Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC_2011_0116

Title: Advice of the Belgian Biosafety Advisory Council on the notification **B/BE/10/V1** of the University of Ghent (UGent) for deliberate release in the environment of genetically modified potatoes resistant to *Phytophthora infestans*.

Context

The notification B/BE/10/V1 has been submitted by the UGent to the Belgian Competent Authority (CA) in November 2010 for a request of deliberate release in the environment of genetically modified higher plants for research and development according to Chapter II of the Royal Decree of 21 February 2005.

The title of the notification is: **Tweejarige veldproef met genetisch gemodificeerde aardappelen met een verminderde vatbaarheid voor *Phytophthora infestans***. The specific purpose of the trials is to evaluate if the GM potato lines that contain the *Rpi* genes, show a decreased susceptibility for *Phytophthora infestans* in comparison to the non-modified parental lines and non-resistant potato lines.

The notification has been officially acknowledged by the CA on 3 November 2010 and forwarded to the Biosafety Advisory Council for advice.
Within the framework of the evaluation procedure, the Biosafety Advisory Council, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier. Five experts from the common list of experts drawn up by the Biosafety Advisory Council and the Biosafety and Biotechnology Unit (SBB) answered positively to this request. The SBB also took part in the evaluation of the dossier.
The experts and the SBB assessed whether the information provided in the notification was sufficient in order to state that the deliberate release of the genetically modified (GM) potato lines would not raise any problems for the environment, animal or human health.

On 17 December 2011, based on a list of questions prepared by the Biosafety Advisory Council, the CA requested the notifier to provide additional information. A new version of the dossier and answers to the questions were received on 4 January 2011 by the Biosafety Advisory Council. On 24 January 2011 the Biosafety Advisory Council did not forward an

advice. Some members of the Council raised additional questions. The CA forwarded the questions to the notifier. Answers were received on 8 February 2011.

For the purpose of the scientific evaluation, the following legislation has been considered:
- Annex II (principles for the risk assessment) and annex III (information required in notifications) of the Royal Decree of 21 February 2005
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.

In parallel to the scientific evaluation, the CA made the dossier available on its website for a one-month public consultation as required in the abovementioned Royal Decree. The CA forwarded the list of questions to the Biosafety Advisory Council. The questions of the public tackling biosafety issues of the GMOs under consideration are taken in consideration in the opinion of the Biosafety Advisory Council. Answers to the questions of the public have been sent to the CA.

Summary of the scientific evaluation

1. Information related to the recipient or parental plants

Potato (*Solanum tuberosum* ssp. *tuberosum*) is mainly a selfing species, but cross-pollination can also occur (OECD, 1997[1]). The pollen is relatively heavy and is spread by wind and insects, especially bumblebees. Flowering, pollen fertility, berry formation and seed set differ according to the *Solanum tuberosum* ssp. *tuberosum* cultivar considered. The cultivar considered here, Désirée, flowers abundantly, produces berries frequently and sets viable true seed.

Solanum tuberosum is sexually compatible with other potato varieties cultivated in Belgium, but not with related wild *Solanum* species occurring in Belgium as *Solanum nigrum* ssp. *nigrum*, *Solanum nigrum* ssp. *schultesii*, *Solanum triflorum*, *Solanum dulcamara* and *Solanum nitidibaccatum* (De Vries et al., 1992[2]; OECD, 1997). Field experiments have shown that cross-pollination reduces rapidly (few meters) with the distance, even for highly male-sterile varieties (Conner, 2006[3]; Petti et al., 2007[4]). True potato seeds can survive in the soil and have been reported to retain their viability over a seven-year rotation in the mild climate of Scotland (Lawson, 1983[5]). Further, potato seeds can be dispersed by small mammals, but rarely by birds as they are poisonous. Plants arising from true seeds have a lower fitness

[1] OECD (1997) Consensus Document on the Biology of *Solanum tuberosum* subsp. *tuberosum* (Potato). Series on the Harmonization of Regulatory Oversight in Biotechnology, No. 8
[2] De Vries F., van der Meijden R., Brandenburg, W.A. (1992) Gorteria, Botanical Files. A study of the real chances for spontaneous gene flow from cultivated plants to the wild flora of the Netherlands. Supplement 1
[3] Conner A.J. (2006) Biosafety evaluation of transgenic potatoes: Gene flow from transgenic potatoes. International Symposium Ecological and Environmental Biosafety of Transgenic Plants, p.125-137
[4] Petti C., Meade C., Downes M., Mullins E. (2007) Facilitating co-existence by tracking gene dispersal in conventional potato systems with microsatellite markers. Environmental Biosafety Research 6, 223-235.
[5] Lawson, H.M. (1983) True potato seeds as arable weeds. Potato Research 26, 237-246.

compared to potato plants grown from tubers. They usually do not persist for more than one or two growing seasons and have rarely been seen outside the fields (Conner, 2006).

The main reproduction in potatoes is by vegetative propagation through tubers (OECD, 1997). Tubers that are not covered by thick layers of soil usually are killed by winter frost. In contrast to true potato seed, tubers do not remain dormant and will sprout the next season.

2. Information on the design and management conditions in the field trial

The notifier announces that the trial will take place during two years (2011 and 2012); each year on a different plot not larger than 1500 m^2. The planting will take place earliest in April-May and harvesting in August/mid October of each year. Preparation and management of the trial site will be according to conventional agricultural practice. Tubers will be removed by hand and transported to the laboratory in closed bags, where they will be destroyed after analyses. The notifier announces that the year following the release, the field plot will remain fallow or will be planted with cereals. Emerging volunteers will be destroyed prior to flower setting. The notifier announces that monitoring will be extended until there is a whole season without any potato volunteers.

