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].

Wheat lines overexpressing the wheat *TaVIT2* gene (with the high-molecular weight glutenin promoter) were previously released in 2019 and 2021 as part of consent 19/R52/02 (https://www.gov.uk/guidance/gm-inspectorate-deliberate-release-inspection-programme). Monitoring records have been completed for the 2019 growing season with subsequent two-year monitoring for 'volunteers' (germinated wheat seedlings). In 2021, the requirement to inspect the trial site and the 20 m border at least once a week during the period of cultivation was not fully met. The circumstances surrounding the oversight were discussed (inadvertently, no replacement sought during May half-term holidays), as was the interpretation of the consent conditions (with advice provided by the Defra Genetic Resources & GM Policy Team). Following this we put in place additional measures to maintain an appropriate frequency of monitoring in the future. To date, all additional monitoring visits have been once every week as required and post-monitoring of the site has began. In both years the Genetic Modification Inspectorate was content that no risks to human health or the environment, posed by the genetically modified organism, were identified.

The *OsNAS2* gene (with the maize *UBIQUITIN1* promoter) was previously released in Australia from 2015-2021 under DIR128 and DIR152 published by the Australian Government, Department of Health, Office of the Gene Technology Regulator.
https://www.ogtr.gov.au/gmo-dealings/dealings-involving-intentional-release/dir-128
https://www.ogtr.gov.au/gmo-dealings/dealings-involving-intentional-release/dir-152

In these documents, the risk management plan is describes as:
"*As the level of risk is assessed as negligible, specific risk treatment is not required. However, as this is a limited and controlled release, the licence includes limits on the size, locations and duration of the release, as well as controls including containment provisions at the trial site; prohibiting the use of GM plant materials in human food or animal feed; destroying GM plant materials not required for further studies; transporting GM plant materials in accordance with the Regulator's guidelines; and conducting post-harvest monitoring at the trial sites to ensure all GMOs are destroyed.*"

The results of the monitoring and risk management plan of DIR128 are reported in the DIR152 aplication (Section 7.1). This states that there were no reports of adverse effects on human health and safety or the environment resulting from the DIR128 release (which included the same maize *UBIQUITIN1* promoter and *OsNAS2* sequence as intended for the current UK release).

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.

The same team of reseachers at the John Innes Centre has previously applied and released GM wheat under consent 19/R52/02. The current application includes the same *TaVIT2* gene and promoter as used in 19/R52/02, and adds a second gene, *OsNAS2*, previously released in Australia under DIR128 and DIR152 (see part A2).

Part A4: Risk assessment and a statement on risk evaluation

Summary
Bearing in mind the limited scope of the trial and that the GM wheat will not be put into the human food chain or fed to livestock, the overall risk of harm to human health or the environmental arising from this trial is assessed as very low.

Environmental risks

The probability of seeds escaping from the trial site or transfer of inserted genetic material to sexually-compatible species outside the trial area is estimated as very low. Commercial wheat cultivars do not establish easily nor 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 procedures to minimise the spread of seeds or pollen will further reduce the probability of these events occurring (e.g 2 m pollen barrier surrounding trial site). Appropriate physical barriers (fenced growing area and full height netted framework over experimental planting) will be employed to prevent access by mammals and birds. 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.

It is highly unlikely that intended or unintended effects of the genetic modification will result in major changes in invasiveness or persistence. The two genes introduced into the plants proposed for release do not confer characteristics that would increase their competitiveness in unmanaged ecosystems. Previous field trials with transgenic

lines overexpressing either *TaVIT2* or *OsNAS2* individually did not identify increase in seed size, plant growth, or vigour. Apart from the expected phenotype, which is increased iron and zinc content in the grain endosperm, plants from the three proposed events (combining both *TaVIT2* and *OsNAS2* genes) are indistinguishable from untransformed controls, when grown in glasshouses or in controlled environment rooms. No other changes to the plant morphology or development are apparent. Plants remain sensitive to all herbicides such as glyphosate or glufosinate. The introduced genes are thus not anticipated to confer any intrinsic advantage compared to conventional wheat cultivars with respect to persistence in agricultural habitats or invasiveness in natural habitats and no emergent hazard is predicted.

