 Total area of all locations used in any given year

The total release area will be up to 1 ha every year from 2012 to 2018.

ECOSYSTEM

E.8 Is the type of ecosystem where the GMP is to be introduced different from the one where the original plant species is normally cultivated?

No.

If so, in what respect?

E.9 What is the distance from officially recognized biotopes and officially protected areas that the GMPs might affect by outcrossing with wild relatives or by dissemination?

The closest officially recognized biotope is the nature preservation area 1.5 km north of the trial location, called "Dinteloordse Gorzen".

The chance that the GMPs might affect the officially protected area mentioned above is negligible.

There are no sexually compatible wild relatives present in the Netherlands. A fertile crossing of potato with the wild related species *Solanum dulcamara* and *Solanum nigrum* occurring in Europe is not possible (McPartlan and Dale, 1994). Hybridizations can occur only with cultivated potato at a maximum distance of a few meters. The chance of the GMP is establishing in uncultivated landscape is negligible, as detailed in the environmental risk assessment.

DESIGN OF THE TRIALS AND THE INTRODUCTION OF THE GMP

E.10 Describe the experimental design of the trials

Big plots, strip plots or randomized block design.

Will there be an isolation distance employed? If yes, specify the distance.

A distance to other commercially grown potatoes of 10 m will be kept on a voluntarily basis.

E.11 Describe the treatment of the field prior to the introduction of the GMP

Preparation and management of the release site will be according to conventional agricultural practice. This includes but is not limited to fertilization, use of fungicides or insecticides. Weeds will be removed manually or chemically.

E.12 What is the number of GMPs to be introduced at each location and in total in each year?

Each year a maximum of 70.000 potato tubers per line will be introduced at the location. In total a maximum of 70.000 tubers per year will be planted at the location.

E.13 What method or methods are used for the introduction of the GMP?

Seed potatoes will be planted directly into the field.

E.14 Describe the methods used to keep out unauthorized persons from the area

At the release site a notice board will be posted to prohibit the entrance of unauthorized persons.

E.15 Describe the methods used to prevent other organisms from entering the area

No specific measures will be taken.

E.16 If you have stated in A.6 that the GMPs are to be used for human and animal consumption trials, please specify here the design and nature of these tests

Not applicable.

TRANSPORT

E.17 Describe the mode of transport and packaging used for the GMPs and their parts

Seed potatoes will be transported from the storage location to the release site and harvested seed potatoes will either be stored at the farm site or be transported from the release site to other storage sites (see E.19). Plant and tubers samples might be taken from the release site for analysis and disposal outside the Netherlands. Seed potatoes and plant material for analysis and disposal will be packaged in labeled and closed containers. The transport to and from the release site will be carried out in appropriate vehicles separately from non-GM potatoes. Storage of the GM potatoes will be clearly separated from storage of non-GM potatoes.

AFTER THE TRIALS

E.18 Describe the treatment of the introduction area after ending the trials, for example the removal of volunteers. Indicate for each treatment how this will take place.

The year following the field trial emerging volunteers will be destroyed by mechanical or herbicide treatment.

E.19 Describe the treatment of the GMPs itself, parts from the GMPs or of derived materials from the GMPs after ending the trials

Harvest will be done manually or mechanically. Harvested tubers will be transported from the release site for storage, analysis or disposal. Tubers might be stored within a closed storage facility at the farm. The destination of transport or the storage of harvested material within the Netherlands will be described in the Field Compliance Notebook. Tubers identified on the release area after harvest, will be collected and inactivated (e.g. via heat). Above ground green parts will be inactivated prior to harvest either chemically or mechanically. Green plant parts will remain at the release site for decomposition.

E.20 Describe the type and amount of waste material produced

The waste material produced is derived from the remains after harvest and sorting. Green parts will remain on the field for decomposition. Waste tubers will be disposed by e.g. steaming.

E.21 Describe the processing and disposal of the waste material formed

Excess tubers will be inactivated (e.g. via heat). Green plant parts will remain at the release site for decomposition.