3. Information related to the genetic modification

The genes introduced for improving resistance to *Phytophthora infestans* are resistance (R)-genes from wild potato species: *Rpi-vnt1.1* from *Solanum venturii, Rpi-sto1* from *Solanum stoloniferum*, *Rpi-blb3* from *Solanum bulbocastanum*. The three genes are members of the NBS-LRR (Nucleotide Binding Site – Leucine Rich Repeat) class and code for proteins that recognise specific proteins – termed elicitor proteins - of *Phytophthora infestans*. Recognition of the elicitor proteins will lead to local plant cell death and will thus prevent further development of the pathogen *Phytophthora infestans*.

The GM potato lines were produced via *Agrobacterium*-mediated transformation of potato plant tissue. Two vectors were used: pBINAW2 without a selection marker and pBINPLUS with a selection marker for selecting of transformed plants. The selection marker used is an antibiotic resistance gene (neomycine phosphotransferase, *nptII*) that confers resistance to kanamycine, neomycine and amikacine. Three types of lines will be tested: lines containing the *Rpi-vnt1.1* gene, lines containing the *Rpi-sto1* gene and lines containing *Rpi-vnt1.1, Rpi-sto1*and *Rpi-blb3*. Lines containing the *Rpi-sto1* gene and the combination of three *Rpi* genes contain the *nptII* selection gene. Absence of vector backbone sequences conferring resistance to antibiotics relevant to human and veterinary therapy, namely *tetA* and *nptIII* has been demonstrated. The information related to the genetic modification is considered as sufficient and in accordance with the guidelines of the SBB (SBB, 2002)[6].

4. Potential risks for the environment, animal or human health associated with the release of the GM potatoes

A concern associated with the release of GM potato is the potential impact of gene flow between GM and non-GM potatoes. Different paths have to be considered: gene flow by pollen, by true seeds and by tubers. The variety under consideration, Désirée, flowers abundantly, is fertile and produces berries with viable seeds.

[6] SBB (2002) http://www.biosafety.be/gmcropff/EN/TP/partC/GuideMGC_PartB_C.htm

The notifier announces to imply an isolation distance of 150 m to commercially cultivated non-GM potatoes which is in line with the recommended distance for field trials in the literature of 20 m (Conner and Dale, 1996[7]). The Biosafety Advisory Council is of the opinion that under the presented trial conditions outcrossing to neighbouring non-GM potatoes is very unlikely, but cannot be fully excluded. Taken into account that current agricultural practices (rotation) in Belgium offer good opportunities to eliminate occasional seedlings arising from true seed, the risk for the environment in neighbouring fields is considered as very low.

As both occasional seedlings as well as tubers may survive in the soil of the field trial and may produce volunteers in subsequent years, the Biosafety Advisory Council welcomes the propositions of the notifier to control volunteers (see point 2) in the field trial.

The possibility of horizontal gene transfer between GM plants and bacteria is considered as a rare event under natural conditions. The European Food Safety Authority (EFSA) is of the opinion that with regard to safety there is no rationale for inhibiting the use of the antibiotic resistance marker gene *nptII* in field trials. For these reasons, the risks associated with the potential transfer of the *nptII* gene are considered very low.

No effect is expected on non-target organisms. Risks for animals and humans are considered as very low. Indeed, the potatoes used in this trial will not be consumed, as the notifier announces to destroy the tubers after harvest. The notifier also announces to safeguard the field trial as the entrance will be prohibited by a fence.

5. Information related to the control, monitoring, post-release and waste treatment`

The Biosafety Advisory Council appreciates the proposed management measures to prevent potential adverse effects to the environment, animal and human health. However, to minimise the spread of transgenes into the environment, the Biosafety Advisory Council proposes additional measures.

[7] Conner A.J. and Dale P.J. (1996) Reconsideration of pollen dispersal data from field trials of transgenic potatoes. Theoretical and Applied Genetics 92, 505-508.

Conclusion

Based on the scientific assessment of the dossier by Belgian experts, on the answers on the additional questions, on data provided in the COGEM advice[8] in The Netherlands, the majority of the members of the Biosafety Advisory Council proposes a conditional positive advice.

The majority of the members of the Biosafety Advisory Council considers the risks for the environment and human health as negligible on the conditions listed hereunder:

a) Field trials are permitted in 2011 and 2012.
b) The area of the trials is restricted to approximately 2500 m^2.
c) Well before the start of the trial, the notifier provides a correct and accurate field design with a clear identification of individual plots and an accurate number of plants per plot. The precise location of the trial shall be given with references to fixed retrievable points.
d) The entrance of the field trial will be prohibited by a fence.
e) It is the responsibility of the notifier to respect the 150 m distance between the field trial and the closest non-GM potato field.
f) The outermost potato ridges of the field trial shall be planted with a non-GM potato variety. Ideally a variety that flowers simultaneously with the GM lines, without producing berries shall be planted. If not available, the non-GM isogenic variety is acceptable. At all sides of the trial area, the width of this surrounding area should be at least 3 m.
g) All personnel working in the trials will be trained to work with GMOs.
h) Harvesting of the whole experimental area (both GM potatoes and border ridges) must be done by hand. People who pick up the tubers must do everything they can to pick up all the tubers, including the smallest ones.
i) The trial area, extended with a strip of 5 m at all sides of the field trial, shall remain fallow for a period of at least 12 months after the harvest of the GM potatoes. At all times during this period, seedlings and emerging tubers must be killed with a systemic herbicide every 2 weeks. After the fallow period the land may be re-cropped with cereals and maize to allow a good control of volunteers with herbicides. Growing potatoes is not allowed during the whole monitoring period.
j) No ploughing is allowed during the whole monitoring period.
k) The monitoring period shall be x years. If during the year x-2, no volunteers appear anymore, the monitoring period may be ended at the end of the year x.

