Department for Environment, Food and Rural Affairs

Part B: Information about the release application to be included on the public register

B1 The name and address of the applicant

Rothamsted Research,
West Common, Harpenden,
Hertfordshire,
AL5 2JQ
UK

B2 A general description of the genetically modified organisms in relation to which the application is being made

The genetically modified plants to be studied in the field trial are wheat (*Triticum aestivum*), variety Cadenza, in which one or more copies of an asparagine synthetase gene, *TaASN2*, has been rendered dysfunctional using the CRISPR/Cas9 system for genome editing. The editing process involved the transformation of the wheat with plasmids containing a gene encoding the guide RNAs for the CRISPR/Cas9 system and a gene encoding the Cas9 nuclease, as well as a selectable marker gene (*bar*) that imparts tolerance to herbicides based on phosphinothrycin. These transgenes are no longer required and are being removed by genetic segregation. Nevertheless, the plants in the first year of the trial will contain one or more of these transgenes, as well as the CRISPR/Cas9-induced 'edits' to the *TaASN2* genes. It is anticipated that by the third year of the trial the plants will be transgene-free. Plants carrying edits in the *TaASN2* gene have reduced concentrations of free (soluble, non-protein) asparagine in the grain. Free asparagine is an amino acid that can be converted to acrylamide, a carcinogenic contaminant, during baking and toasting.

B3 The location at which the genetically modified organisms are proposed to be released

The field trial will be sited within the GM field trial site at Rothamsted Research, Harpenden, UK, grid reference TL1213.

B4 The purpose for which the genetically modified organisms are proposed to be released (including any future use to which they are intended to be put).

This is a research trial to investigate the effect of knocking out the *TaASN2* gene on free asparagine accumulation in wheat grain in the field, and the effect of low grain asparagine concentration on grain yield, grain size (thousand grain weight), grain protein content and quality, the concentrations of other free amino acids, total seed nitrogen and sulphur, Hagberg Falling Number, starch and sugar content. The formation of acrylamide in heated flour produced from grain harvested from the trial

will also be assessed. The number of plants that can be grown in the field as opposed to a glasshouse will also facilitate the identification of plants in which the transgenes have segregated away. At this stage it is not known whether plants carrying the very low asparagine trait would be suitable for commercial cultivation, and there is still uncertainty over how genome edited plants will be regulated in the UK. Decisions on whether the genome edited plants should be made available to breeders for incorporation into commercial breeding lines will therefore be taken at the end of the trial.

B5 The intended dates of the release.

The trial will run for five seasons, from autumn 2021 to summer 2026. The plants will be sown in September/October and harvested in August/September each year.

B6 The environmental risk assessment.

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.

Plants in which the *bar* gene is still present will be resistant to control by herbicides based on phosphinothrycin. However, control by herbicides based on other active ingredients will not be affected. The major trait in the plants is reduced accumulation of free asparagine in the grain. This appears to have a negative effect on germination, and there is some evidence of increased seed size in some of the edited lines. Otherwise the phenotype of the edited lines, including morphology, pollination and seed-set, do not appear to differ from control wheat cv. Cadenza plants. We therefore expect dissemination of pollen and seeds to be the same as for non-transgenic wheat plants, and the survivability of the plants in unmanaged systems to be reduced due to the germination effect.

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

As stated above, germination of the low asparagine lines is poor, and this is likely to confer a selective disadvantage.

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.

Wheat is a self-pollinating crop with very low rates of cross-pollination with other wheat plants. It can be forced, using laboratory techniques, to cross with rye, Triticale and a limited number of other cereals but spontaneous crossing in the field is extremely rare if it occurs at all.

The only wild relatives of wheat commonly found in the UK are in the genera *Elymus* and *Elytrigia* (formerly known as *Agropyron*) and there are no reports of cross-hybridisation between wheat and these genera. The two most common inland species are common couch (*Elytrigia repens* (= *Agropyron repens*)) and bearded couch (*Elymus caninus* (= *Agropyron caninum*)). Other related species, such as sand couch (*Elytrigia juncea* (= *Agropyron junceum*)), sea couch (*Elytrigia atherica* (= *Agropyron pycnanthum*)) and hybrids are largely confined to coastal habitats.

Common couch is quite widespread on the Rothamsted estate whereas bearded couch is confined to woods and hedgerows. Common couch propagates primarily by vegetative reproduction (rhizomes), rather than by sexual reproduction, and in any case, no reports of wheat × couch spontaneous hybrids have been reported.

Nevertheless, the outer edge of the trial will have a 3 m barrier of non-GM wheat to function as a pollen barrier, and no wheat or other cereals will be cultivated within 20 m of the trial. Common couch and other grasses will be controlled along with other weeds in and around the trial site using standard farm practices, and will not be allowed to grow within 20 m of the trial.

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: There are no target organisms.

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.

Wheat plants have a range of pests and fungal pathogens. The main insect pests in the UK are the bird cherry-oat aphid (*Rhopalosiphum padi*), the grain aphid (*Sitobion avenae*) and the rose grain aphid (*Metopolophium dirhodum*), as well as the orange wheat blossom midge (*Sitodiplosis mosellana*) and the wheat bulb fly (*Delia coarctata*). Wheat also interacts with beneficial insects that attack aphid pests, such as *Aphidius rhopalosiphi*.