F. EXPECTED EFFECTS OF THE GMP ON MAN AND THE ENVIRONMENT

F.1 Indicate the potential direct, indirect, immediate and delayed adverse effects of the GMP in relation to human health and the environment

The intended effect of the genetic modification is to improve the resistance to *Phytophthora infestans*. Thus under Phytophhtora pressure resistant potatoes are intended to have a selective advantage in comparison to untreated less-resistant conventional potatoes in the agricultural environment. This is an advantage applicable in the agricultural environment only in those cases where no other plant protection measures against *Phytophthora infestans* are applied. Conventional agricultural practices as well as volunteer management will assure effective control of volunteers emerging on the field and the immediate surroundings. Potato plants are never seen established outside the agricultural environment and resistance to *P. infestans* is not a key determinant for potential invasiveness of potatoes.

The population of *Phytophthora infestans* is assumed to be reduced in the cultivations of the Phytophthora-resistant potato lines. This is acceptable and desired, since also under conventional agricultural practice, via fungicide-treatment of potato fields, the oomycete *P. infestans* is being controlled. The overall impact of fungal tolerant potatoes on target organisms is therefore considered comparable to the impact of fungicide applications on non-genetically modified potatoes conducted according to conventional agricultural practice.

The genetically modified potatoes differ from conventional potato varieties in their tolerance to *Phytophthora infestans* conferred by the introduced resistance genes (R-genes). Potato already contains a large number of resistance genes conferring tolerance against other plant diseases where the majority of those genes belong to the NBS-LRR class. None of these genes are known to exert any toxic or allergenic effects to human health. The R-genes introduced into the genetically modified potatoes are of the NBS-LRR class and thereby are very specific, limited to species or race, in their recognition of target organisms. The mode of action of the R-genes is a hypersensitive response that leads to plant cell necrosis upon infection by the fungus. The introduced R-genes are expressed by their endogenous promoters at extremely low levels that are comparable to those from other endogenous resistance genes. Due to the specificity of the response reaction no effects on other organisms than *P. infestans* are expected other than those that also apply to the interaction with non-genetically modified potatoes under conventional agricultural practice. Due to a reduced need for fungal treatments an increase in the populations of those non-target organisms that respond to the conventional fungal treatments might be expected. Observations on disease and pest susceptibility as an indicator for effects on non-target organisms are included in the monitoring program conducted during the release. The overall impact on non-target organisms is considered negligible.

The introduced selection marker gene is expressed as the enzyme AHAS, which is an enzyme found in all plant species and not known to confer any toxic or allergenic properties. The safety of plants with AHAS-mediated tolerance to imidazolinone has been assessed by Health Canada and the Canadian Food Inspection Agency for imidazolinone tolerant maize, rice, canola, sunflower, lentils and wheat. Imidazolinone herbicide tolerant maize or CLEARFIELD maize has been cultivated in the US since 1992, CLEARFIELD canola since 1996 and CLEARFIELD wheat since 2001. The AHAS enzyme to the level being produced in the genetically modified potato lines confers improved tolerance to herbicides of the imidazolinone group in tissue culture. However, under greenhouse conditions genetically modified potato plants carrying the ahas gene

do not exhibit any tolerance against imidazolinones and thus no tolerance to a commercial spray regime is expected in the field either. Further there would be no selective advantage as imidazolinone herbicides are not approved for use on potato in the Netherlands.

Neither R-genes nor the ahas gene confer characteristics to the genetically modified potato lines that add competitive abilities in unmanaged ecosystems or allow competing against plants of similar type for space. None of the characteristics – tolerance to the fungus *Phytophthora infestans* and tolerance to herbicides of the imidazolinone group in tissue culture - transferred to the potato plants are anticipated to affect pollen production and fertility, seed dispersal or frost tolerance. Seeds and tubers, which might be spread outside cultivated fields, would have no competitive ability in this environment. Potatoes are not persistent outside the agricultural environment and feral potato plants are not occurring in climate regions of the Netherlands. The introduced R-genes and the ahas gene are thus not anticipated to confer any difference compared to conventional potato varieties with regard to persistence in agriculturally utilized habitats or invasiveness in natural habitats. Further the following risk management measures will be applied: conventional agricultural practice including crop rotation, implementation of isolation distances as well as volunteer management will assure effective control of volunteers emerging on the field and the immediate surroundings.

