Advisory Committee on Releases to the Environment

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

Advice of the Advisory Committee on Releases to the Environment (ACRE) to the Secretary of State under S.124 of the Environmental Protection Act 1990

Details of the notification

Notifier:	Rothamsted Research
Notification reference:	14/R8/01
Product:	*Camelina sativa* that has been genetically modified to produce omega-3 long chain polyunsaturated fatty acids in seed oil

ACRE is satisfied that the risks to human health and the environment associated with this proposed release are extremely low. ACRE has not identified any reasons for the trial not to proceed, subject to the following measures being put in place.

1. Planting of a non-modified *Camelina sativa* pollen barrier surrounding each plot of GM camelina (to flower synchronously with the GM camelina, and of a width consistent with that previously used for GM oilseed rape).

2. Alternatively, a separation distance consistent with that used for GM oilseed rape should be maintained between the GM camelina and any wild or cultivated *Camelina* species outside of the trial site. If any of these species are found within the separation distance during the trial, they should be killed by herbicide application or hand-pulling before flowering.

3. During the trial, suitable measures should be in place to prevent seed dispersal by birds.

4. After sowing, any drilling equipment used should be thoroughly cleaned on the edge of the plot to ensure that no seeds remain on the coulters or other parts of the drill.

5. Prior to harvest, the combine to be used should be prepared to minimise any loss of small seeds through augers, sieves etc. The combine should be one designed to minimise admixture between plots and to facilitate cleaning down.

6. After harvesting, the combine should be thoroughly cleaned on the edge of the plot to ensure no seed remains.

7. Each experimental plot should be shallow cultivated in the spring following harvest (to a depth of no more than 5 cm) to stimulate germination of any volunteer seed in the seed bank.

8. Post-harvest, the presence of volunteers should be monitored during the growing season (February until October) at least monthly for the next three years (two if no volunteers are found), and the number of volunteers found should be reported to Defra. After counting, all volunteers should be killed by herbicide application or hand-pulling before flowering.

9. Material intended for the food/feed chain should not be grown on the site until at least the second year after the trial.

Comment

ACRE considered the risks to human health and the environment associated with a proposed release of GM *Camelina sativa* for a research trial. The GM camelina has been modified to produce omega-3 long chain polyunsaturated fatty acids in its seed oils. The applicant (Rothamsted Research) wishes to carry out the research trial in order to test the agronomic and yield performance of the GM camelina under field conditions.

ACRE addressed a number of issues in its safety assessment, including the molecular characterisation of the material for release, an environmental risk assessment, measures that might be necessary to minimise the spread and persistence of the GMO, and any necessary monitoring requirements. To inform its decision-making, Defra consulted external assessors and requested that ACRE consider the representations received. In line with this, ACRE took into account comments from the Food and Environment Research Agency (Fera), Natural England and the Food Standards Agency when preparing this advice. A public consultation on this application was opened by Defra from 24 January until 21 March 2014, and Defra requested that ACRE consider and address any scientific points raised. ACRE's advice is presented below.

Molecular characterisation

The proposed trial involves the release of three GM events, each derived from a different construct. The three GM lines have been transformed with four, five or seven genes involved in the biosynthesis of omega-3 long-chain polyunsaturated fatty acids. These genes originate from sources including algae, moss, oomycetes and fungi, have been codon-optimised for the Crucifereae and chemically synthesised, and are under the control of seed-specific promoters. Each transformation construct also encodes a selectable marker that is produced in the plant, in two cases dsRed[1], and in one case neomycin

[1] Jach et al. (2001) Use of red fluorescent protein from *Dicosoma* sp. (dsRED) as a reporter for plant gene expression. Plant J. 28(4), 483-91

phosphotransferase II (NPTII). Camelina plants were transformed using *Agrobacterium*-mediated transformation.

ACRE noted that the material for release has been well-characterised at the molecular level and that the transgenes have been shown to be stably inherited following normal rules of Mendelian genetics over five generations. ACRE is therefore satisfied that the genetic changes are stable. The applicant had not provided details of the vector backbone or data on integration of the vector backbone into the plants genome. However, ACRE noted that the vectors used were derived from pBIN19, which is well-characterised and the sequence of which is widely available. ACRE agreed that integration of vector backbone would not represent an environmental risk in this case, and as such concluded that sufficient information had been provided to support the risk assessment. ACRE also noted that the applicant had not provided data on transgene expression in reproductive plant parts, including pollen. ACRE considered that transgene expression in these plant parts, should it occur, would be unlikely to represent a risk, and as such agreed that no further information was required to support the risk assessment.