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 plasmid backbone sequences, *nptI* gene, origins of replication, border sequences etc. come originally from *Escherichia coli* and *Agrobacterium tumefaciens*, two common gut and soil bacteria respectively and these sequences are already widespread in the soil metagenome. Although this makes potential homologous recombination events more likely, we estimate the likelihood of horizontal gene transfer as low and the consequences, were it to occur, negligible. The area proposed to be planted with GMOs is small (total area ≤50 m^2) and temporary lasting between 5 to 6 months during the three years (2022-2024).

Although the above-ground plant material will be cleared from the site and as many roots of the GM plants will be pulled as possible, the *nptI* gene contained in the remaining 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 *nptI* gene represents a very small proportion (much less than one millionth) of the total DNA in any one cell of the transformed wheat plants. This excess of competing DNA will significantly dilute the rate of any *nptI* 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 *nptI* 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 *nptI* 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 *nptI* 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

The gene donor organisms are cereals grown as staple crops. The *HMWG* promoter and *TaVIT2* coding sequences are from wheat (*Triticum aestivum*) cultivars. The *UBIQUTIN1* promoter seqeunce is from maize (*Zea mays*) and the NAS2 gene is from rice (*Oryza sativa*).Thus, people and other organisms have a long history of exposure to the gene products. These sequences are not known to be pathogenic or allergenic to humans, and none of the genes under investigation, or the selectable marker genes, are expected to result in the synthesis of products that are harmful to humans, other organisms or the environment. Any unknown hazards arising from the expression and ingestion of foreign proteins will not occur since the wheat plants and grains will not be consumed by humans nor will enter the animal food chain.

Apart from the *TaVIT2* and *OsNAS2* genes, the vector also includes the *nptI* and *Hyg* genes. The source organism for the gene encoding the hygromycin phosphotransferase (*Hyg*) enzyme, *Eschericia coli*, is present in the large intestine of healthy humans and there have been no reports of its adverse effects on humans, animals or plants. The product of the *Hyg* gene, hygromycin phosphotransferase, has been evaluated on numerous occasions by EFSA and found to raise no safety concerns. According to EFSA (EFSA 2009) genes conferring resistance to hygromycin are included in the first antibiotic resistance marker genes (ARMG) group. They state that, “with regard to safety there is no rationale for inhibiting or restricting the use of genes in this category, either for field experimentation or for the purpose of placing on the market.” The *neomycin phosphotransferase I* (*nptI*) gene is under the control of a bacterial promoter and is used for bacterial selection only (i.e. before they are used to transform plant cells). The source organism for the gene encoding this enzyme (*E. coli*) is present in the large intestine of healthy humans and any NPTI ingested is expected to be broken down by digestive enzymes in the stomach and small intestine. The expression of NPTI in plant cells is very unlikely and the gene is already widely present in the environment.

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.

Overall risk is negligible. It is highly unlikely that intended or unintended effects of the genetic modification resulting in increased iron and zinc in the grain will result in major changes in invasiveness or persistence. The genes introduced into the plants proposed for release do not confer characteristics that would increase the competitiveness of plants in unmanaged ecosystems. In field trials of plants containing either of the two introduced genes, or in controlled environment trials with the two genes combined, plants were indistinguishable from non-transformed controls in growth and fertility.

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

Overall risk is negligible. The transferred genes are not anticipated to affect pollen production and fertility nor seed dispersal. If it were to occur, this hazard would be realised only if seeds or pollen possessing genes encoding these traits were to 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 are no sexually compatible species for out-crossing for at least 20 m from the trial site. Seed removal from the site will be rigorously managed. The chances of modified wheat plants establishing themselves outside the trial site are negligible. The plants remain sensitive to all herbicides such as glyphosate or glufosinate, which will readily be used to eliminate them in the field. The introduced genes are thus not anticipated to confer any intrinsic advantage compared to conventional wheat cultivars with respect to persistence in agricultural habitats or invasiveness in natural habitats and no emergent hazard is predicted.