In order to allow extending its knowledge on the subject, the Biosafety Advisory Council wishes:

1. The notifier to transfer in detail at the end of 2011 and at the end of 2012 all trial results to the Biosafety Advisory Council. This report includes the detailed field design

[8] COGEM advies van 26 januari 2010, kenmerk CGM/100126-02

and well identified photographs demonstrating the reaction of all potato genotypes to *Phytophthora infestans.*

2. The notifier to invite the Biosafety Advisory Council to visit the field trial in order to observe the reactions of the GM and the non-GM potatoes to *Phytophthora infestans.* We propose 2 invitations in 2011 and 2 in 2012 on appropriate dates.
3. The permission for the 2012 trial to depend on a positive evaluation of the 2011 trial by the Biosafety Advisory Council; the evaluation will be based on points b-j and 1-2.

This advice is for a field trial with a restricted area only and only concerns the genetic transformations described in the dossier.

p.o. Prof. D. Reheul
President of the Biosafety Advisory Council

Annex I: Minority opinions.
Annex II: Summary Notification Information Format submitted by the notifier in November 2010.
Annex III: Compilation of comments of experts in charge of assessing the dossier B/BE/10/V1 (ref: BAC_2010_1050).

Annex I: Minority opinions

1. Minority opinion expressed by Mrs Philippe Baret and Damien Winandy

Par principe, les dossiers devraient être présentés de telle sorte que le contrôle des risques ne doivent pas faire l'objet d'une série de conditions imposées par le Conseil.

Outre cette question de principe, nous considérons que l'utilisation de gènes de résistance à l'antibiotique *nptII* dans 18 des 26 lignées présente un risque inutile pour l'environnement et la santé humaine alors que des alternatives existent et que la directive 2001/18/CE prévoit l'élimination de ce type de construction depuis fin 2008 dans ce type de dossier. Il n'y a à ce jour pas de consensus scientifique sur l'absence de risques lié à ce type de marqueur de sélection.

2. Minority opinion expressed by Ms Lucette Flandroy

Whereas 2 different files have been considered, my considerations hereunder can apply to files B/BE/10/V1 and B/BE/10/V2.
I do not agree with the positive advice given by the BAC on these files for the following reasons in A) and C) :

A) This type of conditional positive advice, containing a large series of additional requirements from the BAC, raises principles issues:

1. in relation to the legislation that foresees that the notifiers themselves should make risk assessments and should propose measures to avoid or mitigate potential risks.
2. in relation to the confidence that can be given to notifiers that did not make tests or foresee adequate measures or monitoring to detect/avoid several theoretical potential risks.

B) This being said, it is admissible that the correct enforcement of supplementary conditions imposed to give this positive advice could fill up a blank in the risk assessments performed by the notifiers of which I, as well as several other BAC members including the coordinator of this file, have underlined various gaps and contradictions, even if this risk assessment is intended only for a limited field trial and not for putting on the market (N.B: these supplementary conditions should in any case than be very precise: ex.: more precision should be given for the fence around the field).

C) To better fill the gaps in the risk assessment performed by the notifiers, I would in any case require at least the hereunder additional conditions to test the possibility of adverse effects - resulting from unwanted modifications in these GM events through the transgenetic process – and to avoid as much as possible their occurring in case of repetition/extension of field trials:

1. to make quantitative analysis of *solanine* (potentially toxic not only for humans but also for animals for which a fence around the field would not be a sufficient barrier to avoid their entrance on the field) in the GM potato tubers and leaves, compared to the adequate controls, prior to starting the field trials.
2. alternatively, in order to better discourage entrance of humans on the field and in absence of further characterization of the lines under trial, to place a panel signaling

"potential danger/toxicity if transgressing the fence" at several places near the fence around the field.

3. to test and compare pollen dispersal of GM plants with that of the adequate controls (for ex. through pollen captors at some distances around the field) (Pertinence of the case by case approach in absence of further characterization of the lines under trial, even if the literature shows poor pollen dispersal in general at middle distance for several other GM and conventional potato lines).

D) It is obvious that, in case of putting on the market, the antibiotic resistance marker genes should be eliminated from the selected lines, with better scientific proves of removal than those furnished in this field trial file (Pertinence of the case by case approach of the horizontal transfer of genes from plants to microorganisms, especially in case of remaining decaying parts of the plants on the fields. The concerned antibiotics are important for human and veterinary use).

Summary Notification Information Format

A. General information

A1. Details of notification

Notification Number
B/BE/10/V1

Member State
Belgium

Date of Acknowledgement
3 November 2010

Title of the Project
Two year field trial with genetically modified potatoes that are less susceptible to late blight

Proposed period of release:
01/04/2011 to 31/10/2012

A2. Notifier

Name of the Institute(s) or Company(ies)
University of Ghent

A3. Is the same GMPt release planned elsewhere in the Community?
The same lines are also intended to be experimentally released in The Netherlands.

A4. Has the same GMPt been notified elsewhere by the same notifier?
No

B. Information on the genetically modified plant

B1. Identity of the recipient or parental plant

(a) Family name:	*Solanaceae*
(b) Genus:	*Solanum*
(c) Species:	*tuberosum*
(d) Subspecies:	*tuberosum*
(e) Cultivar / breeding line:	Désirée
(f) Common name:	potato

B2. Description of the traits and characteristics which have been introduced or modified, including marker genes and previous modifications
The genetically modified potatoes are less susceptible to late blight as a result of the introduction of one or three resistance genes stemming from wild relatives from the potato originating from the

Andes. All genes belong to the NBS-LRR class, which code for a class of proteins that are very common in plants and are involved in disease resistance. Arabidopsis, for instance, has about 200 of such genes. When the proteins produced by these genes bind to an elicitor produced by a virulence gene of a pathogen a hypersensitivity reaction is triggered, resulting in the death of the cell in which the binding took place. In this way further spread of the pathogen is blocked. The interaction between the genes is very specific. Specific NBS-LRR proteins bind to specific elicitors.

