Advisory Committee on Releases to the Environment

Advice on an application for deliberate release of a GMO for research and development purposes

Applicant: Rothamsted Research

Application: To release wheat lines genetically modified for increased photosynthetic efficiency.

Ref: 16/R8/02

Date: January 24th, 2017

Advice of the Advisory Committee on Releases to the Environment to the Secretary of State under section 124 of the Environmental Protection Act 1990

ACRE is satisfied that all appropriate measures have been taken to avoid adverse effects to human health and the environment from the proposed release. ACRE sees no reason for the release not to proceed according to the following advice.

To minimise the likelihood that GM wheat from this trial will enter the human food or animal feed chains, the applicant should:

1. Ensure that the 20m surrounding the trial site is planted with a non cereal crop and that cereal volunteers are controlled (prior to flowering) in this area during the trial.

2. Plant a wheat pollen barrier to flower at the same time as the GM wheat as an additional precautionary measure.

3. Control *Elytrigia repens* (Couch Grass) using a glyphosate herbicide and hand-weeding if necessary, within the trial site and the surrounding 20m, before flowering and for the duration of the trial.

4. Ensure that any GM or non GM wheat plant material remaining in the area of release at the end of the trial is disposed of appropriately.

5. Ensure that following harvest, the area of release is lightly tilled twice (once after harvest and again in the following spring) to a depth of 5cm to stimulate germination of any wheat plant volunteers. The release areas should be left fallow and monitored for wheat plant volunteers for 2 years following harvest.

6. Record the number of wheat plant volunteers that germinate before

destroying them with an application of glyphosate herbicide or hand pulling them prior to flowering.

7. Ensure that suitable measures (such as those described Rothamsted Research's application) are put in place to keep large birds out of the trial area and that the efficacy of these measures are kept under review.

8. Ensure that machinery used on the site is cleaned thoroughly onsite, including between using it with GM and non GM material, and that clothing and equipment such as vehicles used by personnel on the site are also cleaned thoroughly before leaving the site.

Comment

ACRE considered the risks to human health and the environment posed by the proposed release of wheat that has been genetically modified with the intention of enhancing photosynthetic efficiency and thereby increasing yield. Under glasshouse conditions, these GM plants showed desirable characteristics i.e. increased biomass and dry seed yield. The purpose of this trial is to examine whether the agronomic characteristics of this GM wheat are altered under field conditions.

Key characteristics of this GM field trial with respect to its environmental risk assessment are:-

i) It will be on a very small scale. This application is to release approximately 300 seeds per m^2 over an area of 243 m^2. The applicant has proposed that the release will take place at one site over two years. The trial will be planted in the spring of 2017 and 2018. The plants will be harvested in August or September.
ii) The GM wheat and non-GM wheat grown in this trial will not be put into the human food chain or fed to livestock.

Rothamsted Research (RR) intends to trial 2 GM lines (Sox44 and Sox23); these contain the same gene constructs but different numbers of these constructs (i.e. two and six copies respectively). They both contain the sedoheptulose-1,7-biphosphatase (*SBPase*) gene from the grass *Brachypodium distachyon* and the *bar* gene from the soil bacterium *Streptomyces hygroscopicus*. SBPase is an enzyme that is already present in wheat and in other plants. The aim of this genetic modification is to over-express this enzyme in wheat to increase the regeneration of ribulose 1,5 bisphosphate (RuBP). This is the substrate for RuBisCO and RuBisCO is often rate-limiting for photosynthesis in plants. The *bar* gene encodes phosphinothricin-N-acetyl transferase (PAT) protein that confers tolerance to glufosinate ammonium herbicides. This herbicide-tolerant trait was used as a selectable marker in identifying GM plants during the development stage of this project. The herbicide will not be used in the trial.

The GM lines are also likely to contain the antibiotic resistance marker genes used to select for the constructs in dividing bacterial cells. These are the *nptI* and *bla* genes derived from *E.coli* that confer resistance to Kanamycin and other aminoglycoside antibiotics and to ampicillin respectively.

The GM lines were generated by bombarding immature wheat embryos with two plasmid vectors. The *SBPase* gene was carried on one vector (pBract302) and the *bar* gene on another (pAHC20).

