



**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<sup>1</sup>

**Date:** 5 September 2011

**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 avoid possible adverse effects to human health and the environment, the applicant should:

1. Ensure that the GM wheat produced as a result of this release will not be put into the human food chain or fed to livestock.
2. Plant a wheat pollen barrier to flower at the same time as the GM wheat.
3. 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.
4. Ensure control of *E. repens* (couch grass) using a glyphosate herbicide, within the trial site and the surrounding 20m, before flowering and for the duration of the trial.
5. 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.
6. 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.
7. Treat any volunteers growing in the fallow year with an application of glyphosate herbicide or hand pull wheat plants prior to flowering.

<sup>1</sup>Application reference 11/R8/01 dated 20 June 2011.

8. Monitor the behaviour of non target organisms and terminate the trial if unexpected disturbances in behaviour are observed.
9. Ensure that suitable measures are put in place to keep large birds out of the trial area.
10. 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.

### Comment

1. 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.

2. 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 350 seeds per m<sup>2</sup> over an area of 288m<sup>2</sup>. The applicant has proposed that the release will take place at one site over two years. The trial will be planted will be in March/April and harvested in July/Aug/Sept. The total trial area including control plots, spacers and pollen barriers will be 12,800m<sup>2</sup>.
- ii) The GM wheat produced as a result of this release will not be put into the human food chain or fed to livestock.

3. Two GM lines are intended for release. Both have been transformed with a (E)- $\beta$ -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.

4. 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 of the risk associated if it were assumed that the entire plasmid had been inserted into the wheat genome. The application has been amended to provide this information.

5. 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.

6. 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.

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

8. 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.

9. 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.

10. 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*)- $\beta$ -farnesene pheromone emitted naturally from plants and further information on the levels of (*E*)- $\beta$ -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.

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

12. 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.

13. 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.

14. 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.

15. *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.

16. ACRE considered the post harvest monitoring plans proposed by the applicant. The Committee recommends that volunteer management measures 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 inflorescence.

17. 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.

18. 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 information.

#### **Items arising from public representations**

19. ACRE considered 842 representations received from members of the public on this application with respect to the scientific issues. The Committee considered the comments relating to:

- 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 further information provided by the applicant 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 proposed mitigation measures to prevent cross pollination as described 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.
- 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 release 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 gene transfer to field mice via ingestion of grain and the risk of seed being transferred outside the trial site. The applicant advised that the EBF pheromone is generated within plant chloroplast and that given seed does not contain chloroplast it is highly unlikely that pheromone will be produced by the seeds. The committee were content that the information provided demonstrated that the risks are low. The application has been amended to reflect this information. The committee advises that limited seed would be dispersed off-site by field mice and the viability of any dispersed seed will be limited.
- 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.

20. ACRE is content that all issues raised had been considered thoroughly during the Committee's assessment of the dossier. ACRE is satisfied that all scientific issues raised by the public with respect to this application have been addressed in this advice.