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 resistance to aphids

Ref: 11/R8/01

Date: June 5th, 2013

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. Plant a wheat pollen barrier to flower at the same time as the GM wheat.

2. 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.

3. Ensure control of *E. 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 (and barley) material remaining in the area of release at the end of the trial is inactivated.

5. Ensure that, in the year following harvest of the GM wheat, the area of release is lightly tilled to a depth of 5cm immediately after harvest to stimulate germination of any wheat volunteers and is then left fallow for 1 year following the final harvest.

6. Treat any volunteers growing in the fallow year with an application of glyphosate herbicide or hand pull wheat plants prior to flowering.

7. Take measures to minimise the likelihood that GM wheat plants sown in the autumn will set seed by treating them with broad spectrum herbicide(s) at the end of 2013 and monitoring them for stem extension over the winter and during the following spring.

8. Ensure that suitable measures are put in place to keep large birds out of the trial area.

9. Ensure that machinery used on the site is cleaned thoroughly onsite and between GM and non GM use and that clothing and equipment including vehicles used by personnel

on the site is also cleaned thoroughly before leaving the site.

In addition, the applicant should monitor the behaviour of non target organisms and terminate the trial if unexpected disturbances in behaviour are observed.

Comment

ACRE considered the risks to human health and the environment posed by the proposed release of wheat genetically modified for resistance to aphids. The Committee has addressed a number of points in its safety assessment including scientific issues raised in public representations.

Key characteristics of this release for risk assessment are that:-

i) The trial will be on a very small scale. This application is to release approximately 500 seeds per m^2 over an area of 288m^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 2012 and 2013 and in the autumn of 2013. The spring-sown plants will be harvested in July/Aug/Sept. The autumn sown plants will be destroyed by herbicide before they set seed. The total trial area including control plots, spacers and pollen barriers will be 12,800m^2.
ii) The GM wheat produced as a result of this release will not be put into the human food chain or fed to livestock.

Two GM lines are intended for release. Both have been transformed with a (E)-β-farnesene synthase (EBFS) gene. The line 2803R6P1 has also been transformed with an EBFS and farnesyl diphosphate synthase (FPPS) gene. Both gene sequences are synthetic and optimised for expression in wheat. Plant cells were transformed using micro projectile bombardment with separate plasmid vectors. The vector used carries the antibiotic resistant marker gene *nptI* for selection in bacteria and the *pat* gene that confers resistance to glufosinate ammonium herbicides.

ACRE noted that the applicant had not taken steps to determine whether the vector backbone had been inserted into the transformed plants. ACRE did not request further data on the molecular characterisation but instead asked the applicant to provide a more considered assessment in the application of the risk associated if it were assumed that the entire plasmid had been inserted into the wheat genome. The application has been amended accordingly.

ACRE has previously considered the issue of the presence of antibiotic resistance marker genes, including the statement from the European Medicines Agency (EMA) on the importance of preserving the therapeutic relevance of the antibiotic kanamycin. ACRE is of the opinion that the therapeutic effect of antibiotics that are substrates for NPTI will not be compromised by the presence of the *nptI* gene in GM plants. ACRE's advice on this issue is that (a) the likelihood of transfer of a functional gene from plant material to bacteria is extremely low; (b) bacteria with resistance to these antibiotics are widespread in the environment; and (c) the acquisition of an intact gene is only one of the possible mechanisms by which bacteria may develop resistance. All these points apply to this case of the proposed trial of GM aphid resistant wheat.

With regard to the issue of horizontal gene transfer (HGT) from plants to soil prokaryotes, ACRE is of the opinion that HGT between plants and soil prokaryotes under field conditions is a rare phenomenon. Even if it is assumed that this rare recombination event does occur, the consequences are predicted to be negligible since genes are highly unlikely to recombine as fully functional transcription units, and so would not be expressed. However ACRE did not

consider that the applicants had given sufficient consideration to the risks if horizontal gene transfer were to occur and requested further information from the applicant. The application has been amended to provide this information. ACRE considered the additional information provided was sufficient.

ACRE considered the risks, if the backbone sequence had been incorporated would be negligible and is content with the applicants risk assessment.

The herbicide tolerance trait was used for the production of the transgenic plants in the laboratory and will not be utilised in the field trials. Genes encoding the PAT protein are already widely present in soil bacteria and the use of glufosinate herbicides is rare. ACRE therefore concluded that the use of a herbicide resistance marker gene introduced a negligible risk to the environment.

ACRE is of the opinion that that none of the inserted DNA (two synthetic genes EBFS and FPPS, including *Ubi* promoter and *nos T* terminator regions; selectable markers – *nptI and Bar*) is likely to result in a risk to human health or the environment in the context of the proposed release.