Molecular characterisation

ACRE concluded that the GM plants were developed using standard practice and noted that the transformation vectors are well-known (although it was less usual to bombard two vectors rather than one). ACRE was content that RR's description of how the GM wheat plants were made was clear. RR assumed that all the genetic elements present on the vectors would have been transferred to the GM plants.

Some of the public representations criticised RR's molecular characterisation of the two GM lines for not including information on the structure of the inserts or their location in the wheat genome. These data are not required in applications for small trial releases of GM plants that are not destined for food/feed use unless they are needed to inform the risk assessment. ACRE concluded that sequencing the DNA inserted into the wheat would not be useful in addressing the risk-based questions associated with this trial.

ACRE discussed whether information on location of the inserts in the wheat genome would provide useful data on the biological and agronomic characteristics of these plants compared to gathering data on field performance. ACRE concluded that the former had little relevance relative to the latter. ACRE noted that under glasshouse conditions, the GM plants had increased total biomass and dry seed yield as might be expected from enhancing SBPase expression. Otherwise, RR reported that these plants were indistinguishable in terms of their morphology and development from untransformed controls. The purpose of the trial is to analyse the agronomic characteristics of the GM wheat under field conditions. The GM plants will be monitored between sowing and harvest as part of the experimental design; it is also a standard requirement in consents that are issued for GM field trials. ACRE also discussed the potential for the intrinsic characteristics of wheat to change (e.g. through insertion of DNA in native wheat genes) such that they pose a greater environmental risk e.g. to make wheat a problem weed[1]. ACRE considered that frequent monitoring of the site for unintended and unexpected effects was appropriate given the genetic elements that have been introduced, the scale and design of the trial and the innate biological and agronomic properties of wheat.

The Environmental Risk Assessment

Improving photosynthetic efficiency and thereby increasing the yield potential of this GM wheat is the aim of this research. ACRE concluded that it was very unlikely that increased SBPase expression would lead to increased persistence of the GM wheat seeds in the arable environment i.e. by inducing seed dormancy. ACRE also considered it unlikely that the GM wheat would invade and persist in habitats outside of arable conditions i.e. become a problem weed as a result of this genetic

[1] Chepil W.S. (1946) Germination of Weed Seeds I. Longevity, Periodicity of Germination, and Vitality of Seeds in Cultivated Soil. Scientific Agriculture **26**: 307-346.
Anderson, R. L. and G. Soper. 2003. Review of volunteer wheat (Triticum aestivum) seedling emergence and seed longevity in soil. Weed Technol **17**:620–626.

modification. If RR were to submit an application for wide scale cultivation of these GM plants in the future, data from small-scale field trials on the comparative agronomic and phenotypic characteristics are likely to be required.

Public representations also raised the potential for the glufosinate ammonium-tolerance trait (present in the GM wheat plants) to confer a selective advantage on these plants. This herbicide was used to select for the presence of the pAHC20 plasmid in wheat cells and plants during the development of these GM plants. Glufosinate ammonium herbicides will not be used at the trial site. ACRE also noted that genes encoding the PAT protein (which confers tolerance to glufosinate ammonium herbicides) are already widely present in soil bacteria and glufosinate herbicides are seldom used in the UK.

The majority of public representations reflected concern that growing plants containing antibiotic resistant marker genes would compromise the use of associated antibiotics in human and veterinary medicine. ACRE has discussed the use of resistance marker genes in GM plants on a number of occasions and taken into consideration the statement from the European Medicines Agency (EMA) on the importance of preserving the therapeutic relevance of the antibiotics.

ACRE emphasised that both the *nptI* and *bla* genes are present at high frequency in agricultural soils[2]. Antibiotic resistant bacteria occur naturally in the environment but many are a result of contamination with human and animal excreta in sewage, slurry and manure. Antibiotic resistance in humans and other animals has resulted from the strong selective pressure associated with the substantial use of industrially-made antibiotics in human and veterinary medicine and as food supplements for farm animals.