In a number of the genetically modified lines that will be introduced also the NPT-II antibiotic resistance gene is present. This gene is present as a selection marker for the plant transformation and fullfils no function in the final potao. The rest of the lines is marker free and contain only sequences stemming from wild tuber bearing family member of the potato and are therefore 'cisgenic'.

B3. Type of genetic modification
Insertion of genetic material.

B4. In case of insertion of genetic material, give the source and intended function of each constituent fragment of the region to be inserted
The region to be inserted, which is flanked by the T-DNA borders from the Ti-plasmid of Agrobacterium tumefaciens contains either:

- One resistance gene (Rpi-vnt1, stemming from solanum venturii)
- One resistance gene and a selection marker gene (Rpi-sto1 + nptII, stemming from solanum stoloniferum and from Tn5 respectively)
- Three resistance genes and a selection marker gene (Rpi-vnt1+Rpi-sto1+Rpi-blb3+nptII, stemming from solanum venturii,)

As already indicated under B2 the Rpi-genes contribute to a decreased susceptibility to late blight. All three Rpi-genes involved are under the control of their own natural expression signals.

The npt-II gene expression is driven by the NOS promoter and terminator. The npt-II gene stems from the transposon Tn5. The NOS promoter and terminator originate from *Agrobacterium tumefaciens.* The npt-II gene functions as a selection marker during the transformation and regeneration of the potato plants.

B5. In the case of deletion or other modification of genetic material, give information on the function of the deleted or modified sequences
Not applicable

B6. Brief description of the method used for the genetic modification
The method used for the genetic transformation is based on Agrobacterium tumefaciens cocultivation with potato derived plant tissue. After this cocultivation step where the gene transfer takes place, the transformed cells are:

-Either selected using a positive screen (based on resistance to the antibiotic kanamycin) and induced to regenerate a whole plant.

-Or induced to regenerate a whole plant without using a positive screen. This is done in case of the single gene Rpi-vnt1 construct, that does not harbour a selection marker. All regenerated plants have then been subjected to a PCR to check for the presence of the Rpi-vnt1 insert. Lines that had not been genetically modified were discarded.

C. Experimental Release

C1. Purpose of the release
The purpose of the release is to test the susceptibility of the genetically modified potato lines to late blight under Belgian climatic and soil conditions.

C2. Geographical location of the site
The site of release is located in the municipality of Wetteren.

C3. Size of the site (m2)
The size of the site will be no more than 1500 m^2, including non-genetically modified reference and control lines.

C4. Relevant data regarding previous releases carried out with the same GM-plant, if any, specifically related to the potential environmental and human health impacts from the release

There have been no earlier releases with the same GM plant.

D. Summary of the potential environmental impact from the release of the GMPts

The direct environmental impact from the release is expected to be zero. A decreased susceptibility of potato to late blight using natural resistance genes, which are under the control of their own natural expression signals, which stem from wild Solanum species, and of which similar genes are already present in conventional varieties that are on the market, does not lead to any environmental impact. The resistance is also so specific (specific resistance proteins react with very specific elicitors resulting from specific avirulence genes of *Phytophthora infestans*), that the interaction with other fungi (such as *Alternaria*) or fungi-like organisms is not expected to change in any way.

There will be no spread of genetically modified potatoes from the release, as the distance to other potato fields will be such that no successful hybridization can take place, and also any hybridization is extremely unlikely to result in the formation of a viable genetically modified potato seed. On top of that potato volunteers do not establish and are destroyed in normal agricultural weed killing programmes. In the European Union potato is not able to establish itself in the natural environment and there are no wild relatives with which potato can hybridize.

The presence of the antibiotics resistance gene npt-II also does not lead to any unwanted negative impact on the environment, and we refer for this to the most recent consolidated opinion of EFSA of 2009 concerning the use of the npt-II resistance gene as a selectable marker in plants.

There is an indirect positive environmental impact resulting from the release, as these potato lines will not have to be sprayed with fungicides to control late blight.

E. Brief description of any measures taken for the management of risks

There will be a very careful harvesting of the potato tubers by hand with the goal to prevent any tubers to remain in the soil after the trial. In the years following the trial there will be monitoring to detect any potato volunteers. Detected volunteers will be destroyed using a herbicide. The

monitoring will continue until there has been one full growing season in which no volunteers were detected anymore.

F. Summary of foreseen field trial studies focused to gain new data on environmental and human health impact from the release

In this field trial there will be additional data collection on the susceptibility of the genetically modified lines to *Alternaria* and some harmful insects, in comparison to their parental lines.

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Conseil de Biosécurité

17-01-2011

Secretariaat
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Compilation of comments of experts in charge of assessing the dossier B/BE/10/V1

Coordinator: Prof. P. Baret
Experts: Kürt Demeulemeester (PCA-Beitem), Adinda De Schrijver (WIV-ISP), Patrick du Jardin (ULg-Gembloux Agro-BioTech), Jean Jacquemin (CRA-W Gembloux), Henri Maraite (UCL) and Michel Van Koninckxloo (HEP Hainaut-Condorcet)
SBB: Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman, Katia Pauwels

INTRODUCTION

Dossier **B/BE/10/V1** concerns a notification of the University of Gent for deliberate release in the environment of genetically modified higher plants (GMHP) according to Chapter II of the Royal Decree of 21 February 2005.
The notification has been officially acknowledged on 3 November 2010 and concerns a field trial with potato lines genetically modified to be resistant to *Phytophtora* disease.

