

Advisory Committee on Releases to the Environment

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

Applicant: John Innes Centre

Application: To release genetically modified lines with lower levels of glucosinolates.

Ref: 19/R52/01

Date: 8 April 2019

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 genome edited (GE) *Brassica oleracea* from this trial will enter the human food or animal feed chains, the applicant should:

1. Ensure that *B. oleracea* and *B. napus* volunteer plants growing in trial plots are controlled (prior to flowering) during the trial.
2. Record the number of volunteers that germinate before destroying them by applying herbicide or hand pulling them prior to flowering.
3. Control all plants within the genera *Brassica*, *Eruca*, *Erucastrum*, *Hirschfeldia*, *Moricandia*, *Raphanus* and *Sinapis* (other than those used in the trial) growing in the trial plots and in the surrounding 20m, before they flower.
4. Ensure that all *B. oleracea* and *B. napus* material is disposed of such that GE plants will not persist on the trial site.
5. Ensure that after harvesting the *B. oleracea* and *B. napus* plants, the ground they are grown in is lightly tilled twice (once after harvest and again in the following spring) to a depth of 5cm to stimulate germination of any seed. Each plot should be left fallow and monitored for *B. oleracea* and *B. napus* volunteers for a minimum of 2 years following harvest.
6. Ensure that machinery used on the site is cleaned thoroughly onsite and

that clothing and equipment such as vehicles used by personnel on the site are also cleaned thoroughly before leaving the site.

Comment

ACRE considered the risks to human health and the environment posed by a field trial involving *Brassica oleracea* plants, in which the function of a native gene (*Myb28*) has been disrupted using genome editing. *Myb28* is an important regulator of glucosinolates that are present in *Brassica* species. These compounds are associated with health properties but at higher levels can be harmful. This study is intended to inform the researchers about the effect of the *Myb28* gene under field conditions.

Key characteristics of this field trial with respect to its environmental risk assessment are:-

- i) It will be on a small scale. The genome edited (GE) plants will be grown in area less than 1000 square metres. The John Innes Centre (JIC) is proposing a 3 year trial starting in spring 2019.
- ii) The GM and non-GM Brassica plants grown in this trial will not be put into the human food chain or fed to livestock.

The JIC used the CRISPR-Cas9 genome editing system to introduce small insertions or deletions into the *Myb28* gene in *B. oleracea* plants. In addition to the CRISPR guide RNA and the Cas9 cutter, the original transformants also produced neomycin phosphotransferase II (*nptII*) and spectinomycin adenyltransferase (*aadA*). These confer resistance to antibiotics such as kanamycin /neomycin and spectinomycin/ streptomycin respectively. This antibiotic resistance allows transformed plants and bacteria to be selected from untransformed material. In subsequent generations, the genetic elements coding for these proteins will be bred out of plants selected for use in the field trial. These plants will contain mutations in the *Myb28* gene.

Molecular characterisation

ACRE was content with the JIC's description of how the GE *B. oleracea* plants were made. The JIC will need to provide confirmation that plants used in the trial do not contain exogenous DNA inserted during the transformation process. This is because these genetic elements were not considered in its risk assessment. However, they are often used in transgenic plants and ACRE did not identify a risk associated with the presence.

Some of the public representations criticised the JIC's molecular characterisation of the GE lines for not determining whether DNA introduced into the primary transformants is present in the GE plants used in the field trial.

They also suggest that the JIC should have analysed the plants for off-target effects and interference in the regulation of gene expression. The JIC did

consider the risk of these plants having a greater negative environmental impact as a result of Cas9 cutting DNA sequence that is similar to the *Myb28* target sequence. ACRE agreed that it is highly unlikely that *B.oleracea*'s low potential for invasiveness and persistence would change as a result of additional mutations. The plots in which the GE plants will be grown and the area surrounding these plots will be monitored during and after the trial. Measures to minimise seed survival on the site and cross-pollination with sexually compatible species will also be put in place as a precaution.

ACRE noted that traditional mutagenesis techniques used in plant breeding generate many hundreds of off-target effects. The majority of these are lost when the mutant plants with desired characteristics are 'backcrossed' to lines that have not been mutated.

