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].

Events containing these genes have not previously been released.

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.

Rothamsted Research has received previous consents to release GM wheat: 97/R8/3, 01/R8/4, 11/R8/01, 14/R8/01, 16/R8/02, 21/R8/01

Part A4: Risk assessment and a statement on risk evaluation

Summary
Environmental risks

The probability of seeds escaping from the trial site or the transfer of inserted characteristics to sexually- compatible species outside the trial area is estimated as very low. Commercial wheat varieties do not establish easily or thrive in uncultivated environments and are naturally self-pollinating with out-crossing being a rare event.

Wheat seeds are relatively large and not normally dispersed by wind. Management measures including the use of gas guns and hawk kites will be employed to mitigate the risk of seed removal by birds.

Management procedures to minimise the spread of seeds or pollen will further reduce the probability of these events occurring. There will be no cereals grown for 20 metres from the boundary of the experimental plots and no sexually-compatible wild relatives of wheat exist in the vicinity. If out-crossing to plants outside the trial area where to somehow occur, selection pressure to maintain the genes in the environment would exist only where kanamycin-based herbicides were applied. Even if the up-regulation of PHYB resulted in significantly enhanced photosynthesis, the chances of successful establishment of these wheat plants in unmanaged ecosystems is extremely low.

The risk of non-sexual, horizontal gene transfer to other species is extremely low. In the event of horizontal gene transfer to bacteria, neither the trait gene nor the selectable marker genes would be expected to confer a selective advantage in the field environment under consideration. The area proposed to be planted with GMOs or GMO-segregating azygous plants is small; total area including spacing between plots is less than 500 m^2, and temporary (trial seasons lasting between 11 and 12 months at maximum, running from 2022 to 2027 only).

Although the above-ground plant material will be cleared from the site, the nptII gene contained in the plant root DNA will decompose into the soil. The transgene is fully integrated into the plant DNA and the copy number is low thus the nptII gene represents a very small proportion (much less than one millionth) of the total DNA in any one cell of our transformed wheat plants. This excess of competing DNA will significantly dilute the rate of any nptII natural bacterial transformation. In addition, enzymatic degradation of free plant DNA in the soil and the low level of spontaneous bacterial competence to take up free DNA will significantly reduce the incidence of natural transformation. Although the transfer of functional gene units from plants to soil bacteria is accepted to be extremely low under natural conditions (Schluëter et al 1995, Nielsen et al 1997, EFSA, 2009), it cannot be completely discounted that some bacteria may successfully take up the nptII gene. However, there will be no antibiotics applied to the soil to provide additional selection pressure for the gene to persist in the environment. The source of the nptII gene is the gut bacterium E. coli carrying a plasmid containing the transposable element (Tn 903). R plasmids possessing resistance to aminoglycoside antibiotics are already naturally found in the soil and other environments. The nptII gene encodes the enzyme Aminoglycoside 3'-phosphotransferase which confers resistance to kanamycin and related aminoglycoside antibiotics. Although these antibiotics still have some clinical applications, alternatives are readily available. Taken together, and bearing in mind the limited scope of this trial, the risk of generating of any additional antibiotic resistance within the soil microbial community or risks to human health or the environment if this were to occur as a result of the proposed trial is considered to be extremely low.

Human health risks

Observations on the general plant morphology of controlled environment-grown plants, timing of flowering and germination show that the PHYB-overexpressing transgenic wheat described here is indistinguishable from its non-GM equivalents except for the expected phenotype of enhanced PHYB expression and, under controlled growth conditions, increased total biomass and dry seed yield. No other changes to the plant morphology or development are apparent. The gene donor plants (*Triticum aestivum* and *Zoysia japonica*) are not known to be pathogenic or allergenic and neither the gene under investigation, nor the selectable marker genes are expected to result in the synthesis of products that are harmful to humans, or

other organisms or the environment. Any unknown hazards arising from the expression and ingestion of foreign proteins will not be realised because the wheat plants will not be consumed by humans. Thus the overall risk of harm to human health or the environmental arising from this trial is assessed as very low.