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.

Overall risk is negligible. This hazard would be realised only if seeds or pollen possessing genes encoding these traits were to spread from the trial site and successfully become established in environments were the appropriate selection pressures were present. We are unaware of any selective pressure which would benefit wheat plants with high iron uptake or wheat seeds with high iron content in the endosperm. Dispersal is very unlikely as wheat pollen is relatively heavy so does not travel long distances, it has a short half-life and there are no sexually compatible species for out-crossing for at least 20 m from the trial site. Seed removal from the site will be rigorously managed.

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).**

Overall risk is very low. We outline the potential effects of each gene within the construct in the table below.

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.**

Overall risk is very low. We outline this in more detail in the table below.

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).**

Overall risk is very low. We outline this in more detail in the table below.

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.**

Overall risk is negligible. The wheat grain harvested from the trial will not be used for general human or animal consumption.

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).**

It is very unlikely that changes in biogeochemical processes would occur.

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.**

Overall risk negligible.

	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	**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.**	Increased invasiveness may arise from intended or unintended effects of the genetic modification that resulted in wheat plants with a more weed-like 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 baseline of invasiveness and persistence. Even if intended or unintended effects of the genetic modification resulted in major changes in invasiveness or	It is highly unlikely that intended or unintended effects of the genetic modification of increased plant iron uptake and endosperm iron content will result in major changes in invasiveness or persistence. The gene introduced into the plants proposed for release do not confer characteristics that would increase the	Harvested seeds will be transported from the site in sealed containers. Any equipment used during the growing season, including for planting and harvesting of transgenic material, will be thoroughly cleaned after use and before it is allowed to leave the release site. All machinery (including wheels and tyres) used on the trial site	Overall risk is negligible.

			persistence, it is considered that this would not result in significant environmental harm for agricultural or unmanaged ecosystems. Wheat is a benign plant that can be easily managed by cultivation or herbicides. The magnitude of harm if the hazard was realised is considered to be very small.	competitiveness of plants in unmanaged ecosystems. Neither would the gene enable plants carrying them to out-compete plants of similar type for space. The transferred gene is not anticipated to affect pollen production and fertility nor seed dispersal. If it were to occur, this hazard would be realised only if seeds or pollen possessing genes encoding these traits were to spread from the trial site and successfully become established elsewhere. This is very unlikely as wheat pollen is relatively heavy so	will be cleaned thoroughly before leaving the trial site. We will include a 2m pollen barrier surrounding the site and there is a large buffer zone to minimize the spread of pollen. Surrounding the trial site is a 20 m area in which no cereals will be grown so it will be easy to identify any cereal plants in the surrounding area. Appropriate physical barriers (fenced growing area and full height netted framework over experimental planting throughout the growing season) will be employed to	

				does not travel far, it has a short half-life and there are no sexually compatible species for out-crossing for at least 20 m from the trial site. Seed removal from the site will be rigorously managed. The chances of modified wheat plants establishing themselves outside the trial site are negligible. The transgenic plants proposed for release will also possess two antibiotic resistance genes (*nptI* and *Hyg*) and we have assumed that these are integrated into the plant genomic DNA along with the genes of interest. These anitibiotic	prevent access by mammals and birds.	