Depending on their expertise, the experts were invited to evaluate the genetically modified organisms considered in the notification as regards their potential impacts on the environment, including human and animal health, and information relating to pre- and post-release treatment of the site.
The comments of the experts are roughly structured as in
- Annex II (principles for the risk assessment) of the Royal Decree of 21 February 2005
- Annex III (information required in notifications) of the Royal Decree of 21 February 2005
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.

LIST OF COMMENTS RECEIVED FROM THE EXPERTS

Remark: The comments below have served as basis for a list of questions that the Competent authority forwarded on 16 December 2010 to the notifier with a request to provide additional information. The comments highlighted in grey correspond to the questions addressed to the notifier.

Items left blank have been evaluated by the experts but they had no comments or questions.

Please note that questions on measures to be taken have not been sent to the notifier, as finally it is not up to the notifier which measures need to be implemented. These questions have been taken into account in the discussions of the Biosafety Advisory Council.

The answers of the notifier were received on 4 January 2011 by the Biosafety Advisory Council and evaluated by the experts.

1. INFORMATION RELATED TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

(e.g. reproduction, survivability, dissemination, geographic distribution,...)

Comment: see Q1

The dossier mentions two solanum species: *S. trifolium* and *S. nitidibaccatum* as present in EU, which are not mentioned by the parallel B/BE/10/V2 dossier. The applicant should clarify whether the two additional species are relevant or not by presenting floristic arguments on their distribution in Belgium, and provide any available information on their outcrossing ability with cultivated potato.

2. INFORMATION RELATED TO THE GENETIC MODIFICATION

(e.g. methods used for the modification, description of the vector,...)

Comment 1: see Q1

SASA (*www.**sasa**.gov.**uk***) reports that the variety Désirée forms frequently berries. Frequent berry formation is also reported by NIVAP (Nederlands Potato Consultative Foundation). Varieties with abundant berry formation can give rise to true seed, with results in volunteers in the next culture(s). Therefore, detailed assessment of berry formation during the trial should be done, in order to anticipate the risk of volunteers coming from true seed and to justify that only one year without volunteers is long enough for the post-trial monitoring.
Details on Désirée can be found on the European potato cultivar databases.
See: http://www.europotato.org/display_description.php?variety_name=Desiree

Note coordinator/SBB: The section B.2 (a) (i) on 'modes of reproduction' contains too general information on reproduction and should be adapted to the variety under consideration. The capacity of berry formation of the lines and the chance of volunteers coming from true seed should be document.

Comment 2: see Q11

* Table on insert 3: Rpi-sto1 donor is *S. stoloniferum* and not *S. venturii* as mentioned; Rpi-vnt1 donor is not *S. venturii* and not *S. stoloniferum* as mentioned. *S. venturi* should be *S. venturii*.

Comment 3: see Q3 & Q11

* There is in C3 no description, no reference about the resistance of Solanum species and the genes involved (cloning, structure, homology).
* In the table page 8, there are some mistakes Rpi-vnt1 is associated with S. stoloniferum and sto1 with S. venturi. Sizes of the inserts are also different ?

Comment 4: see Q10

* Point B2a)i) "De aardappel is een insectbestoven zelfbevruchter". This statement is not critical enough with regards to evaluation of potential gene flow through pollen. Although there are few studies clearly demonstrating the relative importance of insects and wind in potato pollination, Eastham &Sweet (2002), quoted by the notifier, state on page 35 (4.3): "Wind is considered a more important vector than insects in effecting pollination. Potato is mainly self-pollinating, with estimates of the rates of cross-pollination under field conditions ranging from 0 to about 20 % .» Even if reduced and highly cultivar & environment dependent, the risk of cross-pollination is a critical issue which needs to be thoroughly addressed. Other evidence of pollen dispersal are provided by Petti, C; Meade, C.; Downes, M. & Mullins, E. (2007, Environmental Biosafety Research 6: 223-235).

Point B4b). The notifier should also take into consideration the publication of Kraus, F.B.; Wolf, S.& Moritz, R.F.A. (2009. Journal of Animal Ecology 78: 247-252) demonstrating a flight range of Bombus terrestris males from 2.6 to 9.9 km.

Note coordinator/SBB: We do not consider it necessary to request the notifier to take the publication of Kraus et al. (2009) into account as for ERA it is more important to take into account the distance where cross-pollination takes place.

3. INFORMATION RELATED TO THE GENETICALLY MODIFIED PLANT

3.1. Information related to the traits and characteristics, which have been introduced or modified

Comment 1:

The GMHP has a build-in kanamycine resistance gene. Kanamycine is an antibioticum used in pig breeding. In Belgium pig breeding is widely spread and intensively practised, resulting in a big amount of manure that's possibly contaminated with kanamycine from treated pigs. Is there any risk that bacterial fauna (in the soil or in the plants) changes trough the combination of kanamycine resistant potato varieties in parcels where pig manure is applied? Could the bacterial equilibrium in the soil be affected with an advantage/increase for kanamycine resistant bacteria?
I would like to refer to a critical Dutch report on this subject from Eijsten and van der Meulen,
See : http://www.gentechvrij.nl/tss/index.php?title=Kanamycine_rapport_kritisch_bekeken

Note coordinator/SBB: Not transmitted considering the EFSA opinion on antibiotic marker genes of 2004.

Comment 2:

The experimental design offers the possibility to test the effect of Rpi-vnt1 and Rpi-sto1 alone and the combined effect with Rpi-blb3 in the lines containing the three resistant genes. The later combination offers a prospect of higher durability.

3.2. Information on the molecular characteristics of the final GMO

(e.g. number of copies of the transgenes,...)