Even though the scientific consensus is that selection pressure on bacteria containing antibiotic resistance genes is the driver of antibiotic resistance gene frequency in the environment, ACRE discussed the potential for bacteria in the environment to be transformed with antibiotic resistance genes from the GM wheat plants. Studies of horizontal gene transfer from plants to bacteria suggest that this phenomenon is extremely rare (Please refer to a review by Keese, 2008[3]). ACRE noted that even if a recombination event were to occur between DNA from a plant and a bacterial genome, in order for the gene to be expressed, it would need to be combined as a fully functional transcription unit in the bacterium, which is unlikely. If it were to occur, it would most likely result from a homologous recombination event at a site in the bacterial genome where a version of antibiotic resistance gene already exists.

ACRE concludes that, as a result of this proposed field trial, the antibiotic resistance marker genes present in these GM plants will not increase resistance to antibiotics used for therapeutic purposes in human and veterinary medical practice.

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[2] Walsh F, Duffy B (2013) The Culturable Soil Antibiotic Resistome: A Community of Multi-Drug Resistant Bacteria. PLoS ONE **8**: e65567.
[3] Keese P. (2008). Risks from GMOs due to horizontal gene transfer. Env Biosafety Research. **7**(3): 123 – 149

Some of the public representations recommended that toxicity and allergenicity studies should be carried out. This is not generally necessary for small-scale trials where material will not enter the food or feed chains unless there is a plausible hypothesis whereby such limited exposure to the plant material could cause harm to humans and other animals. SBPase is an enzyme involved in the light-independent reactions of photosynthesis and it is produced by all plants (as well as in some non-photosynthetic organisms). Therefore, SBPase is consumed by humans and other animals when leafy vegetables and other green plant parts are eaten.

Approximately 20 GMOs have been authorised for feed and food use in the EU, which produce the PAT protein (which confers tolerance to glufosinate ammonium herbicides). In each case, the conclusion of the European Food Safety Authority's (EFSA's) safety assessment was that these GMOs are unlikely to pose a greater risk to human health or to the environment than their non-GM counterparts. As part of this assessment, EFSA has considered the toxicity and allergenicity of the PAT protein. This is reflected in RR's application i.e. that the enzymatic function of PAT is specific to its substrate phosphinothricin, which does not occur naturally in humans. PAT is degraded and inactivated in simulated gastric fluid and is therefore unlikely to retain any enzymatic activity *in vivo*. No sequence homology between the PAT protein and known toxins had been found. The native PAT protein (51% purity) has been tested for acute toxicity in mice and no toxicity has been reported at a dose of 5 g per kg body weight.

The *nptI* and *bla* genes are under the control of bacterial promoters to facilitate the maintenance and replication of plasmid vectors containing the genes of interest in dividing bacterial cells before they are used to transform plant cells. Their expression in plant cells is very unlikely. ACRE also noted that the organism from which these genes are derived (*E. coli*) is present in the large intestine of healthy humans. Specific toxicity data on neomycin phosphotransferase I and beta-lactamase were not provided in the application[4]. However, given that these GM plants are not destined for the food/ feed chain, it is a small-scale trial, the genes are already widely present in the environment and expression of the respective proteins in the GM wheat plants will be minimal, ACRE does not consider that they pose a risk to human health or to the environment.

ACRE members were familiar with a paper cited in some representations about the relatively high levels of synthetic *bla* genes in Chinese rivers, which the authors (Chen *et al.* 2012[5]) attribute to improper disposal of laboratory waste. By way of contrast, ACRE noted a paper by LaPara *et al*. (2015)[6] who did not detect any of these genes in wastewater effluent or river water samples from the upper Mississippi River in the USA. The authors attribute this to stringent regulations on destroying laboratory waste containing recombinant DNA being followed. ACRE noted that this

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[4] Rothamsted Research did provide information on a functionally related enzyme NPTII.

[5] Chen, J.; Jin, M.; Qiu, Z.-G.; Guo, C.; Chen, Z.-L.; Shen, Z.-Q. Wang, X.-W.; Li, J.-W. A survey of drug resistance bla genes originating from synthetic plasmid vectors in six Chinese rivers. Environ. Sci. Technol. **46**: 13448−13454.