The Environmental Risk Assessment

ACRE concluded that it was very unlikely that altered sulphur metabolism would change the characteristics/ biology of the plants in a way that would transform them into a problem agriculture weed or increase their ability to invade and persist in habitats outside of arable conditions. If the JIC were to submit an application for wide scale cultivation of these GE plants in the future, data from small-scale field trials on the comparative agronomic and phenotypic characteristics are likely to be required.

The majority of public representations reflected concern that growing plants containing antibiotic resistant marker genes would compromise the use of associated antibiotics in human and veterinary medicine. However, these genes will not be present in the plants used in the field trial. The transformed lines will be self-crossed in order to segregate progeny containing these genes from progeny containing *Myb28* mutations only. ACRE recommends that the JIC submits the results of tests demonstrating absence of inserted DNA, including vector backbone, to Defra prior to sowing/ transplanting these plants in the field trial.

Nonetheless, ACRE discussed the environmental risks associated with plants containing the antibiotic resistance marker genes *nptII* and/ or *aadA*. Bacteria resistant to kanamycin/ neomycin and streptomycin/ streptomycin occur naturally in the environment^{1,2} 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, ACRE discussed the potential for bacteria in the environment to be transformed with antibiotic resistance genes from plant material.

¹ Walsh F, Duffy B (2013). The Culturable Soil Antibiotic Resistome: A Community of Multi-Drug Resistant Bacteria. PLoS ONE 8: e65567.

² van Overbeek L.S, Wellington E.M.H., Egan S., Smalla, K., Heuer H., Collard J-M., Guillaume G., Karagouni, A.D., Nikolakopoulou T.L. and van Elsas J.D (2002) Prevalence of streptomycin-resistance genes in bacterial populations in European habitats. FEMS Microbiology Ecology, Volume 42, Issue 2, November 2002, Pages 277–288.

Studies of horizontal gene transfer from plants to bacteria suggest that this phenomenon is extremely rare (Please refer to a review by Keese, 2008³). ACRE noted 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.

Edits to the *Myb28* gene are targeted to disrupt its translation into a functional protein. This is expected to affect the levels of glucosinolates in the GE plants. Glucosinolates and their derivatives are the focus of research in plant defense, soil health, and human health (e.g. in dementia and cancer studies). However, some of these compounds have toxic effects (mainly as goitrogens and anti-thyroid agents) in both humans and animals at high doses.

The applicant notes that if the GE plants have a reduced glucosinolate content, this could result in increased herbivory and pest and pathogen attack. Many of the public representations were concerned that pollen from these GE plants could outcross with wild and commercial *Brassica* species, and if the *Myb28* mutation does confer reduced glucosinolate content, this could have a deleterious effect. However, this is a small trial and it will be managed to minimise pollen dispersal. The inflorescences on a small proportion of GE plants will be allowed to produce open flowers and these will be contained within pollen proof bags. There will also be a barrier of *B. napus* plants surrounding trial plots.

ACRE notes that a mutation causing decreased fitness in a wild plant species would not persist in a population. If *Myb28* mutations were introduced into commercial *Brassica* lines using GE, a detailed compositional analysis would be required. However, the objective of the trial is to better understand the role of the *Myb28* gene under field conditions.

Managing the trial site

As ACRE has considered the potential risks of this trial to human health and the environment in the context of it being a small-scale trial from which no material will enter the food or feed chains, the committee considered, in detail, management plans to minimise the persistence of GM material at the trial site and the dispersal of GM material from the site.

Gene flow

B. oleracea var *alboglabra* (the sub-species being used in these experiments) is self-compatible (i.e. sets seed by self-pollination) but cross-pollination with other compatible varieties of *B. oleracea* or, extremely rarely, some other crucifer species is possible. As described above, the applicant plans to remove inflorescences at an early bud stage from the majority of GE plants

³ Keese P. (2008). Risks from GMOs due to horizontal gene transfer. *Env Biosafety Research*. 7(3): 123 – 149

but up to ten plants will be allowed to set seed. Their inflorescences will be covered with pollen-proof bags before the flowers open. However, the JIC will cultivate a barrier of *B. napus* plants and employ a separation distance of 20 metres around the GE plants as a precautionary measure. ACRE recommends that plants of the following genera growing within this area must be removed before they flower: *Brassica*, *Eruca*, *Erucastrum*, *Hirschfeldia*, *Moricandia*, *Raphanus* and *Sinapis*. ACRE recommends that monitoring of this area starts when the GE seed/ plants are sown/ transplanted into plots in each year of the trial and that this continues until mid-October.