				resistance traits will be used only for the *in vitro* selection of transgenic lines during tissue culture. No effect in persistence or invasiveness is expected from any of the elements in the vector backbone (in addition to the *nptI* gene described above). No antibiotics will be used in the field site. The plants remain sensitive to all herbicides such as glyphosate or glufosinate, which will readily be used to eliminate them in the field. The introduced genes are thus not anticipated to confer any intrinsic		

				advantage compared to conventional wheat cultivars with respect to persistence in agricultural habitats or invasiveness in natural habitats and no emergent hazard is predicted.		
b	**Selective advantage or disadvantage conferred to wheat or other sexually compatible plant species.**	Selective advantage or disadvantage may result from the intended traits (increased iron uptake in the plant and increased iron content in endosperm) or as a result of unintended effects of the genetic modification. These hazards could be realised in the receiving environment via dispersal of GM	The basal ability for commercial cereal crop cultivars to survive in uncultivated environments is very low. We anticipate that the conferred trait of increased uptake in the plant and increased iron content in the endosperm will not provide any selective advantage compared to other factors determining a	This hazard would be realised only if seeds or pollen possessing genes encoding these traits were to spread from the trial site and successfully become established in environments were the appropriate selection pressures were present. We are unaware of any selective pressure which would benefit wheat seeds with	Harvested seeds will be transported from the site in sealed containers. Any equipment used during the growing season, including for planting and harvesting of transgenic material, will be thoroughly cleaned after use and before it is allowed to leave the release site. All machinery (including wheels and tyres)	Overall risk is negligible.

		seeds from trial site to the surrounding environment or via out-crossing to sexually-compatible species outside trial site.	plant's ability to survive in unmanaged ecosystems.	high iron content in the endosperm. Dispersal is very unlikely as wheat pollen is relatively heavy so does not travel long distances, it has a short half-life and there are no sexually compatible species for out-crossing for at least 20 m from the trial site. We will also surround the trial with a 2m pollen barrier. Seed removal from the site will be rigorously managed.	used on the trial site will be cleaned thoroughly before leaving the trial site. We will include a 2m pollen barrier surrounding the site and there is a large buffer zone to minimize the spread of pollen. Surrounding the trial site is a 20 m area in which no cereals will be grown so it will be easy to identify any cereal plants in the surrounding area. Appropriate physical barriers (fenced growing area and full height netted framework over experimental planting throughout the growing season) will be employed to	

					prevent access by mammals and birds.	
c	**Potential effect on human or animal health due to introduced wheat *Vaculoar Iron Transporter 2* (*TaVIT2*) gene**	By contact or ingestion of GM plant material.	Although there are no robust toxicity data available for the VIT2 protein, it is considered that the magnitude of harm caused by contact, inhalation or ingestion of these GM plants is negligible. The VIT2 protein is already consumed by humans and other animals when they eat leafy vegetables and other green plant parts. The VIT2 protein occurs naturally in wheat and across many other plants and fungi. In the previous two years of field trials with *TaVIT2*	Some contact between the GM plants and humans or animals is expected. People operating farm equipment and scientists working in the trial site will come into physical contact with the plants. Small mammals such as mice, invertebrates and birds may also come into contact and/or ingest plant material.	(i) The wheat grain harvested from the trial is not intended for general human or animal consumption. (ii) Appropriate physical barriers and/or deterrents will be employed to minimise access by large mammals and birds. (iii) Equipment will be thoroughly cleaned before being removed from the trial site.	Overall risk is very low

			(19/R52/02), no risks to human health posed by the genetically modified organism were identified.			
d	**Potential effect on human or animal health due to introduced rice *Nicotianamine synthase 2* (*OsNAS2*) gene**	By contact or ingestion of GM plant material.	Although there are no robust toxicity data available for the NAS2 protein, it is considered that the magnitude of harm caused by contact, inhalation or ingestion of these GM plants is negligible. The NAS2 protein is already consumed by humans and other animals when they eat leafy	Some contact between the GM plants and humans or animals is expected. People operating farm equipment and scientists working in the trial site will come into physical contact with the plants. Small mammals such as mice, invertebrates and birds may also come into contact	(i) The wheat grain harvested from the trial is not intended for general human or animal consumption. (ii) Appropriate physical barriers and/or deterrents will be employed to minimise access by large mammals and birds. (iii) Equipment will be thoroughly cleaned before being removed from the trial site.	Overall risk is very low.

