Advisory Committee on Releases to the Environment

These minutes are subject to approval following formal adoption at the next ACRE meeting

Minutes of an *ad hoc* meeting of ACRE held on Monday 9 January, 2017

Due to industrial action on London Underground services, ACRE's meeting on Monday 9th January had to be cancelled. There were two items on the agenda:

- An application to field trial wheat plants genetically modified to photosynthesis more efficiently (Ref: 16/R8/02)
- An application for Part B consent to release a genetically modified Adenovirus 4 HIV vaccine for use in a phase I clinical trial. (Ref: 16/R49/01).

As the deadline for a decision on whether to authorise the GM wheat field trial was due by February 1st , ACRE established a group who discussed the application via a telephone conference. On the basis of this discussion, the secretariat was asked to draft preliminary advice, which was then to be considered and agreed by the committee as a whole.

The deadline for ACRE advice on the GM vaccine trial was less pressing and ACRE agreed to postpone its consideration of this application.

Application to field trial wheat plants genetically modified to photosynthesis more efficiently (Ref: 16/R8/02)

The telephone conference involved:

ACRE members: Prof Rosie Hails (Chair), Prof Ian Crute, Dr Peter Lund, Mr Simon Kerr, Dr Andy Wilcox and Prof Jim Dunwell.

Assessors: Dr Karen Pearson SASA
GM Inspectorate: Mr James Blackburn
Defra: Dr Louise Ball (secretary)

Professor Ian Crute declared a potential conflict of interest in that he had been a Director of Rothamsted in the past. The chair concluded that whilst the minutes for the meeting

should record this information, this there was no need for Ian to be excluded from ACRE's considerations.

Rothamsted Research 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 group began by discussing the molecular characterisation of these GMOs, before moving onto consider the environmental risk assessment and finally the measures that Rothamsted would have to adopt in order to minimise the dispersal of seed from the trial site and the persistence of seed at the site. The group took the representations made during a public consultation into account as it addressed each issue.

Molecular characterisation

The group concluded that the information provided on the two GM wheat lines was clear; it noted that they were developed using standard practice. Rothamsted had assumed that all the genetic elements present on the vectors would have been transferred to the GM plants.

Some of the public representations criticised Rothamsted'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. The group noted that these data are not required in applications for small trial releases of GM plants not destined for food/feed use unless they are needed to inform the risk assessment. The group concluded that sequencing the DNA inserted into the wheat would not be useful in addressing the risk-based questions associated with this particular trial.

The group discussed whether information on the 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. It concluded that the former had little relevance relative to the latter. Under glasshouse conditions, the GM plants had increased total biomass and dry seed yield as might be expected from enhancing SBPase expression. Otherwise, Rothamsted reported that these plants were indistinguishable in terms of their morphology and development from untransformed controls. The group noted that the purpose of the trial is to analyse the agronomic characteristics of the GM wheat under field conditions.

The group 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. The group 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.

Environmental risk assessment

The group considered 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 modification.

Public representations also raised the potential for the glufosinate ammonium-tolerance trait to confer a selective advantage on these plants. This herbicide was used during the development of these GM plants but will not be used at the trial site. The group 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 group discussed the potential for plants containing antibiotic resistant marker (ARM) genes to compromise the use of associated antibiotics in human and veterinary medicine. The group noted that both the ARM genes present in these GM wheat plants (i.e. *nptI* and *bla*) are present at high frequency in agricultural soils. It noted that 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, the group 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 and 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 group concluded that, as a result of this proposed field trial, the ARM genes present in these GM plants would not increase resistance to antibiotics used for therapeutic purposes in human and veterinary medical practice.

The group noted that toxicity and allergenicity studies are generally not necessary for small-scale trials where material will not enter the food or feed chains and it did not identify a plausible risk hypothesis whereby such limited exposure to the plant material could cause harm to humans and other animals in this case. The group noted that (i) SBPase is produced by all plants and therefore it is consumed by humans and other animals; (ii) the PAT protein is expressed in a number of GM plants that have been authorised for food and feed use and as such, has been assessed in detail on a number of occasions and (iii) the *nptI* and *bla* genes are under the control of bacterial promoters and as such, their expression in the wheat cells is very unlikely.

A number of representations referred to a paper about the relatively high levels of synthetic *bla* genes in Chinese rivers, which the authors (Chen *et al*. 2012[1]) attributed to improper disposal of laboratory waste. Members of the group were aware of the paper and

[1] 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.

noted a paper by LaPara *et al.* (2015)[2] 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 attributed this to stringent regulations on destroying laboratory waste containing recombinant DNA being followed.

Managing the Trial Site

The group noted that wheat is a self-pollinating crop with very low rates of cross-pollination with other wheat plants. Rothamsted had 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. Rothamsted had also proposed to sow a 3 metre-wide wheat pollen barrier (comprising the same variety as the GM wheat) around the trial. ACRE had recommended a 2 metre-wide pollen barrier in its advice on a previous GM wheat trial at the same site. The group concluded that since the pollen barrier would be an additional precautionary measure to the 20 metre separation distance, there was no reason for ACRE to extend its recommendation for a pollen barrier to 3 metres. The group recommended that the 20m surrounding the trial site should be 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. Previously ACRE had recommended one year of monitoring for wheat volunteers. Whilst the group consider that the survival of seed beyond the next spring is extremely rare, it considered the additional year would be helpful to check that management of the site is effective i.e. that volunteers that do occur are killed and that none escape detection and set seed.

The group recommended that volunteer management measures should be initiated in the autumn. It recommended that shallow light tillage should be carried out immediately after harvest to encourage volunteers and that the area should be left fallow over winter and another shallow, light tillage carried out in the spring. The group recommended that any volunteers detected in this two-year post-harvest period should be recorded and then destroyed before the emergence of inflorescences. The group discussed whether Rothamsted should be required to analyse the volunteers to determine whether they were

[2] 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.

GM, non-GM or hybrids. The conclusion was that this information was interesting but would not provide data that was necessary for the regulation of this trial.

The group noted that there had 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). The group discussed *Elytrigia repens* (couch grass) because it is a common agricultural weed that is a wild relative of wheat and is common in the area surrounding the trial site. No spontaneous couch grass x wheat hybrids have been reported. However, as a precautionary measure the group concluded that couch grass growing within the trial site and in the surrounding 20 m area should be destroyed before it flowers. It recommended that this area should be monitored for the presence of couch grass until mid-October following harvest and in the subsequent 2 years following the final harvest of GM material.

The group considered the measures proposed by Rothamsted to minimise unintentional transfer of material from the trial site. In particular the group discussed the cleaning of machinery that would be used.

The trial site will be surrounded by a 2.4m high chain link fence to prevent the entry of rabbits and other large mammals. The group considered the additional measures proposed by Rothamsted to deter animals including birds and recommended that these should be kept under observation to ensure that 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. The group 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. The group concluded that the impact of this GM seed presence is an economic issue that is beyond ACRE's remit rather than an environmental one.

Defra had received 86 representations during the public consultation on this application. In addition to the many relevant comments, the group noted that there were also comments on issues that are outside of ACRE's remit.

Action: The secretariat to draft advice based on the group's discussion and to circulate it to the whole of the ACRE committee for comment.