Application for consent to release a GMO

Part A2: Data or results from any previous releases of the GMO

Give information on data or results from any previous releases of this GMO by you either inside or outside the European Community [especially the results of monitoring and the effectiveness of any risk management procedures].

Plants transformed with SLJ24904 and SLJ25057 have been tested in the field in Norwich (UK) under consent 17/R29/01. SLJ24904 lines were released in the summer of 2017 and 2018, while SLJ25057 lines were tested in 2018. As expected, they showed susceptibility and full resistance against late blight, respectively. Monitoring during the release did not uncover any unexpected event or hazard and risk management procedures in place where deemed appropriate. Some of those lines, together with new lines generated with plasmid SLJ25057 will be tested under this consent if granted.

Part A3: Details of previous applications for release
Give details of any previous applications to release the GMO made to the Secretary of State under the 2002 Regulations or to another Member State under the Deliberate Release Directive 2001/18/EC.

As indicated above, plants transformed with SLJ24904 and SLJ25057 have been tested in the field in Norwich (UK) under consent 17/R29/01. Please note that SLJ25057 is a simplified version of SLJ24918, carrying fewer elements in the T-DNA.

Part A4: Risk assessment and a statement on risk evaluation

Summary
Environmental risks

Four hundred years of cultivation have established that the potato has limited ability to survive in UK environments except when cultivated. Plants generated from tubers are readily identifiable and easily eliminated either by hand pulling or use of herbicides. Potato plants are not invasive of natural habitats. The pollen of potato normally disperses less than 10 metres, is often infertile and potatoes cannot cross with other crop plants to produce hybrids. A major factor contributing to the lack of pollen dispersal is the fact that flowers of *Solanum spp* produce no nectar, so pollen is the only food reward offered. Consequently, they are not frequently visited by honeybees seeking nectar. In addition, the anthers of these plants require sonication

by insects to release pollen, and thus the spectrum of pollinating insects is restricted. Bumblebees typically forage over 70–631 metres (Osborne et al, 1999), but pollen from one flower is usually deposited only across a limited number of flowers that are subsequently visited. This and factors such as residence time in one crop favours highly localized cross-pollination of plants near the pollen source (Cresswell et al, 2002). Estimates of the rates of cross-pollination under field conditions range from 0 to about 20% (Plaisted, 1980). Other studies have shown that the rates of cross-pollination are 2% at a distance of 3 metres from the crop, reducing to 0.017% at a distance of 10 metres (McPartlan and Dale, 1994).

Based on current knowledge, the overall risk to the environment from transgenic potatoes sited at least 20 metres from other plants with which it is cross-fertile is low to effectively zero. The resistance traits to be expressed are predicted to affect only the target pathogens, *P. infestans* and potato cyst nematodes (if present). The expected environmental impact is negligible and will most probably reduce the level of other agricultural inputs such as use of fungicides or nematicides to control late blight or potato cyst nematodes in potato crops.

Any evaluation of biosafety of transgenic potato crops to animals must be set in the context that these plants are a natural hazard to a range of animals. Their tissues naturally contain steroidal glycoalkaloids such as α-chaconine and α-solanine that are potent neurotoxins, particularly if administered by an intraperitoneal route. Their levels in leaves are normally higher than safe levels accepted in tubers for food.

Human health risks

Lines transformed with SLJ25057 carry late blight resistance (*R*) genes. *R* genes of the NB-LRR class are not new to the human diet, being present in all plants consumed by both humans and animals. The model plant species *Arabidopsis thaliana* is known to possess approximately 200 *R* genes and *R* gene homologues (Meyers et al, 2003), while rice possesses approximately 500 (Zhou et al, 2004). Within the potato genome, a set of 438 NB-LRR-type genes has been predicted (Jupe et al, 2012), and further analysis showed that the doubled monohaploid reference potato genome encodes ~ 750 NB-LRR proteins (Jupe et al, 2013). *R* genes themselves are not toxic even to crop pathogens. They simply serve a recognition function, enabling plants to recognise specific molecules produced by the pathogens, resulting in the triggering of plant defence responses. These plant defence responses are not specific to late blight resistance. They can be triggered upon recognition of any plant pathogen.

Lines transformed with SLJ24904 will carry the *Oc-I∆D86* cystatin gene (Urwin et al, 1995) and a gene coding for a repellent peptide (Winter et al, 2002). The expression of these genes is targeted to the plant root system and confers resistance against potato cyst nematodes (PCN). Cystatins are not new to the human diet being

present in many foods, e.g. rice seeds, maize kernels and chicken egg white (Benchabane et al, 2010; Colella et al, 1989). The lack of toxicity of the cystatin Oc-IΔD86 to mammals has already been established (Atkinson et al., 2004). It is readily degraded by boiling and upon exposure to simulated gastric fluid. Similarly, it is not an allergen (Meredith and Atkinson, 2000). The repellent to be used is not lethal to animals and its effect on nematodes is not via the oral route (Winter et al, 2002; Wang et al, 2011). It merely prevents plant parasitic species from invading roots. The repellent is not stable when heated in conditions equivalent to those required to cook potatoes for safe human consumption, and it is easily destroyed upon exposure to simulated intestinal fluid or nonsterile soil (Roderick et al, 2012). In addition, the peptide sequence is not flagged as a potential allergen (Roderick et al, 2012).