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.

Negligible. Please see table below for detailed assessment.

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

Very low. Please see table below for detailed assessment.

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.

Very low. Please see table below for detailed assessment.

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).

Not applicable.

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.

Not applicable.

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).

Neglible. Please see table below for detailed assessment.

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.

Very low. Please see table below for detailed assessment.

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).

Very unlikely. Please see table below for detailed assessment.

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.

Negligible. Please see table below for detailed assessment.

Step1: ***Potential hazards which may be causedby the characteristics of the novel plant***	**Step 2:** ***Evaluationof how above hazards could be realised in the receiving environments***	**Step 3:** ***Evaluation the magnitude of harm caused by each hazard if realised***	**Step 4:** ***Estimation of how likely/often each hazard will berealised as harm***	**Step 5:** ***Modification of management strategies to obtain lowest possiblerisks from the deliberate release***	**Step 6:** ***Overall estimate ofrisk caused by the release***
Increased invasiveness in natural habitats or persistence in agricultural habitats due to inserted trait.	Increased invasiveness may arise from intended or unintended effects of the genetic modificationthat resulted in wheat plants with a more 'weedy' habit that are better able to establish and thrive in uncultivated environments or to persist in agricultural habitats.	Wheat is an annual species that requires active management to out-compete weedier plants. Left unmanaged, wheat does not establish and survive in nature and thus has a low base line of invasiveness and persistence. Even if intended or unintended effects of the genetic modification resulted in major changes in invasiveness or persistence, itis considered that this would not result in significant environmental harm for agricultural or unmanaged ecosystems. Wheat is a benign plant that can be easilymanaged by cultivation or herbicides. The magnitude of harm if the hazard wasrealised is considered to be very small.	It is highly unlikely that intended or unintended effects of the genetic modification will result in major changes in invasiveness orpersistence. If it were to occur, this hazard would be realised only if seeds or pollen possessinggenes encoding these traits wereto spread from the trial site and successfully become established elsewhere. This is very unlikely as wheat pollen is relatively heavy so does not travel far, it has a short half-life and there areno sexually compatible species for out-crossing for at least 20m from the trial site. Seed removal from the site will be rigorously managed (see step 5). The chances of modified wheat plantsestablishing themselves outside the trial site are negligible.	Harvested seeds will be transported from the site insealed containers. Machinery will be cleaned thoroughly prior to removal from the site. There is a large buffer zone to minimize the spread of pollen. Surrounding the trialsite is a 20 metre area in which no cereals will be grown so it will be easy to see any cereal plants in the surrounding area. Appropriate physical barriers and/or deterrentswill be employed to minimise access by large mammals and birds. Kanamycin herbicides will not be used on the trial site.	Overall riskis negligible.

Selective advantage or disadvantage conferred to wheat or other sexually compatible plant species.	Selective advantage or disadvantage may result from the intended traits (improved photosynthetic rates and toleranceto Kanamycin antibiotics) or as a result of unintended effects of the genetic modification. Thesehazards could be realised in the receiving environment via dispersal of GM seeds from trial siteto the surrounding environment or via out-crossing to sexually-compatible species outside trial site.	The basal ability for commercial cereal crop varieties to survive in uncultivated environments is very low. We anticipate that the conferred trait of improved photosynthetic rates will provide only minor selective advantage compared to other factors determining a plant's ability to survive in unmanaged ecosystems. Thegenetic modification resulting in increased tolerance to kanamycin herbicides has thepotential to confer a major selective advantage only where those herbicides are used routinely.	This hazard would be realised only if seeds or pollen possessinggenes encoding these traits wereto spread from the trial site and successfully become established in environments were the appropriate selection pressures were present. This is very unlikely as wheat pollen is relatively heavy so does not travellong distances, it has a short half-life and there are no sexually compatible species for out-crossing for at least 20m from the trial site. Seed removal from the site will be rigorously managed. The use of kanamycin herbicides in the surrounding agricultural fields may be expected. Overall, the frequency of this hazard resulting in environmental harm is very low.	Harvested seeds will be transported from the site insealed containers. Machinery will be cleaned thoroughly prior to removal from the site. There is a large buffer zone to minimize the spread of pollen. Surrounding the trialsite is a 20 metre area in which no cereals will be grown so it will be easy to see any cereal plants in the surrounding area. Appropriate physical barriers and/or deterrentswill be employed to minimise access by large mammals and birds. Kanamycin herbicides will not be used on the trial site.	Overall riskis very low.