Comment 1:

The number of copies of the transgenes is not clearly/exactly determined in the different modified lines ("minimal 1 and maximal 4"). More exact information would give a better idea of the chance that modified genes could be transferred in the case of recombination with native/cultivated S. tuberosum species.

Comment coordinator/SBB: Exact information on copy numbers is not considered necessary for Standard Part B applications. For this issue we refer to the guidelines of BAC on Molecular Characterisation of GM Plants for a Standard Part B Consent (see: http://www.biosafety.be/gmcropff/EN/TP/partC/Standard_PartB.pdf)

Comment 2:

The presence of the backbone sequences coding for antibiotic resistance genes (*nptIII* and *tetA*) was checked via PCR analyses and found to be absent in the GM potato lines. This is an important issue as these antibiotic resistance genes belong to a group that confer resistance to antibiotics highly relevant for human therapy and therefore should be avoided according to EFSA (2004) and Directive 2001/18/EC in experimental field trials. I leave it up to people specialised in molecular characterisation to determine if the PCR analyses provided is sufficient to prove the absence of these backbone sequences. The use of the *nptII* gene does not pose a safety issue according to EFSA (2004).

Comment 3: see Q4

Considering the medical relevance of the antibiotics for which the *nptIII*, *tetA* and *tetR* genes confer resistance, considering also that plant residues will be left over and not destroyed (releasing potato DNA into the environment), I am of the opinion that the proof of absence of these genes coming from the backbone of the vector must be perfectly convincing, which I assume is not the case. Although the PCR experiments provide arguments for the absence of these vector sequences, a negative result (absence of amplicon) is able to demonstrate the absence of the target sequence in very stringent conditions with appropriate controls. In this case, the fact that some other plants containing backbone sequences actually produced the corresponding amplicons and that an endogenous gene could be amplified in the trangenic DNAs, indicating the quality of the DNA extracts ("positive controls"), does not prove that the particular reactions performed with the primers aimed at amplifying the antibiotic resistance markers (ARM) did function properly (I indeed conclude from the different primer annealing temperatures for the EF1- positive control and for the ARM genes that the amplifications were done in different test tubes).

In consequence, I suggest to ask the applicant to perform a new set of experiments to substantiate the claim of absence of vector sequences. This could typically use Southern blots of transgenic DNA, with both positive (EF1 probe) and negative controls. For this latter, the plant DNA should be spiked with plasmid DNA corresponding to one copy per genome, indicating the capacity of the probes to detect a single copy of any putative insertion of backbone sequence. Only in case that unspiked/spiked DNA provides absence/presence of signals on the same blot, can the conclusion be drawn about the absence of backbone sequences. Alternatively, duplex PCR containing primers for both the backbone sequences and for an endogenous control could also be used (cfr the demonstration of the absence of the aadA gene in the parallel dossier B/BE/10/V2).

3.3. Information on the expression of the insert

(e.g. parts of plants where the insert is expressed, (expected) expression of the insert during the lifecycle of the plant,...)

Comment:
It is surprising that the notifier is not providing data on the sites of expression of the introduced genes.

Note coordinator/SBB: Information on plant parts where trait is expressed, is not considered necessary for Standard Part B applications. For this issue we refer to the guidelines of BAC on Molecular Characterisation of GM Plants for a Standard Part B Consent (see: http://www.biosafety.be/gmcropff/EN/TP/partC/Standard_PartB.pdf)

3.4. Information on how the GM plant differs from the recipient plant

Comment:
Are there specific observations planned to analyse the berry producing capacity of the lines? Mustonen, L.; Peltonen-Sainio, P. & Pahkala, K. (2009, Acta Agricultura Scandinavica. Section B, Plant Soil Science 59:552-558) recommend “accepting only non-berry-producing GM (potato) cultivars for cultivation”.

3.5. Genetic stability of the insert and phenotypic stability of the GMHP

Comment:
The genetic stability of the insert is not demonstrated as the phenotype expression stability. The insert stability can be demonstrated on several generations of vegetatively propagated material. The stability can be investigated by classical Southern method. Southern analysis is not indicated in this dossier. Expression can also be tested.

Note coordinator/SBB: Information on stability is not considered necessary for Standard Part B applications. For this issue we refer to the guidelines of BAC on Molecular Characterisation of GM Plants for a Standard Part B Consent (see: http://www.biosafety.be/gmcropff/EN/TP/partC/Standard_PartB.pdf)

3.6. Any change to the ability of the GMHP to transfer genetic material to other organisms

Comment 1:
See section 2 (comment on kanamycine)

Comment 2: see Q5
Issue is not properly addressed. One cannot solely refer to D.4 in addressing this issue as D.4 covers another risk assessment issue. In this section vertical and horizontal gene flow should be covered.

3.7. Information on any toxic, allergenic or other harmful effects on human health arising from the genetic modification

Comment 1: see Q6 & Q12
The applicant mentions that some of the resistance genes have been introgressed into cultivated potato and argue that there is a history of safe use for these genes. Could the applicant indicate which are these genes?

On the other hand, the NBS-LRR resistance genes are very diverse and their ubiquitous occurrence in plants can not be regarded as an argument that none of the encoded proteins is toxic or allergenic to humans and animals, in the absence of direct testing of the individual NBS-LRR protein considered.
I thus disagree with the argumentation of the applicant, and the management and monitoring of the trial should be conducted in a way that the risk of any unintended consumption by humans and farm animals is totally eliminated. This should be ensured by the proposed trial protocol.

Comment 2:
Seen the lack of knowledge concerning the genetic modifications in the lines to be tested it is difficult to assess this point more precisely.