Lines transformed with SLJ25057 will contain a gene-silencing module. This module only includes potato sequences and its structure is such that it does not code for proteins. Its mode of action is based on using the endogenous post-transcriptional silencing machinery of plants to reduce the expression of the *Ppo* and *VInv* genes in tubers. No toxic or allergenic potential is therefore expected and nucleic acids (such as the endogenous RNA and DNA molecules of plants) are readily degraded by human digestive fluids (Liu et al, 2015). It is also worth noting that transgenic potatoes developed with an equivalent technology have been approved for commercialization in the US.

All the plasmids used to generate the plants included in this application carry the selectable marker gene *CSR*. *CSR* is an allele of the tomato acetolactate synthase (*ALS*) gene that has been cloned under the control of its native regulatory elements. It codes for a variant of the ALS enzyme that is resistant to inhibition by some herbicides (sulfonylureas and imidazolinones). Resistance to ALS-inhibiting herbicides is present in several commercially-available crops, including wheat, soybean, rice, canola and sunflower (Green and Owen, 2011; Hanson et al, 2014). In all of them, resistance is due to mutations in the *ALS* gene. This is also the case for the tomato *ALS* allele introduced in the plants proposed for release. Resistance to these herbicides has been typically achieved by traditional breeding methods but at least one transgenic event that includes a resistant *ALS* allele has been deregulated in the US (Green and Owen, 2011). Therefore, no harmful effects are predicted to arise from the use of this marker gene.

Furthermore, linker sequences used to assemble the plasmids included in this application do not code for proteins and no toxic or allergenic potential is predicted. Several measures have been taken to avoid backbone integration in the transgenic plants to which this application refers. In the unlikely event of backbone sequences being inserted, the only two protein-coding genes present in the vectors' backbones are the marker gene *nptII* and the *ipt* gene.

The marker gene *nptII* (or *aph(3')-IIa*) is under the control of a bacterial promoter and is used for bacterial selection only. It is expressed as an enzyme (aminoglycoside 3-phosphotransferase II or neomycin phosphotransferase II) that inactivates the antibiotics neomycin, kanamycin, geneticin (G418), and paromomycin by phosphorylation. The protein encoded by the gene has been shown to be bio-safe, non-toxic and poses no risk to human or animal health (The EFSA Journal, 2009, 1034: 66-82). No toxicity of the NPTII protein has been observed and this protein is rapidly degraded in simulated digestive fluids. The characteristics of the transgenic protein NPTII involve no outstanding safety issues and derived products are no more likely to cause adverse effects on human and animal health than conventional potato (The EFSA Journal, 2006, 323: 1-20).

The isopentenyl transferase (*ipt*) gene derives from the soil bacteria *A. tumefaciens*. This gene codes for an enzyme that catalyses the synthesis of the cytokinin isopentenyl adenosine, which naturally occurs in plants (Sakakibara et al, 2005). Plants have their own isopentenyl transferase genes for cytokinin production, some of which are expressed in edible parts of crops like maize kernels (Brugiere et al, 2008). In this case, the presence of the *ipt* gene in the vector backbone allows the counter-selection of plants where the backbone has been integrated. If the gene is expressed and the IPT enzyme produced, plants will display an abnormal development in tissue culture conditions and will be discarded (Richael et al, 2008). If the *ipt* gene is not significantly expressed due to positional effects or has been only partially inserted, it is possible that plants where parts of the backbone have been integrated 'scape' the counter-selection step. However, no harmful effects are expected in relation to this gene. The IPT enzyme sequence is not flagged as a potential allergen by Allergenonline (www.allergenonline.com). An '80mer Sliding Window Search' was carried out and it yielded no matches of significant identity. Such search is described as 'a precautionary search using a sliding window of 80 amino acid segments of each protein to find identities greater than 35% (according to CODEX Alimentarius guidelines, 2003)'.

In addition to the absence of known harmful properties of any of the genetic elements present in the modified potatoes, no harmful properties are expected to emerge when the above-mentioned genes and traits are combined. After harvest, tubers will be destroyed or kept under contained conditions for experimental purposes; thus, there will be no risk of the genetically modified material entering the food chain. Finally, this work should be judged in the context of the natural hazard that potato plants pose with their endogenous high levels of natural toxins.

Risk assessment
Conclusions on the Potential Environmental Impact from the Release or the Placing on the Market of GMOs

i. Likelihood of the genetically modified higher plant (GMHP) becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats.

Neither the genes or the gene-silencing module introduced into the potato plants proposed for release confer characteristics that would increase the competitiveness of plants in unmanaged ecosystems. Neither would the genes enable plants carrying them to out-compete plants of similar type for space. None of the transferred genes 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 advantage in this environment. Potatoes are not persistent outside the agricultural environment and feral potato plants do not generally occur in the UK.