[6] LaPara, T.M., Madson, M., Borchardt,S., Lang, K. S and Johnson T. J (2015). Multiple Discharges of Treated Municipal Wastewater Have a Small Effect on the Quantities of Numerous Antibiotic Resistance Determinants in the Upper Mississippi River. Environ. Sci. Technol. **49**: 11509−11515.

issue is relevant to the UK's Genetically Modified Organisms (Contained Use) Regulations 2014, which apply to the use of plasmids with antibiotic resistance genes under laboratory conditions and address the management of waste.

Managing the trial site

As ACRE has considered the potential risks of this trial to human health and the environment in the context of it being a small-scale trial from which no material will enter the food or feed chains, the committee considered, in detail, management plans to minimise the persistence of GM material at the trial site and the dispersal of GM material from the site.

Gene flow

Wheat is a self-pollinating crop with very low rates of cross-pollination with other wheat plants. This is because fertilization often occurs before the florets open, which makes out-crossing unlikely; in addition, wheat pollen is relatively heavy and tends to travel shorter distances than pollen from other grass species. Studies have detected cross-pollination rates of 1–2% between wheat plants in close proximity, but this rapidly decreases with the distance between plants. There are several relevant studies involving GM wheat field trials, most recently those of Foetzki *et al*. (2012)[7] and Miroshnichenko *et al.* (2016)[8].

RR has proposed a separation distance of 20 metres between the plants used in the trial and any other wheat plants or sexually compatible wild relatives to minimise gene flow. ACRE noted that the separation distance required to prevent hybridisation between different wheat varieties when certified seed is produced for marketing purposes is 2 metres. RR has also proposed to sow a 3 metre-wide wheat pollen barrier (comprising the same variety as the GM wheat) around the trial. ACRE recommended a 2 metre-wide pollen barrier in its advice on a previous GM wheat trial at the same site and as this is an additional precautionary measure to the 20 metre separation distance, sees no reason to extend this to 3 metres. In order to maintain the separation distance, ACRE advises that the 20m surrounding the trial site is planted with a non cereal crop and that cereal volunteers are controlled (prior to flowering) in this area during the trial and for two years afterwards.

There have been no reports in the literature of spontaneous hybridisation events between wheat and wild relatives of wheat (that may grow in and around the trial site). *Elytrigia repens* (Couch Grass) is a common agricultural weed that is a wild relative of wheat and is common in the area surrounding the trial site. *E. repens* propagates primarily by vegetative reproduction (rhizomes) rather than by sexual reproduction and no spontaneous couch grass x wheat hybrids have been reported. However, ACRE agrees with RR that, as a precautionary measure, couch grass growing within the trial site and in the surrounding 20 m area should be destroyed

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[7] Foetzki A., Diaz Quijano C., Moullet O., Fammartino A., Kneubuehler Y. and Mascher F. (2012). Surveying of pollen-mediated crop-to-crop gene flow from a wheat field trial as a biosafety measure. GM Crops and Food: Biotechnology in Agriculture and the Food Chain **3**(2), 115–122.

[8] Miroshnichenko D., Pushin A and Dolgov S (2016). Assessment of the pollen-mediated transgene flow from the plants of herbicide resistant wheat to conventional wheat (*Triticum aestivum* L.). Euphytica **209**:71–84.

before it flowers (June – August). ACRE recommends that this area should be monitored for the presence of this weed until mid-October following harvest and in the subsequent 2 years following the final harvest of GM material. It may be appropriate to use mechanical or hand-weeding in addition to the use of herbicides (other than glufosinate ammonium herbicides).

Wheat plant volunteers

Wheat is an annual species and survives from year to year only via seed. Most modern commercial cultivars of wheat have low seed dormancy. The novel trait introduced into these two GM lines would not be expected to induce seed dormancy. For example, it would not be expected to alter seed hormone levels (e.g. abscisic acid or gibberellic acid).

