Advisory Committee on Releases to the Environment

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

Applicant: John Innes Centre

Application: To release genetically modified wheat lines that have been biofortified with higher iron levels in their grain.

Ref: 19/R52/02

Date: April 1st, 2019

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 in the John Innes Centre'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 to enhance the iron content in its grain. In controlled growth conditions, over expression of a gene that affects iron transport within the grain resulted in a greater than 2-fold increase in iron in the white flour faction. Other characteristics such as plant growth, grain number or weight were not altered. The purpose of this trial is to analyse the micronutrient accumulation and agronomic performance of these plants under field conditions.

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

i) It will be on a small scale. Fewer than 1000 GM wheat plants will be grown in each year of the trial. The John Innes Centre is proposing a 3 year trial starting in spring 2019.
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.

The John Innes Centre (JIC) intends to trial GM lines containing a gene that encodes a vacuolar iron transporter. This is under the control of an endosperm–specific promoter; its expression has not been observed in other vegetative tissue during the life cycle of the plant. The GM lines may also contain the antibiotic resistance genes encoding neomycin phosphotransferase I (*npt1*) and hygromycin phosphotransferase (*hyg*). NPT1 confers resistance to aminoglycoside antibiotics such as kanamycin and neomycin. These genes are used in the development of GM plants to facilitate the selection of bacteria and plants (respectively) that have been transformed successfully.

The GM lines were generated by infecting immature wheat embryos with *Agrobacterium tumefaciens* containing the genes of interest.

Molecular characterisation

ACRE was content that JIC's description of how the GM wheat plants were made was clear. The JIC assumed that all the genetic elements present on the vectors would have been transferred to the GM plants.

Some of the public representations criticised the JIC's molecular characterisation of the GM lines for not including information on unintended effects on the genome, epigenome or transcriptome. These data are not required in applications for small trial releases of GM plants unless they are needed to inform the risk assessment. ACRE considered whether this information would provide useful data on the biological and agronomic characteristics of these plants compared to gathering data from the field. As part of this, ACRE discussed what intrinsic characteristics of wheat these alterations would need to change in order for them to confer an environmental risk e.g. to make wheat a problem weed[1].

It is inevitable that there will be differences between plant lines. This is the case for conventional plant breeding as much as it is for GM. Attempting to interpret these differences is challenging and not constructive unless there is an indication of what hazard to look for. Under controlled conditions, the GM plants are indistinguishable from untransformed plants. An objective of the trial is to determine whether this is the case under field conditions. Monitoring of GM plants is a standard requirement in any consent that is issued for a GM field trial. ACRE concluded that in the case of this particular trial, additional data on molecular characterisation would not be helpful in addressing risk-based questions.

The Environmental Risk Assessment

ACRE concluded that it was very unlikely that increased levels of iron in the endosperm of GM wheat seeds would alter the characteristics/ biology of the plants in a way that would transform them into a problem agriculture weeds or increase their ability to invade and persist in habitats outside of arable conditions. If the JIC 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.

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 *hyg* 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

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[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.

[2] Walsh F, Duffy B (2013) The Culturable Soil Antibiotic Resistome: A Community of Multi-Drug Resistant Bacteria. PLoS ONE **8**: e65567.

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.

The public representations also referred to a paper about the relatively high levels of a synthetic antibiotic resistance genes detected in Chinese rivers, which the authors (Chen *et al.* 2012[4]) attributed to improper disposal of laboratory waste. By way of contrast, LaPara *et al.* (2015)[5] 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. The UK's Genetically Modified Organisms (Contained Use) Regulations 2014, apply to the use of plasmids with antibiotic resistance genes under laboratory conditions and address the management of waste.

One public comment also registered concern that the antibiotic resistance genes would transfer to a fungus (*Nosema apis*) in the mid-guts of honey bees. As such infections are not treated with antibiotics, there is no selection pressure for genes that confer antibiotic resistance.

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.

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.

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[3] Keese P. (2008). Risks from GMOs due to horizontal gene transfer. Env Biosafety Research. **7**(3): 123 – 149

[4] 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.

[5] 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.

The Vacuolar Iron Transporter protein, encoded by the gene of interest, is already consumed by humans and other animals when leafy vegetables and other green plant parts are eaten. The *nptI* selectable marker gene is under the control of a bacterial promoter 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 were not provided in the application but the JIC discusses the studies in the peer-reviewed literature on the safety of a functionally related protein, NPTII. 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.

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. Cross-pollination rates rapidly decrease with the distance between plants. There are several relevant studies involving GM wheat field trials including those of Foetzki *et al*. (2012)[6] and Miroshnichenko *et al.* (2016)[7].

The JIC 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.

The JIC does not propose to sow a pollen barrier around the GM plants grown in the trial. However, ACRE recommends that a 2 metre-wide pollen barrier (comprising wheat plants of the same species sown at the same time as the GM plants) as an additional precautionary measure to the 20 metre separation

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[6] 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.

[7] 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.

distance. 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* (common couch grass) is a common agricultural weed that is a wild relative of wheat. *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, as a precautionary measure, ACRE considers that common couch grass growing within the trial site and in the surrounding 20 m area should be destroyed 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.

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).

Mature wheat 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[8] and Ryan *et al.* 2009[9]). 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[10].

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

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[8] 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.
[9] 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.
[10] 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.

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 also advises that the GM plots should be harvested before the non-GM plots. The JIC 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.

Plants will be hand-harvested, conditioned and threshed to supply seeds for analysis or future trials. The JIC states that any remaining grain will autoclaved and then disposed of. ACRE considers that incineration or deep burial at a local authority-approved landfill site using an approved contractor would also be appropriate. 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 will be destroyed using a suitable herbicide or be harvested, as appropriate.

The trial site will be surrounded by a fence and there will be a framework and netting protecting the site from mammals and 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 has advised Defra on management measures that should be adopted at this particular trial site. Consent conditions associated with any GMO trial in England apply to the transport, labelling and destruction of GM material.

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 63 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 if the application included use as human food or animal feed. For example, that increased iron levels in the GM wheat flour may not translate into increased bioavailability for consumers or that increased iron consumption might be harmful for certain groups of consumers. Other comments were more political or economic in nature and are outside of

ACRE's remit; for example, being against the use of genetic engineering and/ or suggesting that traditional breeding methods are more likely to be effective in increasing iron levels in wheat than taking a GM approach.