Wheat can become infected by several fungal pathogens in the UK, including

Septoria tritici (*Mycosphaerella graminicola*), yellow rust (*Puccinia striiformis*) and brown rust (*Puccinia triticina*), as well as take-all disease (*Gaeumannomyces graminis* var. *tritici*). Good phytosanitary practice has been shown to be important in preventing the accumulation of high concentrations of free asparagine in the grain, but the relationship between pathogen infection and asparagine metabolism requires further research if the mechanisms underpinning this observation are to be understood. Even so, we consider it extremely unlikely that reduced free asparagine concentration in the grain will make the wheat more resistant to fungal pathogens.

Wheat also interacts with multiple fungi, bacteria and protists in the rhizospere, but these interactions are not expected to be affected in any way by the traits carried by the plants.

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).

The *gRNA*, *Cas9* and *bar* genes are not expected to result in the synthesis of products that are harmful to human health. The primary trait of reduced free asparagine concentration in the grain will have no effect on human health. 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.

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 *gRNA*, *Cas9* and *bar* genes are not expected to result in the synthesis of products that are harmful to animal health. The primary trait of reduced free asparagine concentration in the grain will have no effect on animal health. Any unknown hazards arising from the expression and ingestion of foreign proteins by domestic or farm animals will not be realised because the wheat plants will not be used for animal feed. The site is enclosed by a fence to deter entry by rabbits or other large mammals, and bird scaring devices including gas guns and hawk kites will be used to keep out birds.

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).

Biogeochemical processes are not expected to be affected by the cultivation of the genetically modified plants.

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 site will be prepared according to standard agronomic practices for winter wheat cultivation. At harvest, a sample of plants may be hand-harvested, conditioned and threshed to supply seeds for research purposes. All such small samples removed from the trial site will be stored in containment prior to use and will eventually be autoclaved before disposal. The remainder of the site will be harvested by the plot combine. Grain that is not required for analysis or to provide seed for future trials will be disposed of by incineration, autoclaving or deep burial at a local authority-approved landfill site by an approved contractor, while any material remaining after analysis will be autoclaved before disposal. All straw will be chopped and left on site. The combine will be cleaned prior to leaving the site so that all traces of plant material from the trial will remain in the trial area. All transport of material will be logged.

Once the trial is concluded, or when the site is moved from one year to the next (Part A1, Section 36), the trial area will remain in stubble for the following two years to enable monitoring of volunteers and a broad spectrum herbicide such as glyphosate will be applied as required.

Any environmental effects resulting from these procedures will be 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	**Increased invasiveness in natural habitats or persistence in agricultural habitats.**	Increased invasiveness may arise from intended or unintended effects of the genetic modification that result 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, 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 were realised is,	It is highly unlikely that intended or unintended effects of the genetic modification will result in major changes in invasiveness or persistence. 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 become established elsewhere. This is very unlikely as wheat pollen is relatively heavy so does not travel far, and it has a short half-life. Cereals and grasses will not be allowed to grow within 20 m of the trial site, and spontaneous crossing between wheat and its closest wild relatives in the UK has not been observed. Seed removal from the site will be rigorously managed. The chances of modified	Harvested seeds will be transported from the site in sealed containers. Machinery will be cleaned thoroughly prior to removal from the site. The large buffer zone will minimize the spread of pollen. Surrounding the trial site is a 20 metre area in which no cereals or wild grasses will be allowed to grow. Appropriate physical barriers and/or deterrents will be employed to minimise access by large mammals and birds. Phosphinothrycin-based herbicides will not be used on the trial site.	Overall risk is negligible.

			therefore, considered to be very small.	wheat plants establishing themselves outside the trial site are considered to be negligible.		
b	**Selective advantage or disadvantage conferred to sexually compatible plant species**	Selective advantage or disadvantage may result from intended or unintended effects of the genetic modification. This hazard could be realised in the receiving environment *via* out-crossing to sexually-compatible species outside the trial site.	The trait of low asparagine concentration in the grain is associated with poor germination and would therefore be likely to confer a selective disadvantage. The genetic modification resulting in increased tolerance to phosphinothrycin-based herbicides has the potential to confer a major selective advantage in agricultural systems where those herbicides are used routinely but will have no substantial effect on a plant's ability to survive in unmanaged ecosystems.	This hazard would be realised only if pollen possessing genes encoding these traits were to spread from the trial site and become established. This is very unlikely as wheat pollen is relatively heavy so does not travel long distances, and it has a short half-life. Cereals and grasses will not be allowed to grow within 20m of the trial site, and spontaneous crossing between wheat and its closest wild relatives in the UK has not been observed. The likelihood of this hazard resulting in environmental harm is, therefore, considered to be extremely low.	There is a large buffer zone to minimize the spread of pollen. Surrounding the trial site is a 20 metre area in which no cereals or grasses will be allowed to grow. Appropriate physical barriers and/or deterrents will be employed to minimise access by large mammals and birds. Phosphinothrycin-based herbicides will not be used on the trial site.	Overall risk is extremely low.
c	**Potential environmental impact due to interactions between the novel**	This hazard could not be realised because there are no target organisms.				No risk.

