



*ADVISORY COMMITTEE ON RELEASES TO THE ENVIRONMENT*

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

**Applicant:** University of Leeds

**Application:** To release potato lines genetically modified for resistance to potato cyst nematodes

**Ref:** 07/R31/01 <sup>1</sup>

**Date:** 29 April 2008

**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 on 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 on human health and the environment, the applicant should:

1. Ensure that the GM potatoes produced as a result of this release will not be put into the human food chain or fed to livestock.
2. Ensure that any GM or non GM potato plant material remaining in the area of release at the end of the trial is inactivated.
3. Ensure that in the two years following harvest of the GM potato tubers, the area of release is left fallow and not ploughed; but use only shallow tillage as required.
4. Treat any groundkeepers and volunteers growing from true seed in the fallow years with an application of glyphosate herbicide or hand pull potato plants prior to flowering.
5. Ensure that during any post-trial monitoring period remaining after the fallow period that a crop is cultivated on the release site which would permit easy identification and control of groundkeepers and volunteers.
6. Control all groundkeepers and volunteers continuously until a period of two years has elapsed during which no groundkeepers or volunteers have been observed. The appropriate herbicides should be used to control potato plants growing from true seed and from groundkeepers prior to flowering. Alternatively, plants growing from groundkeepers may be hand pulled.
7. Ensure a separation distance of 20 metres to non-GM potato plants growing around the trial site to minimise the probability of cross-pollination occurring.

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<sup>1</sup>Application reference 07/R31/1 dated 3 January 2008 taking into account all information and amendments in the applicant's communications received 16 January and 10 April 2008.

## Comment

ACRE considered the risks to human health and the environment posed by the proposed release of potatoes genetically modified for resistance to potato cyst nematodes. 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 a maximum of 12,000 GM potatoes over a 3 year period. The applicant has proposed that the release will take place at one site. The site the GM potato plants will occupy less than 1000 m<sup>2</sup> (0.1 hectare) in an experimental plot of no more than 0.1 hectare in each year of the trial.
- ii) The GM potatoes produced as a result of this release will not be put into the human food chain or fed to livestock.

In reaching its view on the current application, ACRE also considered the results of post-release monitoring reports provided by consent holders from previous trials where GM potatoes were released.

Molecular characterisation of the GM potato lines to be released in this trial confirmed that a gene conferring resistance to the antibiotics neomycin and kanamycin (*nptII*) was present in all the GM lines to be released. ACRE has previously considered the issue of the presence of an antibiotic resistance marker gene in potatoes. ACRE's recently issued advice in relation to other GMOs remains unchanged in the absence of any new evidence. ACRE is of the opinion that the therapeutic effect of antibiotics that are substrates for NPTII will not be compromised by the presence of the *nptII* 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) acquisition of an intact gene is only one of the possible mechanisms for bacteria to develop resistance. These points also apply to the specific case of the proposed trial of GM potato cyst nematode (PCN) repellent potatoes.

ACRE requested further molecular characterisation to demonstrate the absence of sequences from plasmid vectors in GM potatoes. The data provided shows that sequences from the plasmid vectors are absent.

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. Furthermore the absence of prokaryotic features (i.e. plasmid vector backbone sequences), will reduce the probability of HGT. ACRE's advice on the specific issue of HGT of the synthetic PCN repellent gene is that synthetic gene DNA is no more likely to recombine with bacteria or viruses in the soil than any other DNA in the environment. Even if it is assumed that this rare recombination event does occur, the consequences are predicted to be negligible since (a) the gene is highly unlikely to recombine as a fully functional transcription unit (i.e. promoter together with the gene) such that expression of the synthetic peptide would occur; (b) the expression of the synthetic peptide in GM potatoes is driven by plant specific promoters and therefore there is even less chance that expression of the synthetic gene would occur in soil prokaryotes and (c) given the lack of stability of the peptide (see below), the impact of such expression on a recipient organism is unlikely to confer any selective advantage, therefore any perturbations would be of low risk and be transient.

Regarding the issue of persistence of synthetic DNA in the soil, ACRE is of the opinion that the synthetic PCN repellent gene (27 base-pair) DNA sequence would be degraded as any other DNA released into the soil environment. In addition, ACRE is of the opinion that the DNA breakdown products from the repellent gene sequence would not persist in the environment. ACRE advises that although specific and sensitive PCR assays could be designed to detect the synthetic gene from DNA extracted directly from soil, there is no way of distinguishing whether this originates from within GM potato plant residues or if it is free in the soil. Even if it is the latter ACRE is of the opinion that it is extremely unlikely that significant quantities of intact and biologically active synthetic genes will be released from the GM plants and persist in soil in a form that could replicate via horizontal gene transfer. Therefore ACRE advises that no special monitoring and /or clean up procedures are required after the end of the trial. Overall, ACRE concludes that none of the inserted genes (synthetic repellent, cystatin - *Oc-ID86*, selectable marker - *nptII*, constitutive promoter - *CaMV 35S*, root specific promoters - *ARSK-1*, *MDK420*, transcription terminators - *nos*) are likely to pose a risk to human health or the environment in the context of the proposed release.

With respect to potatoes transformed with plant cystatin genes ACRE is satisfied that these are well characterised and that they have been safely released in a previous trial. ACRE is of the opinion that plants containing and expressing the cystatin gene will not harm human health or the environment in the context of this proposed trial.