The advantage conferred by the resistance genes against the target organism *P. infestans* will be applicable only in the agricultural environment and only in those cases where no other plant protection measures against *P. infestans* are applied. Further, this advantage will only be apparent in the event that the local *P. infestans* population is comprised of isolates against which the plants are resistant. Should the local population comprise genotypes which are not recognised either by the introduced *R* genes, or by *R* genes already present in the genome of the potato plant, no increase in survivability will be apparent.

Similarly, the advantage conferred by the resistance genes against the target potato cyst nematodes (PCN) will be applicable only in the agricultural environment and only in those cases where no other plant protection measures against PCN are applied. Further, this advantage will only be apparent if cysts of the PCN *G. pallida* are present in the soil. No difference would be observed if cysts from the PCN *G. rostochiensis* are present, since the Maris Piper parental line is already resistant to that species. Further, there is no evidence that potato cyst nematodes influence the persistence of volunteers. They are introduced animals and so absent from natural habitats and specific parasites of the potato. Therefore potato cyst nematodes resistance is unlikely to make the plants more invasive of natural habitats.

The gene-silencing module present in the plants transformed with SLJ25057 is designed to modify tuber quality traits that are important in post-harvest management and processing of the potato tubers. They are not expected to affect the persistence of the plants in field conditions.

The transgenic plants proposed for release will be resistant to herbicides that contain sulfonylureas or imidazolinones as active ingredients. This trait will be used only for the *in vitro* selection of transgenic lines during tissue culture. The plants remain sensitive to other herbicides such as glyphosate or glufosinate,

which could readily be used to eliminate them in the field. In addition, sulfonylureas and imidazolinones will not be used in the context of this trial.

Finally, in the unlikely event of backbone integration, no effect in persistence or invasiveness is expected from any of the elements in the vector backbone. The *nptII* gene is driven by a bacterial promoter and no antibiotic will be used in the field. The *ipt* gene will be used to counter-select plants where the backbone has been integrated and this will be performed *in vitro*, at the tissue-culture stage. If the *ipt* gene is fully integrated and expressed, the enzyme IPT stimulates the production of natural cytokinins during tissue culture of transformed plants. This induces a characteristic shooting phenotype that allows the easy identification of such plants (Richael et al, 2008).

The introduced genes are thus not anticipated to confer any intrinsic advantage compared to conventional potato varieties with respect to persistence in agricultural habitats or invasiveness in natural habitats and no emergent hazard is predicted for the proposed combinations of genes and traits. To further minimise any risk, the following risk management measures will be applied: implementation of isolation distances of a minimum of 20 metres from any other potato plants not included in the trial and volunteer management to ensure effective control of volunteers emerging on the field and the immediate surroundings (the plot will be left fallow after potato harvest to enable easy identification and removal of groundkeepers). The overall impact is therefore considered negligible.

ii. Any selective advantage or disadvantage conferred to the GMHP.

The intended effect of the genetic modifications described here is to improve the resistance of the recipient plants to *P. infestans* or to potato cyst nematodes (PCN) and in some cases to increase the processing quality of tubers.

Under *P. infestans* and/or PCN pressure, resistant potatoes are intended to have a selective advantage in comparison to untreated non-resistant conventional potatoes included in the trial. This advantage is only applicable in the agricultural environment and only in those cases where no other plant protection measures against *P. infestans* or PCN (such as fungicide or nematicide treatments) are applied. Conventional agricultural practices as well as volunteer management will ensure 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* and/or PCN is not a characteristic that would enhance the invasiveness of potatoes. Further, there's no evidence that PCN limit the distribution or abundance of wild Solanaceae in the UK.

Resistance levels against late blight are predicted to be high in plants carrying the three-*R*-gene stack, based on results from previous trials performed in Norwich. The benefits of this approach have been extensively reported in the scientific literature, including examples of other genetically modified potato plants carrying *R*-gene stacks that have been field-trialled within the European Union (Haverkort et al, 2016; Jo et al, 2016). Further, deployment of a gene-stack conferring resistance against pathogenic nematodes by two different mechanisms has the potential to be a more efficient and durable strategy to control PCN, compared to deployment of individual genes (Fuller et al, 2008). In a similar work, the use of the repellent peptide in combination with a maize kernel cystatin to confer resistance against pathogenic nematodes in plantain (*Musa spp.*) has been successfully tested in the field (Roderick et al, 2012; Tripathi et al, 2015).

The gene-silencing module present in plants transformed with SLJ25057 is designed to modify tuber quality traits that are important in post-harvest management and processing of the potato tubers. They are not expected to affect the fitness of the plants in field conditions. It is worth noting that the enzyme PPO has been linked to plant defence responses, however, silencing of *Ppo* in tubers does not enhance susceptibility to the late blight pathogen (Rommens et al, 2006). Also, commercial potatoes in which the *Ppo* gene has been silenced by the same mechanism don't show increased disease susceptibility. This is probably due to an incomplete suppression of the browning process and to other plant defence mechanisms.