			vegetables and other green plant parts. The NAS2 protein occurs naturally in rice and across many other plants and fungi. In the previous release in Australia (RID128), no reports of adverse effects on human health were reported for the NAS2 protein.	and/or ingest plant material.		
e	**Potential effect on human or animal health due to introduced *hygromycin phosphotransferase* (*Hyg*) gene.**	By contact or ingestion of GM plant material.	The magnitude of harm caused by contact, inhalation or ingestion of HYG in these GM plants is extremely low. The source organism for the gene encoding this enzyme (*E. coli*) is present in the large intestine of healthy humans and there have been no reports of its adverse	Some contact between the GM plants and humans or animals is expected. People operating farm equipment and scientists working in the trial site will come into physical contact with the plants. Small mammals such as mice, invertebrates	(i) The wheat grain harvested from the trial is not intended for general human or animal consumption. (ii) Appropriate physical barriers and/or deterrents will be employed to minimise access by large mammals and birds. (iii) Equipment will be thoroughly cleaned before	Overall risk is very low.

			effects on humans, animals or plants. The product of the *Hyg* gene, hygromycin phosphotransferase, has been evaluated on numerous occasions by EFSA and found to raise no safety concerns. According to EFSA (EFSA 2009) genes conferring resistance to hygromycin are included in the first antibiotic resistance marker genes (ARMG) group. They state that, “with regard to safety there is no rationale for inhibiting or restricting the use of genes in this category, either for field experimentation or for the purpose of	and birds may also come into contact and/or ingest plant material.	being removed from the trial site.	

			placing on the market."			
f	**Potential direct effect on human or animal health due to introduced *neomycin phosphotransferase* (*NPTI*) gene.**	By contact or ingestion of GM plant material.	The magnitude of harm caused by contact, inhalation or ingestion of plant material containing NPTI is extremely low. The source organism for gene encoding this enzyme (*E. coli*) is present in the large intestine of healthy humans and any NPTI ingested is expected to be broken down by digestive enzymes in the stomach and small intestine. The expression of NPTI in plant cells is very unlikely and the gene is already widely present in the environment. Although specific	The frequency of exposure is very low. The promoter driving expression of the NPTI gene is prokaryote-specific so NPTI protein will not be present in the modified plants.	(i) The wheat grain harvested from the trial is not intended for general human or animal consumption. (ii) Appropriate physical barriers and/or deterrents will be employed to minimise access by large mammals and birds. (iii) Equipment will be thoroughly cleaned before being removed from the trial site.	Overall risk is very low.

			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 monitored for adverse effects over the following seven days. The authors concluded that no treatment-related			

			adverse health effects had occurred (Fuchs et al. 1993). NPTII is also classified alongside the *Hyg* gene in the EFSA guidelines.			
g	**Consideration of the potential risk of the *NPTI* gene becoming more prevalent in the soil as a result of the trial**	By decomposition of plant root DNA into the soil and natural transformation of competent microbes that subsequently became established in the soil 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 cannot be completely discounted that some bacteria may successfully take up the nptI gene. However, there will be no antibiotics applied to the soil to provide additional selection pressure	The transgene is fully integrated into the plant DNA and the copy number is low thus the *nptI* 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 *nptI* natural bacterial transformation. In addition, enzymatic degradation of free plant DNA in the soil and the low level of	Seeds and most above-ground plant biomass will be harvested and removed from the site. No antibiotics will be applied to the soil to provide additional selection pressure for the gene to persist in the environment.	The risk of generating additional antibiotic resistance within the soil microbial community is considered to be very low.

			for the gene to persist in the environment. The source of the *nptI* 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 *nptI* gene encodes the enzyme aminoglycoside 3'-phosphotransferase which confers resistance to kanamycin and related aminoglycoside antibiotics. Although these antibiotics still	spontaneous bacterial competence to take up free DNA will significantly reduce the incidence of natural transformation.		