By comparison, relatively little information was provided in the first instance on the repellent peptide encoded by the synthetic repellent gene. The University of Leeds was requested to provide additional data to enable ACRE to evaluate more fully the risks to the environment particularly to non-target organisms. Additional information and a revised risk assessment have been considered by ACRE, and the Committee is now satisfied that the repellent peptide is not toxic to target organisms (PCN) and to a range of non-target organisms representative of the soil environment into which the repellent would be secreted from plant roots. Data were supplied showing that the repellent was predicted to be present in the soil at very low levels. Other data show that it is rapidly destroyed by soil microorganisms and that it does not cause mortality to a variety of non-target organisms (tested on representative oligochaetes, insects, non-target nematodes, planarians, ciliates and a higher plant species). ACRE is satisfied that the evidence shows that the synthetic repellent peptide will specifically exert a repellent effect on the intended target organism PCN in the context of the proposed release.

Cultivated potatoes are a low-risk crop for pollen-mediated gene flow because they are highly self-compatible and cannot cross with other UK wild species to produce viable offspring. A complete chain of events would have to occur in order for a potato containing a GM event to enter the human food chain. First, pollen containing a GM event would have to be produced and this occurs to a variable degree in potato plants since some cultivars produce few flowers. In the University of Leeds trial, the transformed potato cultivar is Desiree which is capable of flowering and setting seed and therefore this can be regarded as possible. The second stage would be the successful transfer of the pollen to a non-GM potato growing in a commercial crop. This relies on pollen being transported by the wind or by insects, pollen dispersal typically tails off with distance from the pollen source. ACRE recognises that rare long-distance cross-pollination events are possible, especially where pollen beetles are common in the release area. However, cross-pollination frequencies reduce dramatically over distance and pollen competition from within a non-GM potato crop reduces the likelihood of successful hybridisation further. Third, even if GM pollen successfully hybridised and resulted in GM seed, the chance of such seed successfully germinating and surviving until harvest as a tuber in a non-GM potato crop is low because potatoes are usually grown in rotations and the volunteers

resulting from true seed are very vulnerable to herbicide applications and crop competition.

The applicant has proposed a separation distance of 20 metres to non-GM potato plants growing around the trial site and ACRE therefore considers that the probability of cross-pollination occurring is minimal.

ACRE notes the information provided on the layout of the release site, which proposes to surround GM potatoes (cv Desiree) with guard non GM potatoes (cv Sante). The aim here is to protect the trial potatoes from the typical field edge effects of wind and rain. Due to the close proximity to the GM potatoes, the University of Leeds propose to treat these guard potatoes as part of the GM trial and dispose of them in the same way as the GM material. ACRE considers that this measure is appropriate to ensure that all GM potato material is inactivated at the end of the trial. The Committee recognises that other details on the layout of the release site are required primarily for enforcement and do not affect the risk assessment of this release. The Committee also notes that the release will be overseen by the GM Inspectorate and that it is appropriate given the experimental nature of the programme of work for details of the plot design to be provided just prior to the time of the release.

The University of Leeds has indicated that the trial will be fenced to prevent entry of wild animals. Potatoes are not generally eaten by wild animals due to the natural presence of toxic chemicals in plant foliage. ACRE sees no reason to fence the trial since this is likely to have a minimal impact in reducing the transfer of tubers away from the trial site by involuntary means.

ACRE considered the post harvest monitoring plans proposed by the applicant. Monitoring of previous releases of potatoes has revealed that groundkeepers may persist for many years after the initial release and in addition to this, potato plants in this trial will be allowed to set true seed. ACRE advises that the ground on which potatoes have been released should remain fallow for two years following the release. This would allow any tubers inadvertently not harvested to produce groundkeepers, and for true potato seed to remain near the soil surface. Additionally ACRE recommends that in the two years following harvest of the potato tubers shallow tillage should be the only form of cultivation used on the release area. Furthermore the Committee considers that University of Leeds should monitor the trial area until it has been clear of potato groundkeepers and volunteers for a continuous period of two years and that crops which facilitate the removal of potato groundkeepers and volunteers should only be grown throughout the remaining post-trial monitoring period.

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

ACRE considered 103 representations received from members of the public on this application with respect to the scientific issues. The Committee considered the comments relating to:

- The presence of the *nptII* antibiotic resistance marker gene. ACRE has considered the issue of the presence of an antibiotic resistance marker gene in potatoes, this is detailed above.
- Safety/specificity of synthetic peptide. ACRE identified this as key information that was initially lacking and requested it from the University of Leeds to enable a more comprehensive evaluation of the risks to the environment, particularly to non-target organisms, to be made. Additional information and a revised risk assessment were supplied and considered by ACRE, and it is now satisfied that the potential risks to the environment are negligible, as detailed earlier in this

advice. The issue of the safety of the breakdown products of the synthetic peptide was raised. ACRE considered this and advises that the breakdown products of a peptide are amino acids and the potential toxicity of the peptide breakdown products is likely to be negligible.

- The impact of the GM PCN resistant potatoes on natural populations of entomopathogenic nematodes. ACRE is satisfied by the evidence that shows that non-target nematodes would not be affected by either cystatin or repellent GM potatoes. The Committee advise that it is unlikely that PCN resistant potatoes would impact on natural populations of entomopathogenic nematodes, primarily because the exudate from potato is highly specific to PCN.
- The potential allergenicity of cystatins. ACRE notes that the cystatin gene is derived from naturally occurring rice, and is present in many other edible plants. The evidence provided shows that cystatin is not allergenic. The Committee considered this case, and concluded that the issue is not relevant because the potatoes from this trial will not enter the human or animal food chain.
- Risk management issues of separation distances, survival of potato tubers and cross pollination. As explained earlier in this advice, cross pollination is just one event in a chain of unlikely events that would be needed to contaminate potato crops outside the trial area. The Committee considers that the other risk management issues raised in these representations did not affect the risk assessment given the scale of the release and the fact that tubers from this release were not destined for the food chain of humans or livestock.

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.