All the plasmids used to generate the plants included in this application carry the selectable marker gene *CSR*. *CSR* is an allele of the tomato acetolactate synthase (*ALS*) gene. It codes for a variant of the ALS enzyme that is resistant to inhibition by some herbicides (sulfonylureas and imidazolinones). Resistance to ALS-inhibiting herbicides is present in several commercially-available crops, including wheat, soybean, rice, canola and sunflower (Green and Owen, 2011; Hanson et al, 2014). In all of them, resistance is due to mutations in the *ALS* gene. This is also the case for the tomato *ALS* allele introduced in the plants proposed for release. Resistance to these herbicides has been typically achieved by traditional breeding methods but at least one transgenic event that includes a resistant *ALS* allele has been deregulated in the US (Green and Owen, 2011). This trait will be used only for the *in vitro* selection of transgenic lines during tissue culture. The plants remain sensitive to other herbicides such as glyphosate or glufosinate, which could readily be used to eliminate them in the field. In addition, sulfonylureas and imidazolinones will not be used in the context of this trial, so no selective advantage will be conferred to this plants.

Finally, in the unlikely event of backbone integration, no detrimental effect is expected from any of the elements in the vector backbone. The *nptII* gene is driven by a bacterial promoter and is only used for bacterial selection. It confers

improved tolerance to the antibiotics neomycin, kanamycin, geneticin (G418), and paromomycin. These antibiotics are not used in agriculture and the *nptII* gene has been approved as safe for use by the European Food Safety Authority. The *ipt* gene will be used to counter-select plants where the backbone has been integrated and this will be performed *in vitro*, at the tissue-culture stage (Richael et al, 2008).

No other emergent advantages or disadvantages are expected from the proposed combinations of genes and traits.

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

Genetic material can be transferred from conventional potatoes as well as genetically-modified potatoes to sexually compatible plants via pollen. Transfer via pollen to other species or wild relatives at or near the release site is very unlikely due to the absence of sexually compatible species. *S. tuberosum tuberosum* does not cross pollinate with other UK crops and has been shown not to hybridise under field conditions to native solanaceous plants, *S. dulcamara* or *S. nigrum* (OECD, 1997 and chapter 4.6 Research Report No 1 Genetically Modified Crops and their relatives - a UK perspective, published by Department of Environment 1994). Therefore, out-crossing 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, monitoring and 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 intrinsic selective advantage or disadvantage is being transferred to those potatoes (see point ii) and because potato plants are propagated vegetatively there is no significant risk of introduction of the GM traits into conventional potato material (true potato seed is not saved by growers).

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

The target organisms of the introduced genes are *P. infestans* or potato cyst nematodes (PCN). The intended effect of the genetic modification is to confer tolerance to those organisms. None of the other introduced sequences, such as the selectable marker gene or the gene-silencing modules, neither the genes present in the vector backbone (and not intended for insertion) have target organisms.

One of the main goals of the trial is to test whether the plants are resistant to circulating *P. infestans* isolates in field conditions. If resistance occurs, it will reduce the late blight population in the trial plants. Under conventional agricultural practice *P. infestans* is also controlled by fungicide treatment of potato fields and thus the outcome of the interaction (i.e. a reduction in the population of *P. infestans*) is a desirable one and does not differ from the outcome of these other practices. The overall impact of *P. infestans* 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.

Resistance to nematodes will not be specifically evaluated in our trial and plants transformed with plasmid SLJ24904 will only be included as controls. If eventually present, the expectation is that PCN multiplication will be reduced, as it similarly happens due to conventional agricultural practices, where nematicides may be used. As stated before for late blight, the outcome of the interaction (i.e. a reduction in the population of PCN) is a desirable one and does not differ from the outcome of these other practices. No negative environmental impact of this is apparent. PCN is not a normal part of the UK fauna and is restricted to fields where it exclusively parasitises potatoes.

v. Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMHP 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.

The late blight resistance genes introduced into the genetically modified potatoes are of the NB-LRR class. Genes of this class recognise specific molecules produced by some plant pathogens (in this case *P. infestans*) and trigger a hypersensitive response, leading to plant cell necrosis, which limits the spread of the pathogen. Due to the specificity of the recognition 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. Pathogens other than the particular races of *P. infestans* to which the introduced genes confer resistance, and that are able to infect the non-transgenic plants grown as part of the trial, will also be able to infect the transgenic plants. Due to a reduced need for fungal treatments, an increase in the populations of those non-target organisms that respond to fungal treatments might be expected.

Regarding the resistance against nematodes, Désirée plants carrying the *Oc-I∆D86* cystatin gene or the repellent-coding gene under the control of the root-specific promoters *ARSK1* and *MDK4-20* respectively, have also been previously tested in field trials in the UK. Results of those trials have been reported in Lilley

et al (2004), Kiezebrink and Atkinson (2004) and Green et al (2012). These and other works performed with similar transgenic lines established considerable advantages of this approach to soil micro-organisms relative to nematicide use, with no detrimental effects on non-target organisms and soil health (Cowgill et al, 2002a, 2002b, 2004; Cowgill and Atkinson, 2003; Celis et al, 2004; Kiezebrink and Atkinson, 2004; Green et al, 2012).