			have some clinical applications, alternatives are readily available.			
h	**Potential effects on human or animal health due to horizontal gene transfer of recombinant DNA**	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 *TaVIT2* and *OsNAS2* genes are not expected to be expressed in bacteria and would have no safety concern if they were given the presence of these genes in many plants and fungi. Horizontal gene transfer of a complete *nptI* fragment could confer functional	The rate of horizontal gene transfer from genetically modified plants 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 and microbial DNA in the soil. If recombinant DNA were to move	The wheat grain harvested from the trial is not intended for general human or animal consumption. No antibiotics will be applied to the soil to provide additional selection pressure for the gene to persist in the environment.	Overall risk is very low.

			antibiotic resistance to receiving bacteria. Some aminoglycoside antibiotics including 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 *nptI* gene is the gut bacterium *E. coli* carrying a plasmid containing the transposable element (Tn 903). R	by 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; ≤50 m^2 and temporary (lasting between 5 to 6 months) during each of the three proposed years.		

			plasmids possessing resistance to aminoglycoside antibiotics are already widespread in the soil.			
i	**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 risks associated with expression of the genes.**	By DNA released from decomposing plant material being taken up into the T-DNA of wild-type *Agrobacterium* and the subsequent expression of functional cassettes in other plants after natural transformation by *Agrobacterium*.	In the very unlikely event that functional *Hyg, TaVIT2* or *OsNAS2* cassettes were integrated and expressed in transformed plant cells that subsequently led to production of functional HYG, VIT2 or NAS2, it is 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	This risk will be managed by not applying antobiotics to the field site. Seeds, the above-ground plant biomass and as many roots of the GM plants will be harvested as possible and removed from the site.	Overall risk is very low.

				circular Ti plasmid with appropriate antibiotic selection (Demanèche et al 2001), no such demonstration has been 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 et al 1978, Mattanovich et al 1989). It is considered highly unlikely that free DNA liberated by degradation of 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 transgene vector		

				used in this trial. In the unlikely event that intact *Hyg*, *TaVIT2* or *OsNAS2* cassettes are recombined into the T-DNA region of a virulent *Agrobacterium* Ti plasmid, this homologous recombination event would inevitably result in all or part of the oncogene set on the T-DNA being lost. 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 germline so no vertical gene transfer of this recombinant DNA is possible.		
j	**Potential effects on biogeochemical processes (changes in soil decomposition of organic material)**	Changes in biogeochemical processes may result from unintended changes in the modified plants or from unintended changes in soil microbes due to horizontal transfer of DNA.	The magnitude of harm is estimated to be extremely low. Biogeochemical processes are not expected to be affected by the cultivation of the transgenic plants.	The frequency of changes to biogeochemical processes is considered to be very low. The area proposed to be planted with GMOs is small; ≤50 m^2 and temporary (lasting between 5 to 6 months) during each of the three proposed years.	None.	It is very unlikely that changes in biogeochemical processes would occur.
k	**Possible environmental impact due to**	This modification may result in higher iron uptake by the plant and higher iron	Negligible. Application of conventional agricultural practice	The likelihood of changes to cultivation practices is considered to be	Conventional agricultural practice.	Overall risk negligible.

	changes in cultivation practice	content in wheat grain endosperm.	will be as for a conventional, non-transgenic crop.	very low. The area proposed to be planted with GMOs is small; ≤50 m^2 and temporary (lasting between 5 to 6 months) during each of the three proposed years.		

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.

There is no confidential information included in this application. All the work reported here has been publically funded, has no associated commercial confidentiality considerations, and has been published open access in Connorton et al 2017, Balk, et al 2019, Beasley et al 2019, or is available online in the DEFRA website (https://www.gov.uk/government/publications/genetically-modified-organisms-john-innes-centre-19r5202) or the Australian government website (https://www.ogtr.gov.au/what-weve-approved/dealings-involving-intentional-release).

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 *TaVIT2* lines has been described in the publication Connorton et al 2017 and the description of the *OsNAS2* lines has been described in Beasley et al 2019. The purpose of the release has not yet been published, but our overall objective of biofortifying wheat flour by combining *TaVIT2* and *OsNAS2* is public knowledge and has been done on collaboration with academic partners in Australia.

References

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