Advisory Committee on Releases to the Environment

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

Applicant: The Sainsbury Laboratory

Application: To release potato lines genetically modified for resistance to potato late blight and to potato cyst nematodes and for improved tuber quality.

Ref: 17/R29/01

Date: April 23rd, 2017

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 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 at least annual shallow tillage in the spring is used to stimulate germination of any true potato seed.

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, 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 four years has elapsed. Report on the number of groundkeepers and volunteers observed during this period. Appropriate herbicides should be used to control potato plants growing from true seed and from groundkeepers prior to flowering

7. Ensure a separation distance of 20 metres to non-GM potato plants growing around the trial site to minimise the possibility of cross-pollination occurring.

Comment

ACRE does not make decisions on whether to authorise the use of GM crops in field trials. It provides scientific advice to the UK regulatory authorities on any risks to the environment or to human health posed by these trials and recommends best practice for managing them, including any conditions that are required to manage a risk of harm.

Key characteristics of this release for risk assessment are:-

i) The trial will take place over four years at one site. It will be a small scale trial with approximately 250 GM potato plants being grown each year in plots of about 100 square metres.

ii) The GM potatoes produced as a result of this release will not be put into the human food chain or fed to livestock.

TSL plans to field trial GM potato plants containing three resistance (*R*) genes either stacked together or present as single genes. These *R* genes confer resistance to different isolates of *Phytophthora infestans*, which causes late blight in potatoes. The *R* genes are derived from wild potato relatives *Solanum venturii* and *Solanum americanum*. Some of the potato lines will contain a cystatin gene and a synthetic gene encoding a peptide that repels potato cyst nematodes. Expression of both genes has been targeted to the roots of the GM potato plants. In some cases these are stacked with *R* genes. Some plants also contain constructs that interfere with the expression of genes involved in the production of polyphenol oxidase (associated with browning of bruised tissue), the production of vacuolar acid invertase (associated with cold-induced potato sweetening) and the production of asparagine (which, in combination with reducing sugars leads to the formation of acrylamide). TSL concludes that vector backbone DNA is unlikely to be present in the GM plants. There are two selectable marker genes on the backbones of the transformation vectors. These encode neomycin phosphotransferase II (NPTII) and isopentenyl transferase (IPT).

ACRE has assessed GM potato plants containing a number of these genes previously and trials involving these plants have been authorised by Defra. ACRE has assessed the use of the *R* genes and genes encoding the cystatin and the synthetic peptide as well as the *nptII* gene previously and not identified a risk of environmental harm, at least in the context of these small-scale trials. ACRE notes that GM potato plants containing the constructs that silence genes to improve potato tuber quality have been, or are, in the process of being deregulated in the USA.

TSL has not yet generated all of the GM plant lines it plans to use in the trial. However, this did not affect ACRE's assessment, which depends on detailed information on the methods and vectors used to transform these GM plants, information on the biology and agronomy of potatoes and information on the scale of the trial and how it will be managed. TSL provided data on expression levels of one of the R genes (*Rpi-amr3i*) but ACRE considered that this was not necessary to inform the risk assessment. TSL also carried out tests to demonstrate

that vector backbone DNA had not been introduced into these plants. It also carried out an assessment of the risk of human and environmental harm if vector backbone DNA were to be present in any of the GM plants. ACRE concludes that in the context of this trial, the risks are negligible and that there is no reason to require that vector backbone DNA is absent from GM plants used in this trial. In addition, ACRE concluded that information on the copy number of the different genetic elements is not helpful in terms of informing the risk assessment in this case.

A number of the public representations expressed concern about the number of genes that will be inserted into some of the GM lines. The concern is that this increases the potential for unexpected interactions that could result in harm to the environment or to human health. ACRE considered the potential for the different gene products or traits to interact and could not identify a plausible scientific hypothesis that would lead to harm. ACRE also considered what characteristics of the potato would need to alter in order to increase the risk of harm associated with this trial. It discussed the variation in the levels of native toxins produced by commercial potato varieties. These provide a natural defence in the field but would need to be characterised before any GM potato could be authorised for food or use as animal feed. ACRE also noted that the trial site will be monitored at least every week during the growing season for unexpected effects. Any effect that could be conceived as harmful would result in the trial being destroyed.

ACRE concludes that TSL has provided detailed information on the transformation vectors, the genetic elements they carry and the methods used to produce the GM potato plants.