The gene-silencing module present in plants transformed with SLJ25057 is designed to modify tuber quality traits that are important in post-harvest management and processing of the potato tubers. They are not expected to affect the interaction of the transgenic plants with other organisms. Similarly, the selectable marker gene that confers resistance to sulfonylureas or imidazolinones, will be used only for the *in vitro* selection of transgenic lines during tissue culture. Herbicides based on sulfonylureas and imidazolinones as active ingredients will not be used in the context of this trial. Furthermore, the plants remain sensitive to other herbicides such as glyphosate or glufosinate, which could readily be used to eliminate them in the field if needed. Even if not intended for insertion, no effect on non-target organisms is expected from the genes present in the vector backbone.

Finally, no emergent hazard is predicted for the proposed combinations of genes and traits and any effects on disease and susceptibility to pests other than the expected on *P. infestans* or PCN will be monitored during the release. The overall impact on non-target organisms is considered negligible.

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

Most of the genetically modified potatoes included in this application are expected to have an increase in their tolerance to *P. infestans* due to the introduced resistance (*R*) genes. Potato already contains a large number of resistance genes of the same kind: within the potato genome, a set of over 400 NB-LRR-type genes has been predicted (Jupe et al, 2012), and ~750 were found in later studies (Jupe et al, 2013). Included in this number are NB-LRR R genes that were originally introgressed from other wild potato species, namely *S. demissum*, during breeding efforts made during the 20th Century. None of the genes are known to exert any toxic or allergenic effects to human health. The *R* genes themselves are not toxic even to *P. infestans*. These *R* genes encode proteins that trigger a hypersensitive response upon recognition of the late blight pathogen, leading to plant cell necrosis. The introduced genes are expressed by their endogenous promoters, thus they are predicted to have extremely low levels of expression, comparable to those from other endogenous resistance

genes. Due to the lack of any identified toxic effects of the NB-LRR class of *R* genes (and their protein products) we do not expect there to be any immediate or delayed effects on human health resulting from direct or indirect human interactions with the modified plants carrying late blight *R* genes.

Some of the transgenic lines included in this application will carry the *Oc-I∆D86* cystatin gene (Urwin et al, 1995) and a gene coding for a repellent peptide (Winter et al, 2002). The expression of these genes is targeted to the plant root system and confers resistance against potato cyst nematodes (PCN). Cystatins are not new to the human diet being present in many foods, e.g. rice seeds, maize kernels and chicken egg white (Benchabane et al, 2010; Colella et al, 1989). The lack of toxicity of the cystatin Oc-I∆D86 to mammals has already been established (Atkinson et al., 2004). It is readily degraded by boiling and upon exposure to simulated gastric fluid. Similarly, it is not an allergen (Meredith and Atkinson, 2000). The repellent to be used is not lethal to animals and its effect on nematodes is not via the oral route (Winter et al, 2002; Wang et al, 2011). It merely prevents plant parasitic species from invading roots. The repellent is not stable when heated in conditions equivalent to those required to cook potatoes for safe human consumption, and it is easily destroyed upon exposure to simulated intestinal fluid or nonsterile soil (Roderick et al, 2012). In addition, the peptide sequence is not flagged as a potential allergen (Roderick et al, 2012).

Some of the plants included in this application will contain a gene-silencing module. This module only includes potato sequences and its structure is such that it does not code for proteins. Its mode of action is based on using the endogenous post-transcriptional silencing machinery of plants to reduce the expression of the *Ppo* and *VInv* genes in tubers. No toxic or allergenic potential is therefore expected and nucleic acids (such as the endogenous RNA and DNA molecules of plants) are readily degraded by human digestive fluids (Liu et al, 2015). It is also worth noting that transgenic potatoes developed with an equivalent technology have been approved for commercialization in the US.

All the plasmids used to generate the plants included in this application carry the selectable marker gene *CSR*. *CSR* is an allele of the tomato acetolactate synthase (*ALS*) gene that has been cloned under the control of its native regulatory elements. It codes for a variant of the ALS enzyme that is resistant to inhibition by some herbicides (sulfonylureas and imidazolinones). Resistance to ALS-inhibiting herbicides is present in several commercially-available crops, including wheat, soybean, rice, canola and sunflower (Green and Owen, 2011; Hanson et al, 2014). In all of them, resistance is due to mutations in the *ALS* gene. This is also the case for the tomato *ALS* allele introduced in the plants proposed for release. Resistance to these herbicides has been typically achieved by traditional breeding methods but at least one transgenic event that

includes a resistant *ALS* allele has been deregulated in the US (Green and Owen, 2011). Therefore, no harmful effects are predicted to arise from the use of this marker gene.

Furthermore, linker sequences used to assemble the plasmids included in this application do not code for proteins and no toxic or allergenic potential is predicted.