All potato varieties contain varying amounts of glycoalkaloids, which can be toxic to humans and animals in large doses. The genetic modification cannot be assumed to influence the content of these compounds to any considerable extent. Performed analyses have not shown any influence on the level of glycoalkaloids. Thus, the genetically modified potato lines with an improved resistance to *Phytophthora infestans* can be assumed to be equivalent to the parental variety and other cultivated potatoes from this aspect.

Potato propagates as tubers and occasionally with seed. As tubers are generally frost sensitive, their ability to survive is dependent on temperature. During mild winters tubers can survive in the soil.

Seeds from potato berries survive the winter irrespective of temperature. The survival of seed plants is dependent on agricultural praxis in succeeding crops. As a rule, they are eliminated by soil preparation, the use of herbicides, and by succeeding crops. This is also the case for plants arising from any overwintering tubers. Seeds and tubers that may spread outside cultivated fields have no competitive ability in that plant community. Surviving, reproductive wild potato plants have never been described.

Genetic material from transgenic potato lines, as well as from other potatoes, may spread by pollen. Transfer of genetic material via pollen to wild relatives at or near the release site is very unlikely due to the absence of sexually compatible species. Therefore outcrossing to those species can be excluded. Transfer of genetic material via pollen to conventional potato varieties is possible; however the proposed risk management measures (e.g. isolation distance, volunteer management) will prevent any unintended pollination. In the unlikely case that pollen is transferred to non-genetically modified potatoes, the consequences are negligible. No selective advantage or disadvantage is being transferred to those potatoes. There is no risk of introduction of the GM traits into conventional potato material as in Europe potato is propagated vegetatively by tubers.

F.2 What is the likelihood that these adverse effects mentioned in F.1 will actually take place?

The likelihood of an occurrence of adverse effects as an outcome of the planned field trials is extremely low.

F.3 Estimate the risk of each adverse effect, taking into account the impact of any risk management measures taken

1. *Increased invasiveness – risk negligible.*

   Surviving, reproductive potato plants are rarely seen outside the field. Establishment of volunteer potatoes is routinely controlled in subsequent crops. The year following the trial, the area will not be planted with potatoes in order to enable managing and tracing of volunteers. Should any be observed, they will be removed at early stages so that they cannot propagate further.

2. *Selective advantage – risk negligible.*

   No advantage through increased resistance to *Phytophthora infestans*. Potato plants are rarely seen outside the field. Resistance to *P. infestans* is not the key determinant for potential invasiveness of potatoes.

   No selective advantage through tolerance to imidazolinone herbicides since no tolerance to a commercial spray regime is conferred to the potato plants.

3. *Out-crossing – risk negligible.*

   In the unlikely case that pollen is transferred to conventional potatoes, the consequences are negligible since potato is a vegetatively propagated crop. For a successful hybridization, sexually compatible plants that flower simultaneously need to grow in close vicinity. If pollination should have been successful, the botanical seeds still need to ripen and survive winter conditions. In case seeds would germinate the resulting plants will be managed like volunteer potatoes. Although extremely unlikely, hybridization will be further prevented by keeping an isolation zone of 10 meters.

4. *Environmental impact on target organisms – not applicable.*

   There is no target organism in the conventional sense. The intended effect is a reduced population of *Phytophthora infestans* in the potato field. This is acceptable and desired also under conventional agricultural practice where fungicide-treatment of potato fields is applied to control *P. infestans*.

5. *Environmental effect on non-target organisms – risk negligible.*

   No change in interaction between due to the introduced traits can be foreseen.

   Any effect on non-target organism due to the introduced trait of *P. infestans* tolerance is anticipated to be comparable to that of non-genetically modified potatoes under conventional agricultural practice. Due to a reduced need for fungal treatments an increase in the populations of non-target organisms might be expected.

6. *Effects on human or animal health – risk negligible.*

   No toxic or allergenic effects are expected due to the introduced traits are expected. No new components are synthesized in the plant due to the genetic modification. Material from field trial not intended for human/animal consumption.

7. *Effects on biogeochemical processes – risk negligible.*

   Any effect is expected to be comparable to that of conventional potato cultivars under conventional agricultural practice. Due to a reduced need for fungal treatments an increase in the populations of soil organisms might be expected.