Potential effect on human or animal health due to the introduced phytochome B sequence	By contact or ingestion of GM plant material.	Although there are no robust toxicity data available for PHYB, it is considered thatthe magnitude of harm caused by contact,inhalation or ingestion of these GM plantsis negligible. PHYB is already consumed by humans and other animals when they eat leafy vegetables and other green plant parts. PHYB is a plant photoreceptor and as such is present in the cells of all photosynthetic plants. In the quantities produced by the GM plants, PHYB is not considered harmful.	Some contact between the GM plants and humans or animals is expected. People operating farmmachinery and scientists workingin the trial site will come into physical contact with the plants. Small mammals such as mice, invertebrates and birds may alsocome into contact and/or ingest plant material.	No plant material from thetrial will enter the food or animal feed chain. Appropriate physical barriers and/or deterrentswill be employed to minimise access by large mammals and birds. Machinery will be cleaned before being removed fromthe trial site	Overall riskis very low.
Potential direct effect on human or animal health due to introduced neomycin phosphotransf erase	By contact, inhalation or ingestion of GM plant material.	The magnitude of harm caused by contact, inhalation or ingestion of plant material containing NPTII is extremely low.The source organism for gene encoding this enzyme (*E. coli*) is present in the large intestine of healthy humans and any NPTII ingested is expected to be broken down by digestive enzymes in the stomach and small intestine. Although specific toxicity data on neomycin phosphotransferase I (also known as aminoglycoside 3'-phosphotransferase type 1) could not be found, there are several studies reported in scientific literature of the safety of a functionally related enzyme NPTII. For example, acute oral toxicity of NPTII was studied in mice that had received an oral dose of 100, 1000, or 5000 mg NPTII/kg bodyweight and subsequently monitoredfor adverse effects over the following	Some contact between the GM plants and humans or animals is expected. People operating farmmachinery and scientists workingin the trial site will come into physical contact with the plants. Small mammals such as mice, invertebrates and birds may alsocome into contact and/or ingest plant material.	No plant material from thetrial will enter the food or animal feed chain. Appropriate physical barriers and/or deterrentswill be employed to minimise access by large mammals and birds. Machinery will be cleaned before being removed fromthe trial site	Overall riskis very low.

		seven days. The authors concluded thatno treatment-related adverse health effects had occurred (Fuchs et al., 1993).			
Consideration of the potentialrisk of the *nptII* gene becoming more prevalentin the soil as a result of the trial	By decompositionof plant root DNA into the soil and natural transformation of competent microbes that subsequently became established in thesoil community.	Although the transfer of functional gene units from plants to soil bacteria is accepted to be extremely low under natural conditions (Schluëter et al 1995, Nielsen et al 1997, EFSA, 2009), it cannotbe completely discounted that some bacteria may successfully take up the *nptII*gene. However, there will be no antibioticsapplied to the soil to provide additional selection pressure for the gene to persist in the environment. The source of the *nptII*gene is the gut bacterium *E. coli* carrying a plasmid containing the transposable element (Tn 903). R plasmids possessing resistance to aminoglycoside antibiotics are already naturally found in the soil and other environments. The *nptII* gene encodes the enzyme Aminoglycoside 3'- phosphotransferase which confers resistance to kanamycin and related aminoglycoside antibiotics. Although these antibiotics still have some clinical applications, alternatives are readily available.	The transgene is fully integrated into the plant DNA and the copy number is low thus the *nptII* gene represents a very small proportion(much less than one millionth) of the total DNA in any one cell of our transformed wheat plants. This excess of competing DNA will significantly dilute the rate ofany *nptII* natural bacterial transformation. In addition, enzymatic degradation of free plant DNA in the soil and the lowlevel of spontaneous bacterial competence to take up free DNAwill significantly reduce the incidence of natural transformation.	Seeds and other above- ground plant biomass will be harvested and removed from the site. No antibioticswill be applied to the soil to provide additional selectionpressure for the gene to persist in the environment.	The risk of generatin gof any additional antibiotic resistance within the soil microbial communit yis considere dto be very low.