Several measures have been taken to avoid backbone integration in the transgenic plants to which this application refers. In the unlikely event of backbone sequences being inserted, the only two protein-coding genes present in the vectors' backbones are the marker gene *nptII* and the *ipt* gene.
The marker gene *nptII* (or *aph(3')-IIa*) is under the control of a bacterial promoter and is used for bacterial selection only. It is expressed as an enzyme (aminoglycoside 3-phosphotransferase II or neomycin phosphotransferase II) that inactivates the antibiotics neomycin, kanamycin, geneticin (G418), and paromomycin by phosphorylation. The protein encoded by the gene has been shown to be bio-safe, non-toxic and poses no risk to human or animal health (The EFSA Journal, 2009, 1034: 66-82). No toxicity of the NPTII protein has been observed and in simulated digestive fluids this protein is rapidly degraded. The characteristics of the transgenic protein NPTII involve no outstanding safety issues and derived products are no more likely to cause adverse effects on human and animal health than conventional potato (The EFSA Journal, 2006, 323: 1-20).

The isopentenyl transferase (*ipt*) gene derives from the soil bacteria *A. tumefaciens*. This gene codes for an enzyme that catalyses the synthesis of the cytokinin isopentenyl adenosine, which naturally occurs in plants (Sakakibara et al, 2005). Plants have their own isopentenyl transferase genes for cytokinin production, some of which are expressed in edible parts of crops like maize kernels (Brugiere et al, 2008). In this case, the presence of the *ipt* gene in the vector backbone allows the counter-selection of plants where the backbone has been integrated. If the gene is normally expressed and the IPT enzyme produced, plants will display an abnormal development and will be discarded (Richael et al, 2008). If the backbone *ipt* gene is not significantly expressed due to positional effects or has been only partially inserted, it is possible that plants where parts of the backbone have been integrated 'scape' the counter-selection step. However, no harmful effects are expected in relation to this gene. The IPT enzyme sequence is not flagged as a potential allergen by Allergenonline (www.allergenonline.com). An '80mer Sliding Window Search' was carried out and it yielded no matches of significant identity. Such search is described as 'a precautionary search using a sliding window of 80 amino acid segments of each protein to find identities greater than 35% (according to CODEX Alimentarius guidelines, 2003)'. Finally, integration of coding sequences from *Agrobacterium*

spp. into plant genomes is a phenomenon that occurs in nature. For example, it has been recently described that the cultivated sweet potato's genome contains *Agrobacterium* T-DNA sequences with expressed genes (Kyndt et al, 2015).

In summary, none of the introduced genes encode for products that are known to be allergenic or toxic to humans either by ingestion or by contact. In addition to the absence of known harmful properties of any of the genetic elements present in the modified potatoes, no harmful properties are expected to emerge when the above-mentioned genes and traits are combined. The plants are not for human consumption and measures taken with regard to planting, harvest, storage and transportation of the plant material will minimize any contact with humans. The field trial will be isolated from human thoroughfares and after harvest, tubers will be destroyed or kept under contained conditions for experimental purposes. Thus, there will be no risk of the genetically modified material entering the food chain. Therefore the overall impact on human health is negligible. Finally, any evaluation of biosafety of transgenic potato crops to humans must be set in the context that these plants are a natural hazard as they naturally contain steroidal glycoalkaloids. The total content of such glycoalkaloids in tubers of varieties to be used for food should not exceed 20 mg / 100 g fresh weight (Krits et al, 2007).

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

The GM potatoes will not be used for animal feed. Potatoes are not grazed on by animals due to the toxic nature of alkaloids in the green parts of the plant, which are features of non-transgenic potato plants. Measures to be taken during the proposed trial will in any case protect the trial against damage by wild animals (e.g. fences) and also ensure that potato seed stock and plant material are harvested, stored, transported or disposed of (e.g. cleaning of machinery, packaging) in such a way to prevent contact with animals. Therefore the overall impact on animal health is negligible.

viii. 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).

The resistance (*R*) genes against late blight encode receptors that will recognize specific elicitors injected by the pathogen into the plant cell. This recognition will, through a signalling network, trigger both local and systemic defence responses. The local response aims at trapping the pathogen in the cells by localized cell death thus stopping further penetration and spread. Based on this mechanism of

response none of the newly-expressed *R* proteins are expected to be exuded from the plants to the soil. Due to a reduced need for fungal treatments an increase in the populations of other foliar pathogens and soil organisms might be expected.

Regarding the resistance against nematodes, previous works performed with similar transgenic lines established considerable advantages of this approach to soil micro-organisms relative to nematicide use, with no detrimental effects on non-target organisms and soil health (Cowgill et al, 2002a, 2002b, 2004; Cowgill and Atkinson, 2003; Celis et al, 2004; Kiezebrink and Atkinson, 2004; Green et al, 2012).

Further, the gene-silencing module present in some of the plants is designed to modify tuber quality traits that are important in post-harvest management and processing of the potato tubers. It is not expected to have any detrimental effect on soil health. Similarly, the selectable marker gene that confers resistance to sulfonylureas or imidazolinones, will be used only for the *in vitro* selection of transgenic lines during tissue culture. Herbicides based on sulfonylureas and imidazolinones as active ingredients will not be used in the context of this trial. Even if not intended for insertion, no effect on biogeochemical processes is expected from the genes present in the vector backbone either.