	plant and target organisms					
d	**Potential environmental impact due to interactions between the novel plant and non-target organisms**	Changes in the plants' interactions with non-target organisms could result from intended or unintended effects of the genetic modification. This could have an environmental impact if changes in interactions with non-target organisms resulted in the plants being better able to thrive in uncultivated environments or to persist in agricultural habitats.	Wheat plants have a range of pests and fungal pathogens. The main insect pests in the UK are the bird cherry-oat aphid (*Rhopalosiphum padi*), the grain aphid (*Sitobion avenae*) and the rose grain aphid (*Metopolophium dirhodum*), as well as the orange wheat blossom midge (*Sitodiplosis mosellana*) and the wheat bulb fly (*Delia coarctata*). Wheat also interacts with beneficial insects that attack aphid pests, such as *Aphidius rhopalosiphi*. We consider it extremely unlikely that the genetic modification will affect these non-target organisms in any way. Wheat can become infected by several fungal pathogens in the UK, including *Septoria tritici* (*Mycosphaerella graminicola*), yellow rust (*Puccinia striiformis*) and brown rust (*Puccinia triticina*), as well as take-all disease (*Gaeumannomyces graminis*	Impacts of this hazard on the environment would only occur if seeds or pollen from the GM plants were to spread from the trial site and become established. Pollen spread is very unlikely because wheat pollen is relatively heavy and does not travel long distances, and it has a short half-life. Cereals and grasses will not be allowed to grow within 20 m of the trial site, and spontaneous crossing between wheat and its closest wild relatives in the UK has not been observed. Seed removal from the site will be rigorously managed. The likelihood of this hazard resulting in environmental harm is considered to be extremely low.	Harvested seeds will be transported from the site in sealed 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 trial site is a 20 metre area in which no cereals or grasses will be allowed to grow. Appropriate physical barriers and/or deterrents will be employed to minimise access by large mammals and birds, to prevent them spreading seeds. Phosphinothrycin-based herbicides will not be used on the trial site.	Overall risk is extremely low.

			var. tritici). Good phytosanitary practice has been shown to be important in preventing the accumulation of high concentrations of free asparagine in the grain, but the mechanisms underpinning this observation require further research. Even so, we consider it extremely unlikely that reduced free asparagine concentration in the grain will make the wheat more resistant to fungal pathogens. Wheat also interacts with multiple fungi, bacteria and protists in the rhizospere, but these interactions are not expected to be affected in any way by the traits carried by the plants.			
e	**Potential effect on human or animal health due to the introduced genes**	By contact or ingestion of GM plant material.	The *gRNA*, *Cas9* and *bar* genes are not expected to result in the synthesis of products that are harmful to human health. The primary trait of reduced free asparagine concentration in the grain will have no effect on human or animal health. Any unknown hazards with respect to human health arising from	Some contact between the GM plants and humans is inevitable. People operating farm machinery and scientists working in the trial site will come into physical contact with the plants. However, it is extremely unlikely that they will ingest any plant material. It is more likely that small mammals	No plant material from the trial will enter the food or animal feed chain.	Overall risk is extremely low.

			the expression and ingestion of foreign proteins will not be realised because the wheat plants will not be consumed by humans.	such as mice, invertebrates and birds may come into contact and/or ingest plant material.		
f	**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 GM plants.	The likelihood of changes to biogeochemical processes is considered to be very low. The area proposed to be planted with GMOs is small (a total of 50 1.8 × 6m plots) and temporary (lasting 10-12 months in each year of the five growing seasons of the trial).	No specific management strategies are planned to address this risk.	Overall risk is extremely low.
g	**Possible environmental impact due to changes in cultivation practice**	The cultivation practices applied to the trial are likely to have negligible environmental impact.	The magnitude of any effects arising from changes in cultivation practice will be negligible.	The frequency that this hazard may be realised is low. The trial comprises only 50 × (6 × 1.8m) plots, and will be sown for only five growing seasons.	None.	Overall risk is extremely low.

B7 The methods and plans for monitoring the genetically modified organisms and for responding to an emergency.

The site will be monitored regularly (at least weekly) during the growing period (September/October to August/September) and after the termination of the trial during the following two years. Records will be kept of each visit. The GM plants can be identified by polymerase chain reaction using primers specific for the *Cas9*, *gRNA* and *bar* genes. Primers have also been developed to detect the presence of the edited *TaASN2* genes. This will become important when the plants are transgene-free.

In the unlikely event that the integrity of the site is seriously compromised, the trial will be terminated and all plants (including GM and control wheat plots, and pollen barrier rows) will be destroyed using a suitable herbicide or by harvesting as deemed appropriate. All harvested material will be removed from the site and disposed of by incineration, autoclaving or deep burial at a local authority-approved landfill site by an approved contractor. Transportation of waste materials will be in secure containers and all transport of material will be logged. The phone numbers of all key staff will be available to site security and farm.