Potential effects on human or animal health due to horizontal gene transfer of recombinantDNA	By contact, ingestion or infection with bacteria that had received recombinant DNA via horizontal gene transfer.	The magnitude of harm caused by contact, ingestion or infection with bacteria that had received the recombinant DNA via horizontal gene transfer is low. The PHYB gene is not expected to be expressed in bacteria and would have no safety concern if they were. Horizontal gene transfer of a complete *nptII* fragment could confer functional antibiotic resistance to receivingbacteria. Some aminoglycoside antibioticsincluding kanamycin are important for clinical treatment, especially for second line treatment for multi-resistant tuberculosis (kanamycin) and in gut irrigation in, for example, encephalopathy (neomycin). However, this resistance is already widespread in the environment. The source of the *nptII* gene is the gut bacterium *E. coli* carrying a plasmid containing the transposable element (Tn 903). R plasmids possessing resistance to aminoglycoside antibiotics are already widespread in the soil.	The rate of horizontal gene transfer from genetically modifiedplants to other species is accepted to be extremely low (EFSA, 2009). However, the presence of plasmid backbone sequence and origins of replication which are derived from *E. coli* and *Agrobacterium tumefaciens*, increase the chances of homologous recombination between plant andmicrobial DNA in the soil. If recombinant DNA were to moveby horizontal transfer to soil bacteria, it is unlikely to significantly increase the prevalence of resistance to aminoglycoside antibiotics in the environment. The area proposed to be planted with GMOs is small;a total of 48 3mx1m plots and temporary (lasting between 11 and 12 months).	No plant material from thetrial will enter the food or animal feed chain. No antibiotics will be applied to the soil to giveselective advantage.	Overall riskis very low.
Consideration of the risk of horizontal gene transfer into wild-type *Agrobacterium* species in the soil that could infect and transfer DNA to other plant species including risksassociated with expression of	By DNA released from decomposing plant material being taken up into the T-DNA of wild-type *Agrobacterium* and the subsequent expression of functional cassettesin	In the very unlikely event that functional *nptII* and PHYB cassettes were integrated and expressed in transformed plant cells that subsequently led to production of functional NPTII or PHYB , itis theoretically possible that this may enhance the fitness of the transformed cells in these plants but only if the appropriate environmental selection pressures were present.	Horizontal gene transfer between plants and wild-type *Agrobacterium* species, and the subsequent infection of other plant species with recombinant DNA is considered an exceedingly small risk. Although transformation of wild type *Agrobacterium tumefaciens* has been reported in laboratory experiments using pre-inoculated sterile soil and high concentrations of circular	This risk will be managedby controlling weeds and not spraying with kanamycin-based herbicides. Seeds and other above-ground plant biomass will be harvested and removed from the site.	The risk ofthis is extremely low

the genes.	other plants after natural transformation by *Agrobacterium*.		Ti plasmid with appropriate antibioticselection (Demanèche et al 2001), no such demonstration hasbeen reported in the field or with linearised plant DNA with or without selection. Even in optimised laboratory conditions, electroporation or freeze-thaw methods are required to effectively transform *Agrobacterium spp* (Holsters 1978, Mattanovich et al 1989). It is considered highly unlikely that free DNA liberated by degradationof GM wheat roots in the soil would become stabilised in wild- type *Agrobacterium* and capable of autonomous replication. This could theoretically occur if the transgene insert liberated by decomposing roots was taken up by wild type *Agrobacterium* either as an intact plasmid or as a DNA fragment and subsequently incorporated into the resident Ti plasmid by for instance, homologous recombination. The former would stabilise only if the host *Agrobacterium* cell shared the same IncR compatibility group as the pSa origin of the transgenevector used in this trial. In the unlikely event that intact *nptII or* PHYB cassettes are recombined into the T-DNA regionof a virulent		