Environmental risk assessment

ACRE considered the environmental risk assessment provided by the applicant, and whether there were any areas of uncertainty or additional concerns to be addressed. Overall, ACRE was satisfied with the information provided and the applicants assessment of the risks associated with the release. ACRE is content that the proposed release does not represent a risk to the environment or to human health.

ACRE agreed with the applicant that the biology and ecology of *C. sativa*[2] suggests it has a low baseline of invasiveness and does not compete well with surrounding vegetation.

ACRE agreed that the genetic modification was unlikely to alter this or confer any selective advantage. ACRE noted that the flora of the Rothamsted site has been well-characterised and that the applicant has a good knowledge of plant species present on the experimental farm. However, ACRE felt that the applicant's assessment of the likelihood of compatible species being present in the vicinity of the trial site was limited, and recommended that further information was provided, particularly with respect to the presence of other *Camelina* species that may have the potential to cross-hybridise[3] and the common weed *Capsella bursa-pastoris* (Shepherd's Purse). *C. bursa-pastoris* can cross-hybridise with *C. sativa*, although these hybrids were found to be sterile[4]. However, ACRE noted that only a

[2] Plant and Biotechnology Risk Assessment Unit, Canadian Food Inspection Agency Ottawa, Ontario (2012). The Biology of *Camelina sativa* (L.) Crantz (Camelina). http://www.inspection.gc.ca/plants/plants-with-novel-traits/applicants/directive-94-08/biology-documents/camelina-sativa-l-/eng/1330971423348/1330971509470

[3] Séguin-Swartz, et al. (2013) Hybridization between *Camelina sativa* (L.) Crantz (false flax) and North American Camelina species. Plant Breeding 132, 390-396

[4] Julié-Galau et al. (2014) Evaluation of the potential for interspecific hybridization between *Camelina sativa* and related wild Brassicaceae in anticipation of field trials of GM camelina. Transgenic Research 23, 67-74.

small number of biotypes have been tested. ACRE agreed that i) it is unlikely that *Camelina* species with the potential to cross-hybridise are present in the vicinity of the trial ii) it appears that less closely related Camelineae are generally not able to successfully cross-hybridise with *C. sativa*, and iii) if cross-hybridisation did occur (for example with *C. bursa-pastoris*) this would be very unlikely to produce fertile offspring, confer a selective advantage or represent an environmental hazard. In conclusion no risk was identified. The applicant updated their dossier to include the further information suggested.

As with invasiveness, ACRE felt that persistence and survivability were unlikely to be altered in the GM camelina. ACRE noted that there was some uncertainty over the baseline persistence of *C. sativa* in UK conditions, and this is reflected in the recommended monitoring measures. The application indicates the applicant's intention to avoid re-using experimental plots, ACRE considered that this would be sensible to avoid interference of volunteers in subsequent experimental plots, but did not feel that re-using plots would affect the risk assessment. ACRE also noted that as a small-seeded crop, camelina seeds could be dispersed via a number of routes, including birds and small mammals, as well as molluscs such as slugs[2]. Although it was not considered an environmental risk, ACRE recommended that the applicant acknowledge molluscs as a route of dispersal in their dossier. This was done by the applicant.

The risk of toxic, allergenic or harmful effects to humans or animals of incidental exposure to the material proposed for release were considered. ACRE agreed with the applicant that the risks of any adverse effects are very low.

The risks associated with horizontal gene transfer of transgenes into soil prokaryotes were also considered. ACRE was content with the applicant's assessment of the risk, which was in line with its previous advice[5] in concluding that horizontal gene transfer from plant material into soil prokaryotes is extremely rare, that if recombination did occur it is unlikely that transferred material would recombine as functional transcriptional units, and that even if functional genes were transferred, the hazard associated with this is minimal. As such, ACRE agreed that the risks associated with horizontal gene transfer are very low.