8. *Environmental impact due to changes in cultivation practice – risk negligible.*

   Cultivation will be under conventional agricultural practice. Potential positive effects on the population of soil organisms could be expected.

F.4 If a risk management measure is proposed in F.3, please describe this measure

Occurrence of volunteer potatoes can be limited by careful harvesting and handling. As it is impossible to remove every tuber, the area will not be planted with potatoes the year following the trial in order to enable managing and tracing of volunteers. Establishment of volunteer potatoes is routinely controlled in subsequent crops. Should any be observed, they will be removed at early stages so that they cannot propagate further.

Hybridizations are prevented by keeping an isolation distance of 10 m to the next conventional potato field and by eliminating volunteer potato plants from the isolation zone.

The trial site will be signposted in order to prevent accidental, unintended consumption or removal of tubers.

F.5 Indicate which location-specific aspects are taken into account in the risk analysis. If there are no location-specific aspects, provide a risk analysis for the complete Dutch territory.

No location-specific aspects are taken into account since the location does not exhibit any unique characteristics that are different from any other agriculturally used land in the Netherland.

The risk analysis provided is valid for the complete Dutch territory. No risk was identified in the risk assessment.

F.6 Estimate the overall risk for the intended activities with the GMP for human health and the environment

The overall conclusion of the information given above is that neither cultivation nor transports, analyses or other handling of the transgenic material can be assumed to confer any relevant risks to humans, animals or the environment.

G. PROPOSED MEASURES FOR CONTAINMENT AND RISK MANAGEMENT

G.1 What measures will be taken to prevent the dissemination of the GMPs?

An isolation distance of 10 m between the GM potato lines and conventional commercial potato cultivation will be observed throughout the testing season. The release site will be monitored for volunteers in the year following the field trial and any emerging volunteer potato plants will be destroyed. After harvest only those crops which allow monitoring for volunteers will be cultivated on the release site.

G.2 Describe all other measures taken to prevent effects of the GMPs on human health and the environment.

During transport and handling the potatoes will be clearly labeled, separated from conventional potatoes and packaged in closed containment. Any equipment or machinery used for planting and harvesting will be cleaned on site. Any excess potato material (tubers after planting, after harvest) will be inactivated (e.g. via heat treatment).

H. PROPOSED METHODS OF OBSERVATION DURING AND AFTER THE TRIALS

H.1 Draw up a monitoring plan, specifying how any effect of the GMPs on human health and the environment will be detected during and after the trials. Give a description of the methods used for this detection or observation.

The monitoring plan was designed taking into account the environmental risk assessment. The monitoring plan aims at early observation and identification of adverse effects on human health and the environment that were not anticipated in the environmental risk assessment. All observations will include the trial area, the isolation zone (10 meters) and the immediate field margin.

Case-specific monitoring is not considered necessary because the environmental risk assessment did not identify risk that would require specific attention. Changes in tolerance to *P. infestans* are an intended effect and will be observed independently from the proposed monitoring plan.

Assumptions of risk assessment - General surveillance	**Observations to be made / measures to be taken by the notifier**
No differences regarding general plant characteristics (e.g. size, shape, flowering, development)	Observations of general plant characteristics and agronomic performance
No differences in disease and pest susceptibility	Observation of changes in susceptibility to insects and pests
No difference in competitive behaviour; no selective advantage/disadvantage due to the introduced genes	Monitoring for volunteers
Limitations of the potato to the release site	Restricted access and monitoring of implementation of risk management measures (protocol book)

Baselines

The performance of the genetically modified potato lines will be compared to the performance of the recipient and other standard varieties grown in parallel at the same release site.

Time period

During the course of the entire vegetation period (from about April to October) of the potato lines the area of release will be visited by the ESO and trained personnel to observe the release at defined intervals (at least once every two weeks). The ESO or trained personnel will observe the area post-release at defined intervals for the duration of volunteer monitoring program.

Responsibilities

The notifier is responsible for the monitoring plan. Case-specific and general surveillance will be carried out by BASF Plant Science and contracted individuals including ESO and trained personnel.

Area

It is the site of release and the individual release plots that will be monitored.

Inspections

The area of release will be visited by the ESO and trained personnel. Inspections may also performed by the responsible authority.