Volunteers growing from shed seed

B. oleraceae is a biennial or perennial herb that survives from year to year via seed only. The applicant will adopt measures to minimise seed set. However, it acknowledges that mature seed could be produced where flowers have developed on axillary branches. If not managed, this could over-winter in the soil and germinate the following spring as volunteers.

A barrier of *B. napus* plants will be grown around the plots to (a) minimise edge effects that could interfere with the experiment and (b) to attract insects that have pollinated any remaining, uncovered flowers on the GE *B. oleraceae* plants. Studies of gene flow between *B. napus* and *B. oleracea* plants suggest that spontaneous hybridisation is extremely rare but not impossible. These include field studies in the UK⁴. Therefore, whilst gene flow between the GE *B. oleracea* plants and the *B. napus* plants in the barrier around them is unlikely (particularly given the measures employed to minimise pollen dispersal), ACRE recommends that the JIC takes measures to prevent *B. napus* volunteers containing the edited gene persisting on the site. ACRE recognises that mutations in the *Myb28* gene could occur naturally.

ACRE recommends that *B. oleracea* volunteer management measures in plots where GE and non-GE *B. oleracea* plants have been grown should be initiated in the autumn. ACRE advises that shallow, light tillage should be carried out immediately after harvest to encourage volunteers. These plots should be left fallow over winter and another shallow, light tillage should be carried out in the spring. The plots should be monitored for volunteers in this year and the following year during which time it should remain uncropped. Any volunteers detected in this two-year post-harvest period should be recorded and then destroyed before the emergence of inflorescences. There are little empirical data on the persistence of *B. oleracea* under UK conditions.

⁴ Scheffler J.A. and Dale P.J. (1994). Opportunities for gene transfer from transgenic oilseed rape (*Brassica napus*) to related species. *Transgenic Research* 3(5):263-278
Wilkinson M.J., Davenport I.J., Charters Y.M., Jones A.E., Allainguillaume J., Butler H.T., Mason D.C. and Raybould A.F. (2000). A direct regional scale estimate of transgene movement from genetically modified oilseed rape to its wild progenitors. *Molecular Ecology* 9(7): 983-991.
Ford C.S., Allainguillaume J., Grilli-Chantler P., Cuccato G., Allender C.J. and Wilkinson M.J. (2006). Spontaneous gene flow from rapeseed (*Brassica napus*) to wild *Brassica oleracea* *Proceedings of the Royal Society B*. 22;273(1605):3111-5
Ford C.S., Allainguillaume J., Fu T.Y., Mitchley J. and Wilkinson M.J. (2015). Assessing the value of imperfect biocontainment nationally: rapeseed in the United Kingdom as an exemplar. *New Phytologist* 205(3):1342-9.

Therefore, ACRE recommends that further monitoring should be considered depending on these results.

The same approach should be taken to minimise *B. napus* seed entering the seed bank. However, the JIC should only continue to monitor for, and control, *B. napus* volunteers after monitoring for *B. oleracea* volunteers has finished, if these volunteers are hybrid plants and contain a *Myb28* mutation.

Seed movement

ACRE considered the measures proposed to minimise unintentional transfer of material from the trial site. All machinery should be cleaned thoroughly on the site between uses and before leaving the site. The JIC 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.

A small number of GE plants will be allowed to set seed (in pollen-proof bags). The remainder of the GE and non-GE plant material, once analysed, will be destroyed by autoclaving, incineration or deep burial at a local authority-approved landfill site using an approved contractor. If the integrity of the site is seriously compromised, the trial will be terminated and all plants will be destroyed using a suitable herbicide or be harvested, as appropriate.

Seed from the *B. napus* plants should be destroyed by autoclaving, incineration or deep burial at a local authority-approved landfill site using an approved contractor. The straw can be chopped and left on the site.

ACRE concludes that this particular field trial is extremely unlikely to have an adverse effect on human health and the environment but has recommended management measures that will minimise (i) the dispersal of GE material from the trial site and (ii) the persistence of GE material at the trial site.

Items arising from public representations

Defra received 48 representations during the public consultation on this application. As these covered most of the issues that ACRE considered in its assessment, they are included in the respective sections of this advice. In addition to these, there were representations against GMOs more generally. Some considered the research of value but recommended that it should be carried out in a controlled environmental facility.