ACRE specifically considered the risks associated with the use of the *npt*II antibiotic resistance marker gene, in particular whether the therapeutic effects of antibiotics that are substrates of NPTII could be compromised by its presence in GM camelina in this proposed release. Consistent with its previous advice[5], and the opinion of EFSA[6], ACRE noted that the *npt*II gene occurs naturally in soil bacteria, that the likelihood of transfer of a functional gene from plant material to soil bacteria is extremely low, and that acquisition of an intact gene is only one mechanism by which bacteria may develop resistance. The *npt*II

[5] Advice of ACRE on application 09/R31/01 from University of Leeds to release potato lines genetically modified for resistance to potato cyst nematodes. Published 22nd Jan 2010.

[6] Scientific Opinion of the Panel on Genetically Modified Organisms (GMO) and the Panel on Biological Hazards (BIOHAZ) on a request from the European Commission on the use of antibiotic resistance genes as marker genes in genetically modified plants. The EFSA Journal (2009) 1034, 1-81.

gene is not expected to confer any selective advantage to plants or soil bacteria under the trial conditions. As such, ACRE considered that the environmental risks associated with the use of this gene are very low. ACRE agreed the *dsRED* gene, which is naturally occurring, is also not likely to confer any selective advantage to plants or soil microorganisms in the unlikely event of horizontal gene transfer, and so does not represent a significant risk to human health or the environment.

Minimising dispersal and persistence

As described above, ACRE agreed that the risks to the environment and human health associated with this release are extremely low. Even in the absence of any significant risks, Defra has requested that ACRE advise on proportionate measures that could be taken to restrict the GMO to the site and duration of the trial.

In this context, ACRE considered what proportionate measures should be put in place in order to minimise the spread and persistence of the GM camelina proposed for release. ACRE agreed that maintaining a separation distance consistent with that used previously for GM oilseed rape between the GM camelina and any wild or cultivated *Camelina* species (particularly *C. sativa*, *C. alyssum* and *C. microcarpa*) outside of the trial site would be a suitable measure to minimise the likelihood of cross-hybridisation. Alternatively, the applicant could put in place a 'pollen barrier' of non-modified *C. sativa* surrounding the GM camelina, to minimise the likelihood that pollen would be transferred from the trial site. To be effective, the pollen barrier should flower at the same time as (and so should be of the same variety and be sown on the same day as) the GM camelina. To increase the evidence base for environmental risk assessment of any future proposals for wider cultivation of GM camelina, ACRE suggests that the applicants may wish to collect data relating to the rate of cross-pollination between the GM camelina and that forming the pollen barrier, if it is practicable to do so.

To minimise the likelihood of seed dispersal from the trial site, ACRE made a number of recommendations. ACRE acknowledged that birds represent one possible route of seed dispersal, and recommended that suitable measures should be put in place to keep them out of the trial site (such as those suggested by the applicant). Camelina seeds are very small and this should be taken into account when selecting, checking and cleaning equipment used for sowing and harvesting. Prior to harvest the combine to be used should be prepared to minimise any loss of small seeds through augers, sieves etc. The combine should be one designed to minimise admixture between plots and to facilitate cleaning down. After harvest, the applicant should ensure that the combine is cleaned completely such that all seed is removed before leaving the trial site, and cleaning of the combine should take place on the edge of the newly harvested plot. The applicant's proposal to harvest material from the trial slightly early to minimise seed shedding was noted; ACRE agreed that this measure was not necessary and could potentially affect the results of the trial, and as such suggests that this is not carried out.

ACRE noted comments on this application from Fera indicating that waste seed and plant material from the trial should be disposed of by incineration or deep burial at a local authority-approved landfill site using an approved contractor.

ACRE also considered measures that could be put in place to minimise the persistence of material within the experimental plots. It is recommended that after harvest, the plot should be left fallow until the following spring, at which point the whole plot should be shallow cultivated to a depth of no more than 5 cm (and preferably less) to stimulate germination of any volunteer seed in the seed bank.

To minimise the likelihood of any material from the trial entering the human food or animal feed chain, ACRE recommends that the trial site is not used to cultivate crops for the food/feed chain until at least the second year after the trial is completed (subject to the monitoring outlined below).

