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

B1 The name and address of the applicant

Wild Bioscience Ltd

Milton Park,

Oxfordshire.

OX14 4SA.

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

The additional gene added to these plants encodes a genetic regulator of photosynthesis. This sequence is 99.8% identical to a native sequence already encoded in the wheat genome. The plants also contain a selectable marker gene which originates in bacteria. The nptII gene gives the plant resistance to kanamycin herbicides and was used in the selection of transgenic plants. Except for the enhanced expression of the photosynthesis regulator gene, copies of which are present in all conventional wheat varieties and all plant more broadly, and de-novo expression of the nptII gene, all aspects of the GM plants to be field tested, including morphology, pollination and seed-set appear to be identical to non-transgenic control wheat plants.

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

The location of the field trial is a fenced agricultural area forming part of an experimental farm at Rothamsted Research Harpenden, UK. The area for the proposed field trial, including controls and spacing between GM plots will not exceed 500m^2.

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).

A key determinant of crop yield is the rate of photosynthesis. We have genetically modified wheat plants to test whether we can increase their efficiency to convert energy from sunlight into biomass under field conditions. We have studied these genetically modified plants under controlled environment conditions and showed several significant differences in specific aspects of photosynthesis compared to non-GM controls. Field trials will determine whether this approach can be developed into new higher yielding crop varieties, but these specific plants will not be commercialized.

B5 The intended dates of the release.

The field trial start date will be in Spring 2023 and the plants will be harvested in August or September. We have applied to conduct the experiment starting early 2023 and finishing in 2027, which would enable us to collect multiple years of replicated field trial data.

B6 The environmental risk assessment.

The GM wheat lines are indistinguishable from their non-GM equivalents except for the expected phenotype of enhanced expression of a photosynthetic regulator and, under controlled environment conditions, possessing increased total biomass and dry seed yield. No other changes to the plant morphology or development are apparent. The photosynthetic regulator gene sequence originates from wheat (*Triticum aestivum*) and the selectable marker gene originates from *Escherichia coli*, a common gut bacterium already widespread in the soil metagenome. Thus, the gene under investigation, nor the selectable marker gene, 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 be realised because the wheat plants will not be consumed by humans.

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 without-crossing being a rare event. Wheat seeds are relatively large and not normally dispersed by wind. Management measures including netting when the wheat is in ear and 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 the photosynthetic regulator 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 is small; total area less than 500 m^2, and temporary (each season lasting between 11 and 12 months, to be concluded 2027).

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. The overall risk of harm to human health or the environmental arising from this trial is assessed as very low.

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

The release site will be visited by trained laboratory personnel who are working on the project at no less than weekly intervals during the growing season of the trial. Any unexpected occurrences that could potentially result in adverse environmental effects or

the possibility of adverse effects on human health will be notified to the Defra immediately. Should the need arise to terminate the release at any point the emergency plans detailed below will be followed.

At the end of each season, the plot will remain in stubble and monitored for volunteers during the remainder of the year and the following season. Any volunteers identified will be destroyed by broad spectrum herbicide treatment (e.g., glyphosate) or removed by hand and destroyed.

Following completion of the multi-year trial the release site will remain fallow for a further season to enable easy identification of volunteers. The site will be inspected regularly, and any volunteers identified will be immediately destroyed either by application of a systematic broad leaf herbicide.

Emergency procedures: In the unlikely event that the integrity of the site is seriously compromised or in other emergency situations, the trial will be terminated, and all plants destroyed using a suitable herbicide or burning on site as deemed appropriate. Should the release site be subject to vandalism, care will be taken to ensure that all uprooted plant material within and outside of the trial site is identified and destroyed accordingly as described above.