Late blight resistance

These *R* genes are members of the nucleotide binding site-leucine rich repeat (NB-LRR) family. This type of gene is already abundant in potato and other plant genomes. Many non-GM potato varieties cultivated in Europe already contain genes of this class, derived from another potato wild relative, *Solanum demissum*. Introduction of these additional *R* genes to the GM potatoes is intended to increase the range of late blight pathotypes to which potato is resistant. There is no evidence of *R* genes of

the NB-LRR family conferring toxic or allergenic properties. The corresponding promoter and terminator regions from the potato wild relatives have been transferred along with the *R* genes.

Potato cyst nematode (PCN) resistance

Some of the GM potato lines used in this trial will produce a PCN-repellent peptide that disrupts the chemoreception that PCN require to locate host plants and a cystatin that limits the growth of PCN by inhibiting digestive enzymes (cysteine proteinases). GM potatoes containing these genes have been trialled in England previously without unintended adverse effects. Many of the public representations express concern about the use of the repellent, particularly its potential to harm other soil organisms. The results of previous trials and laboratory experiments are presented in peer-reviewed papers by Lilley et al (2004)[1], Kiezenbrink and Atkinson (2004)[2] and Green et al (2012)[3]. These show that the peptide

[1] Lilley C.J, Urwin P.E, Johnston K.A, Atkinson H.J. (2004) Preferential expression of a plant cystatin at nematode feeding sites confers resistance to *Meloidogyne incognita* and *Globodera pallida*. Plant Biotech J. 2: 3–12.
[2] Atkinson HJ, Keizenbrink DT (2004) Determining risks to soil organisms associated with a genetically modified (GM) crop expressing a biopesticide in its roots. Available at: http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=11999.
[3] Green.J. Wang D, Lilley C.J, Urwin P.E, Atkinson H.J. (2012) Transgenic Potatoes for Potato Cyst Nematode Control Can Replace Pesticide Use without Impact on Soil Quality. PLoS ONE. 7(2): 1- 9. e30973

and the cystatin offer potential for controlling PCN without impacting on the non-target nematode soil community.

ACRE noted that it is unlikely that PCN will be present at the trial site and that Maris Piper potatoes are resistant to the PCN, *Globodera rostochiensis*.

Gene-silencing (RNA interference)

Two of the transformation vectors contain fragments of three native potato genes that when expressed, are designed to silence the polyphenol oxidase gene (*Ppo*), the asparagine synthetase-1 gene (*Ast1*) and the vacuolar acid invertase gene (*Vlnv*) in the potato tubers. The aim is to produce potato tubers that do not brown on bruising and which are less likely to blacken or produce acrylamide upon cooking. A number of public representations were concerned that there is no specific guidance from the European Food Safety Authority (EFSA) on using gene silencing in GM crops. ACRE noted that 'Flavr Savr' tomatoes first sold in 1994 were developed using this technology[4] and that other GM crops have been authorised for food and feed use in the EU since then. These include, MON 87705 and 305423 soybean. These GMOs produce seeds with lower levels of saturated and polyunsaturated fatty acids and higher levels of a healthier monounsaturated fatty acid. ACRE considers that the risk assessment approach for identifying and characterising risk in the EU is appropriate for assessing GMOs developed using gene silencing.

Gene silencing does not result in novel proteins. The main concern is that genes other than the target genes will be affected. These GM potatoes will not enter the food/ feed chain; they would need to be assessed and authorised for this use. Therefore, ACRE considered what characteristics of these GM potatoes could be altered to result in environmental harm when grow on a small-scale under the conditions of this trial. ACRE noted that potatoes produce glycoalkaloids, which are natural toxins that provide protection from herbivores and fungi. The levels can vary in commercial varieties and would be checked as part of the GM food/ feed safety assessment. ACRE noted that TSL will monitor the trial site (at least) weekly during the trial and will have a legal obligation to inform regulators of unintended effects. ACRE did not identify any plausible risks of harm that TSL should monitor for specifically.

Antibiotic resistance marker genes

TSL's use of the *nptII* antibiotic resistance marker gene was raised in most of the public representations on this application. TSL is using *nptII* gene expression to select for the presence of two of its plant transformation vectors in bacterial cells. NPTII confers resistance to the antibiotics neomycin and kanamycin, which are included in the medium on which the bacteria are cultured. By including the *nptII* gene in the backbone of these vectors and selecting for GM plants that do not contain vector backbone, TSL intends to use GM potato plants in the trial that do not contain the *nptII* gene. This is likely to be helpful if any of these GM potato lines are developed for commercial cultivation in the future. ACRE has considered the consequences of the *nptII* gene being present in GM plants used in small-scale trials on a number of occasions previously, including after a statement by the European Medicines Agency (EMA) on the importance of preserving the therapeutic relevance of the antibiotics kanamycin and neomycin. ACRE remains 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 (i) the likelihood of transfer of a functional gene from plant material to bacteria is extremely low and that (ii) bacteria with resistance to these antibiotics are widespread in the environment.