Monitoring

In order to determine whether the proposed measures are operating effectively, ACRE suggests that the applicant should carry out the following monitoring for at least three years after harvesting, or two years if no volunteers are recorded. Volunteers of *C. sativa* growing on experimental plots and in the surrounding area should be allowed to emerge, before being counted at least monthly (during the growing season). Data on numbers of volunteers should be provided to Defra annually. Any volunteers identified should be destroyed by herbicide application or hand-pulling before flowering.

Items arising from public representations

One public representation was received. ACRE considered comments relating to:

Seed dispersal by wildlife

As described above, ACRE considered that the invasiveness and persistence of the GM camelina was unlikely to be affected by the genetic modification, as was the allergenicity, toxicity and potential for harmful effects on human or animal health. As such, ACRE considers that the risks associated with the proposed release are extremely low and did not propose any risk management measures. As requested by Defra, ACRE has recommended proportionate measures to minimise the dispersal of material from the trial site. This includes measures to minimise the likelihood of seed dispersal by the most significant potential routes, i.e. removal by birds and via equipment used on the site. ACRE advised that although it is possible that a small amount of seed might be removed from the site by wildlife such as small mammals or molluscs (as is the case in any field trial), this would not pose a risk to the environment or human health.

ACRE considers it unlikely that the altered nutritional profile of the GM camelina would significantly affect its attractiveness to wildlife, and advises that if this were the case, this would be unlikely to affect the (low) ability of the GM camelina to outcompete surrounding vegetation. As such, ACRE was content that this did not represent an environmental risk.

Pollen dispersal, outcrossing and hybridisation

ACRE agreed with the applicant's assessment that the species that are most likely to be sexually compatible with *C. sativa* (including *C. sativa* itself) are unlikely to be found on the Rothamsted estate. As described above, ACRE considers that cross-hybridisation with less closely related Camelineae is unlikely or expected to be at a very low level, and if this were to occur, the genetic modification would not be expected to confer a selective advantage or enhance persistence in the environment. ACRE has proposed proportionate measures to minimise the likelihood of cross-hybridisation with the species it believes have the most potential to cross-hybridise based on available evidence.

ACRE considered the proposed trial design, and agreed that as the risks associated with the trial are extremely low, the proposed risk management measures are not necessary. ACRE was content that none of the proposed measures would lead to additional risks.

Characterisation and stability of material for release

ACRE considered the molecular and phenotypic characterisation of the material proposed for release. As described above, ACRE considered that expression of the transgenes in pollen (or other plant parts), if it were to occur, would not represent a risk to the environment or human health, and as such did not request further information from the applicant.

The applicant has provided details of the DNA sequences that have (or could have) integrated into the plants genome, and ACRE has not identified any elements of concern. The information provided by the applicant indicates that the GM camelina for release is morphologically indistinguishable from untransformed controls, with no difference in seed set, seed size, fertility or vegetative performance detected. The applicant's characterisation of the GMOs for release and experience of handling them under glasshouse conditions strongly suggest that in the context of this trial they would be unlikely to cause adverse effects.

Unintended effects on toxicity and allergenicity

ACRE considered that the transgenes transferred to the GM camelina are unlikely to affect the toxicity and allergenicity of the camelina to humans or animals via incidental exposure, and as detailed above any significant unintended effects would likely have been detected during the crop development process. If in the future an application was made for wider commercial cultivation of this GM crop in the UK for use in the human food chain or animal feed, a more detailed assessment would be undertaken, to include consideration of unintended effects on food safety.

Food safety and future use

Food safety is outside the scope of this assessment. Material from the proposed trial is not authorised to enter the food or feed chain. The applicant has amended their application to fully reflect this and ACRE is content with that amendment. The proposed trial is for

research purposes, and any future applications of the technology are outside the scope of this assessment.

Antibiotic resistance marker genes

ACRE considered the risks associated with the use of the *npt*II antibiotic resistance marker gene in one of the lines of GM camelina proposed for release to be extremely low. As detailed above, ACRE and EFSA have both concluded that the use of *npt*II in GM crops is extremely unlikely to compromise the therapeutic use of any antibiotic or result in an adverse effect on human health or the environment.

ACRE is grateful for the representation submitted during the public consultation and is content that all of the issues relevant to this trial have been considered during the Committee's assessment of the dossier.

3 April 2014