The GM wheat lines were produced by transforming Cadenza, which is a UK milling variety that can be sown either as a spring or winter wheat. Mature seeds may fall to the ground prior to, or at, harvest time and if not managed, they may over-winter in the soil and germinate the following spring as volunteers. There are several relevant publications, of which the most detailed are two specifically designed to consider longevity of spring wheat in the seed bank in the context of GM (Kristi *et al*. 2007[9] and Ryan *et al.* 2009[10]). These studies conclude that survival of buried seed beyond the next spring is extremely rare and longer term persistence in a field is most likely to occur from seed produced from volunteers that escape detection in the following season and then set seed. This conclusion is supported by the more recent study by Kalinina *et al*. in 2015[11].

ACRE recommends that volunteer management measures should be initiated in the autumn. ACRE advises that shallow light tillage should be carried out immediately after harvest to encourage volunteers. The area should be left fallow over winter and another shallow, light tillage should be carried out in the spring. The area should be monitored for volunteers in this year and the following year during which time it should remain uncropped. Any volunteers detected in this two-year post-harvest period should be recorded and then destroyed before the emergence of inflorescences.

Seed movement

ACRE considered the measures proposed to minimise unintentional transfer of material from the trial site. All machinery should be cleaned thoroughly on the site between uses and before leaving the site. ACRE advises that a plot combine should be used to harvest the plants as a commercial combine would be more difficult to clean. ACRE also advises that the GM plots should be harvested before the non-GM

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[9] Kristi A. De Corby, Rene C. Van Acker, Anita L. Brûlé-Babel, and Lyle F. Friesen (2007). Emergence Timing and Recruitment of Volunteer Spring Wheat**.** Weed Science **55**(1): 60-69.

[10] Ryan L. Nielson, Marc A. McPherson, John T. O'Donovan, K Neil Harker, Rong-Cai Yang, and Linda M. Hall (2009). Seed-Mediated Gene Flow in Wheat: Seed Bank Longevity in Western Canada. Weed Science **57**(1): 124-132.

[11] Olena Kalinina, Simon L. Zeller, Bernhard Schmid (2015). Persistence of seeds, seedlings and plants, performance of transgenic wheat in weed communities in the field and effects on fallow weed diversity. Perspectives in Plant Ecology, Evolution and Systematics **17**: 421–433.

plots. RR should put in place procedures for personnel visiting the site to ensure that material is not transferred from the site via clothing or equipment including vehicles.

A sample of plants will be hand-harvested, conditioned and threshed to supply seeds for future trials or other research purposes. Other harvested material, including that from the pollen barrier rows, will be disposed of by incineration or by deep burial at a local authority-approved landfill site using an approved contractor. The straw will be chopped and left on the site. If 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 be harvested, as appropriate.

The trial site will be surrounded by a 2.4m high chain link fence to prevent the entry of rabbits and other large mammals. RR has also proposed management measures including netting when the wheat is in ear and the use of gas guns and hawk kites to deter birds. ACRE is content with these proposed measures and recommends that these are kept under observation as the trial is ongoing to ensure they are effective.

A majority of representations from the public cited three incidents in the USA where GM wheat plants had been found a number of years after field trials involving GM wheat had finished. ACRE noted that in the USA the development of GM wheat had progressed to large-scale trials where a large amount of GM wheat seed was used. ACRE concluded that the impact of this GM seed presence is an economic issue that is beyond ACRE's remit rather than an environmental one.

ACRE concludes that this particular field trial is extremely unlikely to have an adverse effect on human health and the environment but recommends management measures that will minimise (i) the dispersal of GM material (including transgenes) from the trial site and (ii) the persistence of GM material at the trial site.

Items arising from public representations

Defra received 86 representations during the public consultation on this application. As these covered most of the issues that ACRE considered in its assessment, they are included in the respective sections of this advice. In addition to the many relevant comments, there were also comments on issues that are outside of ACRE's remit. These concern topics that are not relevant to the environmental risk assessment of this particular trial. Some of these comments would have been relevant had the application been for commercial-scale cultivation and/ or food and feed use. Others were more political in nature and are outside of ACRE's remit; for example, they questioned whether GM techniques should be used in the development of plant varieties and more specifically whether it is necessary to develop wheat with enhanced photosynthetic efficiency using this technology. They also questioned whether there would be a market for this product.