			Agrobacterium Ti plasmid, this homologous recombination event would inevitably result in all or part of the oncogene set on the T-DNA beinglost. Thus, even if this modified *Agrobacterium* successfully infected and transferred its T-DNA to wounded plant tissue, it is highly unlikely that a crown gall or hairy root phenotype would form. Whether disease symptoms were evident or not, the plant cells transformed by this wild-type *Agrobacterium* cell would be vegetative not germ-line so no vertical gene transfer of this recombinant DNA is possible		
Potential effects on biogeochemical processes (changes in soil decompositionof organic material	Changes in biogeochemical processes may result from unintended changes in the modified plants orsoil microbes due to horizontal transferof DNA.	The magnitude of harm is estimated to beextremely low. Biogeochemical processesare not expected to be affected by the cultivation of the genetically modified plants.	The frequency of changes to biogeochemical processes is considered to be very low. The area proposed to be planted with GMOs is small and temporary.	None.	It is very unlikely that changes in biogeoch e mical processes would occur.
Possible environmental impact due to changes in cultivation practice	This modification may result in higher yields.	The magnitude of any changes due to changes in cultivation practice will be negligible.	The frequency that this hazard may be realised is low. The number of plants with higher PHYB expression is small (less than half of the total growing area) and will be sown for a small number of growing seasons, ending 2026 (each season lasting between 11 and 12 months).	None.	Overall risk negligible.

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.

This is privately funded research that is core to Wild Bioscience's business model. Specific mention of gene names (PHYB) and promoter choices (PCK) are therefore commercially sensitive, and subject to a patent application (WO 2021/234370 A1).

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.

A description of the GMO and the purpose of the release have not yet been published.

References

Fuchs, R.L., Ream, J.E., Hammond, B.G., Naylor, M.W., Leimgruber, R.M. and Berberich, S.A., (1993). Safety assessment of the neomycin phosphotransferase-II (NPTII) protein. Bio/Technology 11, 1543-1547.

EFSA, 2009. Statement of EFSA on the consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the “Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants” and the Scientific Opinion of the GMO Panel on “Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants”. The EFSA Journal 1108, 1-8. DOI: 10.2903/j.efsa.2009.1108 http://www.efsa.europa.eu/en/efsajournal/pub/1108

Nielsen, K.M., Gebhard, F., Smalla, K., Bones, A.M. and Van Elsas, J.D. (1997) Evaluation of possible horizontal gene transfer from transgenic plants to the soil bacterium Acinetobacter calcoaceticus BD413. Theor. Appl. Genet. 95, 815-821. DOI: 10.1007/s001220050630
http://link.springer.com/article/10.1007/s001220050630

Schluëter, K., Fuëtterer, J. and Potrykus, I. (1995) Horizontal gene transfer from a transgenic potato line to a bacterial pathogen (Erwinia chrysanthemi) occurs - if at all - at an extremely low frequency. Biotechnology 13, 94-98.

Demanèche S, Kay E, Gourbière F and Simonet P 2001 Natural transformation of Pseudomonas fluorescens and Agrobacterium tumefaciens in soil. Appl. Environ. Microbiol. 67, 2617–2621. doi: 10.1128/AEM.67.6.2617-2621.2001

Holsters M, de Waele D, Depicker A, Messens E, van Montagu M, Schell J. 1978. Transfection and transformation of Agrobacterium tumefaciens. Mol Gen Genet. 163(2):181-7. OI: 10.1007/BF00267408
http://link.springer.com/article/10.1007/BF00267408

Mattanovich D, Rfllcer F, da Cflmara Machado A, Laimer M, Regner F, Steinkellner H, Himmler G and Katinger H 1989. Efficient transformation of Agrobacteruim spp. by electroporation. Nucleic Acids Research 17(16):6747.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC318385/
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC318385/