The transgenic plants will be destroyed on completion of the trial and will not enter the food or animal feed chains. ACRE considered that as EBFS and FPPS both occur naturally in the environment expression of these genes are unlikely to pose a risk to human health or animal health. The committee did, however, request further information on the levels of the (*E*)-β-farnesene pheromone emitted naturally from plants and further information on the levels of (*E*)-β-farnesene produced in the semiochemical trials to provide context for the environmental risk assessment. The application has been amended to include this information. ACRE considered the additional information provided by the applicant and concluded that it demonstrated that the levels to be emitted from the trial will be within a previously trialled range.

The trial site will be surrounded by a 2.4m high chain link fence to prevent the entry of rabbits and other large mammals.

ACRE considered the risk of the EBF pheromone to non target organisms. The Committee agreed that the changes in behaviour that the EBF pheromone will instigate are highly specific to aphids and their natural predators but that further information on the proposed monitoring of adverse effects that would potentially cause the trial to be halted was needed. The application has been amended to provide this information. The committee were content with the further information provided.

ACRE discussed the potential for birds to disperse seed from the site outside the trial area, potentially over long distances. At ACRE's request the applicant provided additional information on the measures proposed to keep large birds off the site particularly during sowing and when the wheat is in ear. The application has been amended to include this information. ACRE is content with the additional measures proposed and has recommended that these are kept under observation as the trial is ongoing to ensure they are fully effective.

Wheat is a self pollinating crop with very low rates of cross-pollination with other wheat plants. The applicant has proposed a separation distance of 10 metres between GM and non GM control plots within the trial site as part of the experimental design to reduce the interference between plots. The applicant proposes a 3m wheat pollen barrier will surround the trial. ACRE advised that a 2m wide pollen barrier surrounding the trial would sufficiently reduce the likelihood of cross pollination occurring and recommended that to be effective it must be flowering at the same time as the GM wheat. ACRE advised that whilst it is unlikely that autumn sown plants will

flower before they are destroyed in the winter, this cannot be ruled out. In the unlikely event that this were to occur, a non-GM wheat pollen barrier would be in place and very little, if any, non-GM wheat outside of the trial site is likely to be in flower at the same time.

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. ACRE considered the measures proposed by the applicant to control *E.repens* and recommended that these should include use of a glyphosate herbicide, within the trial site and the surrounding 20m, before flowering of the weed (June – August) and until mid October following harvest and the subsequent 12 month period following the final harvest of GM material. It may be appropriate to use mechanical or hand-weeding in addition to the use of herbicides. ACRE advised that, even if the GM wheat sown in the autumn were to flower, it is extremely unlikely that this would overlap with couch grass flowering.

ACRE considered the post-harvest monitoring plans proposed by the applicant. The Committee recommends that volunteer management measures associated with a spring-sown trial should be initiated in the autumn rather than waiting until the following spring. ACRE advises that shallow light tillage should be carried out immediately after harvest to encourage volunteers and that the site should remain uncropped for a year. Any volunteers should be destroyed before the emergence of inflorescences.

ACRE advised that the applicant should treat the autumn sown GM wheat plants with a broad spectrum herbicide (not glufosinate-based) before the end of 2013. ACRE advised that the applicant monitor for stem extension in the GM wheat over the winter and until the end of May 2014. If such re-growth is detected, it should be eradicated to ensure that the plants do not survive and set seed in the following year. During this period, the applicant would also detect any autumn-sown seed that germinates in the following year.

ACRE considered the measures proposed to minimise unintentional transfer of material from the trial site. The Committee recommends that only one combine should be used on the trial site and that the GM plots are harvested first. All machinery should be cleaned thoroughly on the site between uses and before leaving the site. The applicant 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.

For spring-sown plant material, the applicant proposed to dispose of the GM grain to deep landfill using an approved contractor and leave all straw chopped on site and in the unlikely event of site security being compromised proposed burning material on site. ACRE advised the applicant to reconsider alternative methods of waste disposal in the event of a breach of security and recommended that all material is removed from the site for disposal. The application has been amended to reflect this.

ACRE advised that autumn-sown plant material should be left on the plot and that the plot should not be cultivated until June 2014 (or later, depending on the outcome of monitoring).

Items arising from public representations

Defra received 842 representations during the first public consultation of this application in 2011. It received a further 216 representations when it consulted the public on an extension to the trial in 2013. ACRE was asked to consider scientific issues raised in these consultations. ACRE considered comments relating to:

- *Molecular characterisation data*

➢ ACRE considers that the information provided by the applicant on the GM events was sufficient to carry out an environmental risk assessment. There were a number of representations that related to food safety. However, management measures will be implemented to prevent the GM wheat entering the food/ feed chain. The applicant has described the methods involved in producing the GMOs and the genetic elements used in this process. Where data had not been provided to demonstrate that particular genetic elements had not integrated into the wheat genome, the applicant was required to assume that they had and to carry out a risk assessment accordingly.