Data collection and evaluation

BASF Plant Science will be responsible for all records of observations and analyses performed in accordance with the monitoring plan. Data will be collected and analyzed according to specifications by BASF Plant Science in accordance with international guidelines (e.g. UPOV for general plant characteristics). Field notebooks are kept during the period of release.

Reporting

Information regarding any unexpected occurrences of relevance regarding potential adverse effects on the environment and human health directly related to the genetically modified potato lines will be communicated to the appropriate Authority and required measures will be implemented accordingly. A report summarizing the observations during the field trial will be submitted annually.

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Appendix 1

POINTS TO CONSIDER IN THE CONCLUSION ABOUT THE POSSIBLE ENVIRONMENTAL EFFECTS OF THE GMP INTRODUCTION

1. Likelihood of the GMP becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats.

The characteristics which have been inserted into the genetically modified potato plants are not anticipated to cause them to be more persistent or invasive than conventional potato cultivars. It is not evident which selection advantage the genetically modified potatoes should possess, that could influence the low competition strength and thereby increase persistence or invasiveness compared with conventional potato cultivars. It is not expected that the introduced sequences will affect tuber formation, seed setting, flower development or polling formation. Neither resistance genes nor the ahas gene confer characteristics to the GM potato that add competitive abilities in unmanaged ecosystems or allow competing against plants of similar type for space.

During cultivation of the genetically modified potato lines in the greenhouse and in earlier field tests no changes of the respective characteristics were observed. No particular structures are formed that would enhance invasiveness, no particular tolerances e.g. to abiotic stress has been noticed that would increase persistence. All greenhouse and field observations so far confirm that the behavior of the modified lines is identical to that of the recipient clones. The resistance against Phytophthora is no characteristic which is crucial for the competition strength.

The genetically modified potato lines with increased resistance against *Phytophthora infestans* differ from conventional potato varieties by introduction of the respective resistance genes originating from the wild potato *Solanum bulbocastanum*. In natural habitats, potatoes do not have a chance to establish even without infection pressure by Phytophthora. Resistance against Phytophthora has to be regarded as selection relevant only in agriculture with existing infection pressure.

In addition, potato lines express the ahas gene which confers tolerance against imidazolinone herbicides during selection of transgenic shoots in cell culture. Genetically modified potato plants, which express the ahas gene, were examined in different field trials in Germany, in the Netherlands, in Sweden, and in the Czech Republic. In all cases no differences were observed concerning the examined characteristics compared to the recipient clones.

2. Any selective advantage or disadvantage conferred to the GMP.

If an infestation with *Phytophthora infestans* occurs in the field, genetically modified potatoes with increased resistance against Phytophthora will probably have a selective advantage compared to untreated conventional varieties, provided that no adequate fungicides against Phytophthora will be applied.

The tolerance against imazamox in tissue culture also does not result in any selective advantage or disadvantage under field conditions.

3. Potential for gene transfer to the same or other sexually compatible plant species under conditions of planting the GMP and any selective advantage or disadvantage conferred to those plant species.

A transfer of genetic material could in principle occur via pollen. Potatoes might to a limited extend hybridize with plants of the same species, provided that the plants are in immediate vicinity and that flowering of both plants coincides. By the measures taken during the release, e.g. a 10 m distance to or absence of conventional commercial potatoes, the possibility of a gene transfer can be almost excluded. Since no selective advantage or disadvantage is expected for the genetically modified potato lines, this would apply also to any descendants in the improbable case out-crossing would occur.

A fertile crossing of potato with the wild related species *Solanum dulcamara* and *Solanum nigrum* occurring in Europe is not possible (McPartlan and Dale, 1994). No viable seeds or plants can be formed (OECD, 1997).

4. Potential immediate and/or delayed environmental impacts resulting from direct and indirect interactions between the GMP and target organisms, such as predators, parasitoids, and pathogens (if applicable).

There is no target organism in the conventional sense. The mode of action of the introduced resistance genes is a hypersensitive response of the plant upon infection by *Phytophthora infestans* leading to plant cell necrosis. *Phytophthora infestans* is not a direct target of the introduced genes or of their gene products.

5. Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMP with non-target organisms, (also taking into account organisms which interact with target organisms), including impact on population levels of competitors, herbivores, symbionts (where applicable), parasites and pathogens.

There are no particular interactions foreseen, neither direct or indirect nor immediate or delayed. The introduced traits do not have any obvious effect on plant-visiting organisms, therefore it is expected that the interaction between genetically modified potato lines and non-target organisms will be comparable with that of any other potato.

It is expected that introduction of the resistance genes will reduce the ability of *Phytophthora infestans* to infect the genetically modified potatoes which will result in a decrease of the Phytophthora population on the field trial site. This effect is both intended and desired and is comparable with the effects of the conventional plant protection measures, which are normally used in order to control *Phytophthora infestans*.

NBS-LRR gene products are located in the cytoplasm of cells. In order to trigger the plant's defense response an organism needs to introduce elicitor proteins into the cytoplasm. This can only be done by pathogens of potato, not by loosely associated microorganisms. There is no evidence suggesting soil-dwelling or mycorrhizal fungi to transfer proteins to the cytoplasm of plants they are interacting with. NBS-LRR genes are highly specific in their response to pathogens. In general they respond only to specific races of one specific pathogen carrying a specific elicitor gene.

6. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMP and persons working with, coming into contact with or in the vicinity of the GMP release(s).

None anticipated. The introduced traits do not raise particular safety concerns during the handling of the crop and its produce. No particular safety indications need to be provided. The potato plants are not for human consumption and measures taken with regard to planting, harvest, storage and transportation will minimize any contact. Staff will be instructed not to take away and/or consume any of the experimental material. Accidental, unintended consumption is highly unlikely. The trial site is signposted.

The risk alkaloids pose to humans is well known to Dutch consumers as they hold for all cultivars grown here for the last hundreds of years. The planned genetic changes will not alter this well-known potential toxicological interaction with mammals.

The transgenic potato lines comprise the resistance genes Rpi-blb1 and Rpi-blb2 originating from the wild potato *Solanum bulbocastanum*. The potato genome already contains resistance genes of the NBS LRR class. Most of the today known plant resistance genes belong to the NBS-LRR gene family and the respective proteins are characterized by a similar protein structure. Additionally, also conventional potato cultivars contain resistance genes, which are derived from the wild potato *Solanum demissum* (Wastie, 1991). No member of the NBS-LRR protein class is a known toxic or allergen.

The gene product of the introduced selection marker is the enzyme AHAS, which occurs also in bacteria, other microorganisms, and plants. A single amino acid exchange in the AHAS enzyme modifies the binding site for imidazolinone herbicides thereby generating the tolerant phenotype. Different studies showed that imidazolinone tolerant AHAS enzymes exhibit the same catalytic characteristics as the native AHAS enzymes – except for the tolerance against imidazolinone herbicides. The AHAS protein does not exhibit toxic or allergenic characteristics. The safety of plants which are resistant to imidazolinones was evaluated by Canada Health and the Canadian Food Inspection Agency for corn, rice, rape, sunflower, lenses and wheat. Corn with resistance to imidazolinone herbicides (CLEARFIELD corn) is cultivated in the USA since 1992, CLEARFIELD oilseed rape since 1996 and CLEARFIELD wheat since 2001.

7. Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any products derived from it, if it is intended to be used as animal feed.

The introduced traits do not raise particular safety concerns for animal health. The genetically modified potatoes are not intended to be used as animal feed except for potential small-scale animal feeding trials under contained use conditions.

8. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).

With the exception of an increased resistance to *Phytophthora infestans* no other changes are expected. All results confirm that the plants perform in all respects comparable to the recipient material. No effects on biogeochemical processes are anticipated other than such which apply also to conventional potato cultivars.

9. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMP where these are different from those used for non-GMPs.

The genetically modified potato plants will be planted, cultivated, managed and harvested like any other starch potato under conventional agricultural practice. The methods are well known and established. As such no change in effect is expected.

The ahas gene confers tolerance to the herbicide imazamox during the selection process for transformed plants, and no field tolerance to imidazolinones is intended. The trait is not expected to induce any effect in the field.

The inoculation with *Phytophthora infestans*, if necessary, will be carried out according to conventional methodology.