Thus, no detrimental effects on biogeochemical processes are anticipated for the plants described in this application other than those that may also apply to non-modified potato varieties under conventional agricultural practise. The overall impact on biogeochemical processes is negligible.

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

The trial will be conducted according to conventional agricultural practice except for a reduction in fungicide treatments in order to evaluate the efficacy of the introduced resistance genes against *P. infestans*. Differences in the scale of fungicide treatments are also standard practice either in conventional or organic agriculture or in plant protection trials conducted according to applicable agricultural practice. Alterations in fungicide use are likely to have implications on organisms associated with the plants, either present in the soil or on the plant leaves, possibly increasing the populations of both foliar pathogens, other than *P. infestans*, and soil organisms. Therefore, overall impact on the environment is negligible and is comparable to the effect of the cultivation of non-genetically modified potatoes with a potentially positive impact on soil and plant-associated microflora.

	Step1: ***Potential hazards which may be caused by the characteristics of the novel plant***	**Step 2:** ***Evaluation of how each hazard could be realised in the receiving environments***	**Step 3:** ***Evaluation of the magnitude of harm caused by each hazard if realised***	**Step 4:** ***Estimation of how likely/often each hazard will be realised as harm***	**Step 5:** ***Modification of management strategies to obtain lowest possible risks from the deliberate release***	**Step 6:** ***Overall estimate of risk of harm caused by the release for each hazard***
a	**Increased invasiveness in natural habitats or persistence in agricultural habitats.**	None of the genes introduced confer characteristics that add intrinsic competitive abilities in unmanaged ecosystems or allow the plants to compete against plants of similar type for space. None of the characteristics transferred to the potato plants are anticipated to affect pollen production and fertility, seed	*Negligible*. The introduced traits do not confer intrinsic competitive abilities in natural or agricultural habitats.	*Very unlikely.* Surviving, reproductive potato plants are rarely seen outside the field.	Conventional agricultural practice, volunteer management (monitoring for volunteers and removal/destruction of volunteers in the field), isolation distance and crop rotation.	Overall impact is negligible.

		dispersal or frost tolerance.				
b	**Selective advantage: improved resistance to *P. infestans.***	The intended effect of introducing late blight resistance genes is to improve the resistance against *P. infestans*. Therefore, a selective advantage is conferred in comparison to untreated non-resistant conventional potatoes if the pathogen is present in the field.	*Moderate.* Under *P. infestans* pressure, resistant potatoes are intended to have a selective advantage in comparison to untreated non-resistant conventional potatoes in the agricultural environment. This is acceptable and desired also under conventional agricultural practice, where it is usually achieved by fungicide treatment of potato fields.	*Likely.* The advantage is applicable only in the agricultural environment and only in those cases where no other plant protection measures against *P. infestans* are applied. Potato plants are rarely seen outside the field. Resistance to *P. infestans* is not the key determinant for potential invasiveness of potatoes.	Conventional agricultural practice and volunteer management (monitoring for volunteers and removal/destruction of volunteers).	Overall impact is negligible.

c	**Selective advantage: improved resistance to potato cyst nematodes**	The intended effect of the introduced cystatin- and repellent-coding genes is to increase resistance against potato cyst nematodes (PCN). Therefore, a selective advantage is conferred in comparison to untreated non-resistant conventional potatoes if the pathogen is present in the field.	*Negligible*. If PCN were present in the field, an increased resistance against this pathogen is acceptable and desired also under conventional agricultural practice, where nematicides and other forms of nematode control are applied.	*Very unlikely.* There is no evidence of presence of PCN in the experimental field.	Conventional agricultural practice and volunteer management (monitoring for volunteers and removal/destruction of volunteers).	Overall impact is negligible.
d	**Selective advantage: resistance to sulfonylureas and imidazolinones provided by the selectable marker gene (*CSR*)**	Herbicides based on sulfonylureas or imidazolinones will not be used in the context of this field trial.	*Negligible.* Plants containing the *CSR* selectable marker can be readily eliminated by other effective herbicides, such as glyphosate.	*Very unlikely.* Surviving, reproductive potato plants are rarely seen outside the field and plants containing the *CSR* selectable marker gene can be readily eliminated by other	Conventional agricultural practice and volunteer management (monitoring for volunteers and removal/destruction of volunteers). Herbicides based on sulfonylureas or	Overall impact is negligible.

				effective herbicides, such as glyphosate.	imidazolinones will not be used in the context of this field trial.	
e	**Selective advantage or disadvantage conferred to sexually compatible plant species**	Potato is a vegetatively propagated crop and none of the traits confer an intrinsic selective advantage in the agricultural environment under conventional agricultural practice.	*Negligible.* Neither of the traits confers an intrinsic selective advantage in the agricultural environment under conventional agricultural practice.	*Very unlikely.* Pollen transfer to other cultivated potatoes is possible, but unlikely due to short distance of pollen flow. There are two wild *Solanum* species in the UK but their cross fertilisation with potato crops has not been recorded. In the unlikely case that pollen is transferred to non-genetically modified potatoes, the consequences are negligible since potato is a vegetatively propagated crop. True potato seed is	Conventional agricultural practice, volunteer management (monitoring for volunteers and removal/destruction of volunteers in the field), and isolation distance to other potato crops.	Overall impact is negligible.