3.8. Information on the safety of the GMHP to animal health, particularly regarding any toxic, allergenic or other harmful effects from the genetic modification, where the GMHP is intended to be used in animal feedstuffs

3.9. Mechanism of interaction between the genetically modified plant and target organisms (if applicable)

3.10. Potential changes in the interactions of the GMHP with non-target organisms resulting from the genetic modification

Comment 1: see Q7
There is mentioned that there's no interaction between susceptibility for late blight (P. infestans) and for early blight (Alternaria). Could this be supported by some (scientific) references?
Own observations show that some organic grown potato varieties (with moderate to good P. infestans resistance) are rather susceptible for Alternaria (e.g. cv. Biogold).

Comment 2:
Besides scoring damage resulting from *Phytophthera infestans* (target organism) infestation, also damage from *Alternaria*, aphids and Colorado beetles will be scored during the field trial.

Comment 3: see Q7
Is the notifier in a position to exclude any interaction of the Rpi-gen products with elicitors of other organisms than *Phytophthora infestans*?

3.11. Potential interactions with the abiotic environment

Comment:
I want to note that the BASF notification states: "Further the release will provide an opportunity to investigate any potential interactions with abiotic environment via recording and comparing tuber yield data". A similar investigation will not be done by the VIB.

3.12. Description of detection and identification techniques for the GM plant

Comment: see Q8
Comments on Bijlage Gedetailleerde beschrijving van de genetische modificatie – C.3 In de plant aanwezige sequenties:
* In the PCR protocol it should be better indicated which steps need to repeated 35 times

* In the PCR mix primer numbers are mentioned which do not appear in table 4. Can it be clarified if the PCR protocol, namely 0.3 µl of primer, applies only to this pair of primers or to all the primer combinations mentioned in table 4. Please also note that no primer numbers are given in table 4.
* In the PCR protocol, mix volumes are incorrect: total should be 14 µl and not 15 µl.

3.13. Information about previous releases of the GM plant, if applicable

Comment:
At which stage aberrant lines are eliminated? There's no exact information about the exact aberrant lines found in the earlier Dutch trials.

4. INFORMATION RELATING TO THE SITE OF RELEASE

(e.g. description of the site ecosystem, presence sexually compatible species, proximity of protected areas,...)

Comment:
The distance of 150 m to other potato plots is in the line of distances previously recommended. Nevertheless, recent data on bumblebee foraging (Wolf, S. & Moritz, R.F.A. 2008. Foraging distance in *Bombus terrestris* L. (Hymenoptera: Apidae. Apidologie 39: 419-427) reporting mean foraging distances of workers of 267 m (max. 800m) may raise concern about risk of spread beyond the 150 m foreseen in the trial.

Note coordinator/SBB: Not spread is a risk, but the impact of spread.

5. INFORMATION RELATING TO THE RELEASE

(e.g. purpose of release, dates and duration of the release, methods for preparing and managing the release site, number of plants,...)

Comment 1:
What happens with the harvest of the non-modified surrounding potato plants? (cv. Bintje).

Comment 2: see Q1
- The capacity of berry formation of the lines should be documented.

Note coordinator/SBB: The section B.2 (a) (i) on 'modes of reproduction' contains too general information on reproduction and should be adapted to the variety under consideration. This point is vague in the notification. There should be a sentence clearly stating that Désirée produces true seed. The capacity of berry formation of the lines and the chance of volunteers coming from true seed should be document.

- It is not clear how the 'cultivatorbewerking' could warrant the purpose of bringing the tubers to the surface rather than burying them deeper?

Note coordinator/SBB: Deze "lichte" bewerking wordt toegepast om te vermijden dat de aardappelen ondergeploegd zouden worden.

- The time frame and execution of post crop inspection should be more precisely specified and documented in the logbook in order to allow independent inspection by the authority

6. INFORMATION RELATED TO THE RISKS FOR THE ENVIRONMENT

6.1. Information on the likelihood for the GMHP to become more persistent than the recipient or parental plants or more invasive

Comment:
See comments under point 4.
- G1b) The maximum size of the tubers left in the soil at harvest should be specified.

6.2. Information on the selective advantage or disadvantage conferred to the GMHP

Comment:
Depending on the occurrence of berries with fertile seed, the monitoring of volunteer potato plants needs to be pursued beyond the year following the trial. See comment on point 5.
There is an inconsistency in point G2 mentioning 'the volgende jaar' and point G4 stating 'de volgende jaren' with a more precise description. The later is more adequate.

6.3. Information on potential of gene transfer to other sexually compatible plant species under conditions of planting and its consequences

Comment: see Q13
Missing reference of the cited research on the lack of cross fertilisation when distance is above 20m.

6.4. Information on the environmental impact resulting from direct and indirect interactions of the GMHP with target organisms

Comment 1: see Q14
To fight early blight (Alternaria), 2 applications of fungicides will be applied in the trial. The most adequate/performant early blight fungicides are based on active ingredients azoxystrobin (Amistar) and boscalid + pyraclostrobin (Signum / Terminett). Those active ingredients have NO action against P. infestans. Therefore the mentioned "advantage" of controlling eventual P. infestans resistant stems via Alternaria control is not at order and a false argument. Properties late & early blight fungicides:
See http://www.kennisakker.nl/files/Kennisdocument/Phytophthora_2010-web.pdf

Comment 2:
See comments for points 5. and 6.2.

6.5. Information on the environmental impact resulting from direct and indirect interactions of the GMHP with non-target organisms, including herbivores, parasites, symbionts...

Comment:
This point is not specifically addressed by the notifier. Its relevance for a trial on a small area is low.