➢ The molecular characterisation data provide one layer of evidence in an environmental risk assessment. Information on the phenotypic characteristics of the GMO are also relevant. The GM wheat lines grown in the trial originated from single transformation events. To generate sufficient seed for this trial many generations of plants were grown under contained use conditions in glass houses. They did not show characteristics that would indicate a hazard to human health or the environment.

- *Unanticipated effects of particle bombardment on the host plant*.
  ACRE considered the risk of mutations to the plant and were content that any significant mutations caused by DNA breakage and insertion would be identified during the development process.

- *The use of synthetic genes.*
  ACRE advised that the use of synthetic genes in genetic modification was common and within accepted practice.

- *The presence of the nptI antibiotic resistance marker gene*
  ACRE considered the use of the *nptI* marker gene and its existing prevalence in the environment. The Committee were content that the amendments to the application provided a full analysis of the risks. This is detailed above.

- *The risk of horizontal gene transfer.*
  ACRE notes that the transfer of the *EBFS* and *FPPS* genes into soil microorganisms is extremely unlikely and even if this were to occur soil bacteria would not be capable of producing the EBF pheromone. The committee is content that the further information provided by the applicant considers this risk fully. This is detailed above.

- *The use of a herbicide resistance marker.*
  ACRE considers that the risks associated with the introduction of the herbicide resistance marker gene are negligible. The committee advises that genes encoding the PAT protein are already widely present in soil bacteria (detailed above) and that glufosinate herbicides will not be used in this trial.

- *Risks posed by cross pollination and contamination*
  ACRE has considered the measures proposed to minimise cross pollination (please refer to ACRE's advice above). The committee considers the proposals appropriate as wheat is largely self pollinating and very short separation distances are typically used in commercial wheat seed production.

ACRE has provided specific advice (detailed above) regarding the management of couch. However, these measures are precautionary as it is extremely unlikely that couch will cross with wheat to produce fertile hybrids under field conditions. Under laboratory conditions, techniques that reduce the barriers to hybrid formation are used and even then, their production is challenging. In its application, Rothamsted has identified other wild relatives of wheat commonly found in the UK. These are in the genera *Elymus* and *Elytrigia* (formerly *Agropyron*). However, there are no reports of cross-hybridisation between wheat and these genera and apart from common couch, none of the species will be present at the trial site.

- *Unanticipated impacts on target and non target organisms*
  ACRE considered the risk to target and non target organisms. The committee were content that the further information provided by the applicant (detailed above) demonstrated that the risks are low.

- *Unanticipated impacts on soil dwelling organisms that could result from the breakdown of EBF in the soil*
  ACRE considered the risk to soil dwelling organisms. The committee advises that the volatile nature of EBF means that it is highly unlikely to persist in the soil and that the levels of acetone that will be released during the breakdown of the pheromone will not be significant.

- *Toxicity to field mice and the potential for seed transfer*
  ACRE considered the risk of toxicity to field mice via ingestion of grain and the risk of seed being transferred outside the trial site. EBF is known to occur naturally in a range of plants including in wild type wheat as trace amounts. Over 400 plant species, including several edible plants, are known to produce EBF. EBF is highly volatile and breaks down rapidly to benign oxidation products. In the quantities produced by the GM plants the risk of harm to small mammals or other non-target organisms from eating or inhaling EBF from this trial is extremely small. The committee was content with the information provided by the applicants. The committee also advises that seed dispersal off-site by field mice and the viability of any dispersed seed will be limited.

- *Potential impacts on predator and parasite populations*
  The GMOs are designed to affect aphid and parasitoid behaviour in the GM wheat. A number of representations were concerned about the wider impact of this trait. ACRE considered the potential for natural variation in the populations of pests/ predators/ parasites noting the dynamic complexity of influencing factors. ACRE concluded that the temporal and spatial scale of this trial limits its potential to impact populations of arthropods in the landscape when the GM wheat is growing and especially after the trial has finished. One representation recommended research to investigate the impact on bird species that feed on aphids as part of their diets. ACRE considers this unnecessary for this scale of trial particularly since the researchers are looking to determine whether the GM wheat results in detectable changes in aphid behaviour under field conditions.

  The likelihood that aphids would become habituated to the pheromone was another concern raised in representations. However, ACRE considered that a lack of effectiveness of the trait (for whatever reason) does not constitute an environmental risk.

Many of the representations raise points that are outside of ACRE's remit, which is the scientific assessment of the risks posed by this GM wheat trial. Some of these relate to ethical issues but many raise concerns about the commercial cultivation of these GM wheat lines. If in the future GM plants with this trait were notified for commercial cultivation and food/ feed use in the EU, the data required would be more consistent with that requested in many of the representations e.g. data on genotypic and phenotypic stability, data from detailed toxicity studies and information relevant to greater environmental exposure (e.g. potential impacts on biogeochemical processes). ACRE is grateful for the representations submitted during the public consultation and is content that all of the issues relevant to the nature and scale of this trial have been considered during the Committee's assessment of the dossier.