				not saved by growers.		
f	**Potential environmental impact due to interactions between the novel plant and target organisms**	The intended effect of the transferred resistance genes is to reduce the infection and reproductive success of *P. infestans* and/or PCN, thereby reducing the local population of those pathogens. As both *P. infestans* and PCN cause damaging crop diseases, this effect is beneficial.	*Moderate*. The intended effect of the genetic modification is to confer tolerance against the target organisms *P. infestans* and PCN if present in the field. This is desired also under conventional agricultural practice, where it is usually achieved by the use of fungicides and/or nematicides.	*Likely*. If present in the field, the population of the target organisms is expected to be reduced. This is desired also under conventional agricultural practice, where it is usually achieved by the use of fungicides and/or nematicides.	Monitoring plan including observations on disease and pest susceptibility, including any unintended or unexpected effects. Impact on *P. infestans* populations will be monitored as one of the main aims of the field trial.	Overall impact is negligible.
g	**Potential environmental impact due to interactions between the novel plant and non-target organisms**	No detrimental effect on non-target organisms is expected from the introduced genes. Any effect is anticipated to be comparable to that of non-genetically modified potatoes	*Negligible.* No detrimental effect on non-target organisms is expected from the introduced genes.	*Unlikely*. Any effect on non-target organism is anticipated to be comparable to that of non-genetically modified potatoes under conventional agricultural practice. Due to a reduced	Monitoring plan including observations on disease and pest susceptibility, including any unintended or unexpected effects.	Overall impact is negligible.

		under conventional agricultural practice.		need for anti-fungal treatments an increase in the populations of non-target organisms sensitive to fungicides might be expected.		
h	**Potential effect on human or animal health due to the introduced genes**	No detrimental effect on human or animal health is expected from the introduced genes.	*Negligible.* The introduced genes are not known to confer toxic or allergenic properties. The promoters used are predicted to drive expression of the introduced genes at a very low level and in some cases this expression will be targeted to specific parts of the plants.	*Very unlikely*. Material from the field trial is not intended for human/animal consumption.	Measures with regard to planting, harvest, storage and transportation minimize the contact with humans and animals.	Overall impact is negligible.
i	**Potential effects on biogeochemical processes (changes in soil decomposition of organic material)**	No detrimental effect on the soil is expected from the introduced genes.	*Negligible.* Soil fertility is not expected to be affected any differently due to the cultivation of the	*Unlikely*. Any effect is expected to be comparable to that of non-genetically modified potatoes under conventional	Conventional agricultural practice.	Overall impact is negligible.

			genetically modified potato plants as compared to conventional potatoes.	agricultural practice. Due to a reduced need for fungicide treatments, an increase in the populations of soil organisms sensitive to fungicides might be expected.		
j	**Possible environmental impact due to changes in cultivation practice**	Potential positive effects on the population of other foliar pathogens and soil organisms, due to a reduction in fungicide treatments.	*Negligible.* Application of conventional agricultural practice will be as for a conventional, non-transgenic crop, other than a reduction in anti-fungal treatments against *P. infestans*.	*Likely*. Potential positive effects on the populations of foliar pathogens other than *P. infestans* and on soil organisms sensitive to fungicides.	Conventional agricultural practice.	Overall impact is negligible. Potentially, there may be a positive impact on foliar and soil microflora.

Part A5: Assessment of commercial or confidentiality of information contained in this application.
Identify clearly any information that is considered to be commercially confidential. A clear justification for keeping information confidential must be given.

Not applicable.

Part A6: Statement on whether detailed information on the description of the GMO and the purpose of release has been published
Make a clear statement on whether a detailed description of the GMO and the purpose of the release have been published, and the bibliographic reference for any information so published.
This is intended to assist with the protection of the applicant's intellectual property rights, which may be affected by the prior publication of certain detailed information, e.g. by its inclusion on the public register.

None of the transgenic lines proposed for release in this application have been the subject of any publication. However, with exception of the *Rpi-amr1e* gene, the cloning and functional characterization of the two other late blight resistance genes (*Rpi-vnt1.1* and *Rpi-amr3i*) have been previously reported (Foster et al, 2009; Jones et al, 2014; Witek et al, 2016). Similarly, the nematode resistance genes encoding the modified rice cystatin and the repellent peptide have been the subject of several publications, including: Urwin et al (1995), Lilley et al (2004), Winter et al (2002), Lilley et al (2011) and Green et al (2012). Finally, the use of silencing modules targeting the *Ppo* and *VInv* genes for the improvement of tuber quality has been already presented in several publications (Rommens et al, 2006; Ye et al, 2010; Rommens et al, 2008).

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