Some of the GM potatoes lines will contain the tomato acetolactate synthase (*als*) gene as a plant selectable-marker to distinguish the GM potatoes from untransformed potatoes in tissue culture. This gene encodes a variant of the ALS enzyme that confers resistance to sulfonylurea and imidazolinone herbicides. There are a number of crops developed through traditional breeding methods that have resistance to sulfonylurea and imidazolinone herbicides as a result of mutations in the ALS gene including oilseed rape and wheat varieties. These plants will be sensitive to other herbicides such as glyphosate or glufosinate, which could, if necessary, be used to eliminate them in the field trial. Sulfonylurea and imidazolinone herbicides should not be used at the trial site. The potential for horizontal gene transfer (HGT) from plants to soil bacteria has been discussed above with respect to the *nptII* gene.

TSL is also using a gene encoding isopentenyl transferase (*ipt*) to identify GM potato plants containing vector backbone. The *ipt* gene is present in two of the plant transformation vectors, just outside of the left borders. IPT is a cytokinin and GM plants producing it will have a characteristic shooting phenotype, which is easily detected and the plants discarded.

Pollen-mediated gene flow to other plants

Cultivated potatoes are a low-risk crop for pollen-mediated gene flow because they are highly self-compatible and cannot cross with other wild or ornamental species in the UK to produce viable offspring. Successful transfer of pollen from the GM potatoes to a non-GM potato growing in a commercial crop relies on pollen being transported by the wind or by insects. ACRE recognises that rare long-distance cross-pollination events are possible, especially where pollen beetles are common in the area of the trial site. 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. 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.

Therefore, ACRE considers that the proposed separation distance of 20 metres to non-GM potato plants growing around the trial site is appropriate.

ACRE notes the information provided on the layout of the release site, where it is proposed to surround GM potatoes with guard non-GM potatoes. The aim 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 TSL should 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 the measures proposed by TSL to inactivate material from the trial site are appropriate. Other details on the layout of the trial site are required primarily for enforcement and do not affect the risk assessment. ACRE also notes that the trial 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.

ACRE considered the post-harvest monitoring plans proposed by the applicant. Monitoring of previous releases of potatoes has revealed that groundkeepers may persist for several years after the initial release. These derive from tubers or fragments of tubers left in the soil after harvesting. Where there is sufficient space between plots, the use of a mechanical lifter is likely to be more efficient in removing tubers. Hand-pulling will be preferable if tubers from surrounding plants have the potential to ricochet off the machinery because of their proximity. ACRE considers that potato plants arising from true seed are unlikely in agriculturally managed situations.. However, as a precautionary measure, ACRE advises that the trial site be managed so that any potential for true seed to persist and germinate into GM plants in future years is also minimised. ACRE advises that the trial site should be harvested, and potato tops removed from the field, as soon as soon as practical after results have been obtained in order to minimise maturation and any potential shedding of true seed. In addition ACRE advises that the ground on which potatoes have been released should remain fallow for two years following the release and not ploughed. This would allow true potato seed and tubers to remain near the soil surface and produce volunteers. Light tillage should be carried out annually in the spring to stimulate germination of true potato seed but no other form of cultivation should be used on the release area. After two years, crops that facilitate the removal of potato groundkeepers and volunteers should be grown throughout the remaining post-trial monitoring period. ACRE considers that TSL should monitor the trial plots for potato groundkeepers and volunteers for at least four years after they are harvested. At the end of four years, the results should be examined to determine whether monitoring can stop.

Items arising from public representations

Defra received 119 representations from members of the public on this application. ACRE considered points that had relevance to the safety of this particular trial. One of these representations was submitted on behalf of thirty three organisations.

ACRE considered the comments relating to the potential for this particular trial to cause harm to human health and the environment. This does not include a food safety assessment as any consent that is issued for this trial will not permit material from the trial to enter the food or animal feed chains. ACRE has not addressed issues that are not safety concerns such as whether the GM potatoes will be of benefit to consumers, will be economically viable and whether there are existing non-GM varieties with resistance to potato blight and PCN.

Some of the representations raise concerns about the commercial cultivation of these GM potatoes, for example whether farmers might use sulfonylurea and imidazolinone herbicides. If these GM potatoes were notified for commercial cultivation in the future, such concerns would be considered. ACRE is required to advise on the risks posed by this particular trial and agrees that these herbicides should not be used on the trial site.

ACRE is grateful to those who have submitted comments and has used them in considering the safety of this trial.