6.6. Information on possible effects on human health resulting from potential direct and indirect interactions of the GMHP and persons working with, coming into contact with or living in the vicinity of the GMHP release

Comment:
A priori no specific risk of handling the GMO crop are expected from the introduced genes. Only resistance to late blight and basic characteristics excluding consumption tests are planned. Seen the

absence of knowledge concerning gene expression sites and of possible side effects basic precautions, such as wearing gloves in manipulating the crop, are advised.

6.7. Information on possible effects on animal health and consequences for the food/feed chain resulting from consumption of the GMO and any product derived from it, if it is intended to be used as animal feed

6.8. 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)

Comment:
See comment for 6.5.

6.9. Information on environmental impact of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHPs

Comment:

The techniques used for this trial are not expected to have any specific environmental impact.

7. INFORMATION RELATED TO CONTROL, MONITORING, POSTRELEASE AND WASTE TREATMENT

7.1. Precautions taken

Comment 1:
It's not clear if haulm killing will have place prior before harvesting. By haulm killing, not only the foliage will be destructed, but is also favours the loosening of the tubers from the stolons. In this trial, foliage will stay at the field after harvesting; by haulm killing the risk of tubers still attached to the remaining foliage will be much lower.

Note coordinator/SBB: Misschien minder relevant bij handmatig oogsten zoals in het geval van dit dossier, maar meer relevant in geval van machinaal oogsten?

For transporting the harvested tubers, double layer bags are recommended, in order to prevent loss of tubers during transport (risk of damage to single layer bag/sacs).

Note coordinator/SBB: Is vermeld in B/BE/10/V2, maar inderdaad niet in dit dossier.

Comment 2:
The precautions taken (150 m isolation distance, and 4 rows of maize plants around the plot) are more than sufficient to avoid vertical gene flow by pollen. Connor & Dale (1996) referred to in the Technical Dossier of B/BE/10/V1 summarised available data on cross-pollination, showing that no cross-pollination was detected at 20m. Petti *et al*. (2007) proposed a 30 m isolation distance for potato field trials, based on their data obtained with a high fertile Désirée as pollen donor and a male sterile pollen receptor. Hence an isolation distance of 150 m will be sufficient to avoid vertical gene flow. In addition, the 4 rows of maize planted around the plot will serve as a buffer and reduce the distance of pollen flow. The other measures taken to minimise and prevent gene dispersal are considered sufficient.

7.2. Information on methods for post-release treatment of site

Comment 1:
It's not clear which herbicides will be used to control volunteer plants in the succeeding crop(s). Volunteer control by herbicides used in wheat, mais or sugarbeets are not always 100% effective against volunteer potatoes. Often they suppress (temporarely) potato growth. Monitoring of the treated volunteer plants is recommended. Another alternative is using systemic herbicides locally applied to the volunteer plants (e.g. dipping glyfosaat), which has the additional advantage that tubers are also affected.

Note coordinator/SBB: Monitoring is considered in the notification and emerging volunteers will be destroyed by herbicide treatment. In contrast to BASF notification, it is not specified in VIB notification that a systemic herbicide, such as glyphosate, will be used.

Comment 2:
See comments for Point 6.2.

7.3. Information on postrelease treatment methods for the GM plant material, including wastes

Comment 1: see Q9
Shredding of potato tubers should happen very accurately. Limited shredding is not completely effective in preventing regrowth of the tubers. Cutting seed potatoes (in 2 or 3 pieces) is a common practice for early varieties in order to have more stems or earlier emergence of the potato crop.

Comment 2: see Q9
The destruction of the tuber -heat or mechanical- as well as the waste disposal should be more clearly specified.

7.4 Information related to monitoring plans and the detection techniques

Comment 1:
The site should be monitored also the second year after the trial, even when no volunteer plants are detected in the first year after the trial.
After plowing, tubers left on the field can be buried into the soil and stay there intact for more than one year. Those tubers can still become volunteer plants in the second succeeding crop after the trial, when they come to the surface by plowing the soil for the second time. Problems with volunteer plants in the second year after potato crop is know in practice, especially for some varieties, e.g. cv. Asterix.

Note coordinator/SBB: Ploughing will not be done for this reason. A "cultivator treatment" is proposed in the notification.

Comment 2:
Although not mentioned here, a field notebook will be kept during the period of release (see bijlage proefprotocol) for more information.

7.5. Information on the emergency plan(s) proposed by the notifier

Comment 1:
In case of severe problems with the trial, the possibility of premature termination of the complete trial has to be considered. These possibility is not mentioned in the emergency plan.

Note coordinator/SBB: This issue is covered under point 7 of the Proefprotocol

7.6. Information on methods and procedures to protect the site

8. OTHER INFORMATION

8.1 Do you have any other questions/comments concerning this notification that are not covered under the previous items?

Comment: see Q11
* There are some disparities in Bijlage
In B3 there are some mistakes Rpi-vnt1 is associated with S. stoloniferum and sto1 with S. venturi
Sizes of the inserts are also different.
* In the plasmid figures, total size of the plasmid do no correspond to the addition of the indicated fragment sizes
* In the PCR protocols, mix volumes are incorrect
* There are 26 lines on trial in Table 1, in Table 3 and 5, 29 lines are listed (3 with additional inserts) and in the plot design, 27 transgenic lines are in trial. Line numbers in trial should be unified and clarified.

Note coordinator/SBB: In table 3 it is indicated that with an asterisk that these 3 additional lines are not taken up in the field trial. So, this point was not raised.

References (not present in notification)

EFSA (2004) Opinion of the Scientific Panel on GMOs on the use of antibiotic resistance genes as marker genes in GM plants. The EFSA Journal (2004) 48, 1-18.

Petti C, Meade C, Downes M and Mullins E. (2007). Facilitating co-existence by tracking gene dispersal in conventional potato systems with microsatellite markers. Environmental Biosafety Research 6: 223-235.

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