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:	19/R8/01
Product:	*Camelina sativa* genetically modified with the aim of producing plants with some, or all, of the following traits: (i) omega-3 and omega-6 long chain polyunsaturated fatty acids that are not otherwise present in higher plants (ii) increased oil production (iii) lower sinapine (and other sinapoyl ester) content and (iv) increased oleic acid levels

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. After careful consideration of the present application, ACRE suggests similar measures are put in place, namely:

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(s) or hand-pulling before flowering. Glufosinate ammonium herbicides should not be used.

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 the beginning of December) at least monthly for a minimum of two years. Monitoring may cease a) if no volunteers are identified in the second year of monitoring or b) after the first volunteer-free year. 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.

10. Waste seed and plant material (including destroyed volunteers) from the trial should be disposed of by autoclaving, incineration or deep burial at a local authority-approved landfill site using an approved contractor.

Comment

Rothamsted Research (RR) has described clearly and comprehensively the methods and genetic elements it is using in its field research on GM *Camelina sativa* lines developed to produce more oil and /or oil with specific attributes. RR has risk assessed a small-scale field trial that involves either growing plants containing these elements (in the case of transgenic plants) or plants in which the genetic modification has been introduced using some of these elements (in the case of genome edited plants). The aim of these genetic modifications is to confer the following traits (noting that that each plant will not necessarily express all of these traits):

- increased seed oil production
- the production of omega-3 long chain polyunsaturated fatty acids in seeds
- increased oleic acid content in the seed oil.
- a reduction in sinapine and total sinapine ester content in seeds

In its assessment, ACRE considered information on the genetic modifications, focussing on data that provides the basis for an environmental risk assessment, it then considered whether there are any plausible environmental risks taking into account the scale and design of the trial. It also considered management measures to minimise the spread and persistence of the GMOs and monitoring requirements.

ACRE took into account scientific points raised in public representations. For the main-part, these will be covered as the advice addresses the main issues; any additional points are discussed separately at the end.

Molecular characterisation

RR transforms *C. sativa* with genes of interest by dipping plants into solutions of GM *Agrobacterium*. The seeds from these plants are germinated and markers are used to identify transgenic plants. The selectable marker genes that RR use to select for transformants encode green or red fluorescent proteins or phosphinothricin acetyl transferase (which provides resistance to glufosinate, a broad-spectrum systemic herbicide). The selectable marker genes are under the control of constitutive promoters from the cassava vein mosaic virus or from *Agrobacterium tumefaciens*.

Increased oil production

RR has developed GM *C. sativa* plants with increased oil content in their seeds under glasshouse conditions; it would like to test these in the field. RR's approach involves introducing a combination of genetic modifications that increase the yield as well as improving the attributes of the oil. In plants, fatty acids are synthesised in plastids and then exported to the cytosol and endoplasmic reticulum for editing and assembly into specific lipids. The seeds of RR's GM *C. sativa* lines express an acyl-acyl carrier protein thioesterase from *Arabidopsis thaliana* (thale cress), which affects the type of fatty acids exported from plastids. They also express a modified form of a lysophosphatidic acid acyltransferase, which catalyses the formation of phosphatidic acid, an important intermediate in lipid biosynthesis. RR will use an RNA interference (RNAi) approach to suppress the expression of a fatty acid desaturase (*FAD 2*) and a fatty acid elongase (*FAE 3*) gene in these plants. This is achieved by introducing fragments of these genes, in a reverse orientation, into the *C. sativa* lines.

Some of the representations registered concern that the RNA molecules produced by the GM lines would interfere with the transcription of genes other than the *FAD2* and *FAD3* genes. These did not specify a pathway by which the suppression of genes with similar genetic sequence could result in environmental harm. ACRE considered that unintended effects are unlikely to change *C. sativa* from being an annual species that requires active management to out-compete weedier plants to a problem weed in agronomic systems or to an invasive species in unmanaged ecosystems. ACRE concluded that gene flow from *C. sativa* to other species is unlikely. However, ACRE has recommended management measures to minimise gene flow from the trial sites as a precautionary measure (please refer to the next section of ACRE's advice).

The production of omega-3 long chain polyunsaturated fatty acids in seeds

Defra has authorised RR field trials involving GM *C. sativa* lines producing novel omega-3 long chain polyunsaturated fatty acids [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] in 2014, 2016 and 2018[1]. These are nutritionally important oils. Virtually all polyunsaturated fatty acids (PUFAs) originate from primary producers and can be modified by bioconversions as they pass up the food chain. The PUFAs produced in these plants are produced by marine microbes and are present in fish oils.

In this trial, RR also proposes to include GM *C. sativa* lines containing transgenes encoding the synthesis of another class of fatty acids, the non-methylene-interrupted polyunsaturated fatty acids (NMI-PUFAs) such as sciadonic acid and juniperonic acid. These are omega-6 fatty acids. Higher plants do not produce EPA or DHA but several species of coniferous plants (such as pine, juniper and redwoods) produce NMI-PUFAs.

The genes encoding these fatty acids are under the control of seed specific promoters. Many of the public representations were concerned that these promoters might be not be specific and that transgene expression could occur in vegetative tissue as well as in seed. In particular, these noted recommendations from Colombo *et al* (2018)[2] for independent verification that promoters driving the expression of genes encoding the biosynthetic pathway of EPA and DHA are seed-specific and that toxicity tests should be carried our involving plant tissue and crop pests. Representations also cited a study by Hixton *et al* (2016)[3] which concluded that "the presence of EPA and DHA in diets of larval *Pieris rapae* (the cabbage white butterfly) may alter adult mass and wing morphology; therefore, further research on the environmental impacts of EPA and DHA production on terrestrial biota is advisable." In general, there concern that the presence of novel omega 3-fatty acids might alter terrestrial ecosystem dynamics.

ACRE agreed that the introduction of such novel compounds into the terrestrial food web on a larger scale would need to be considered in detail. However, in the case of these research trials, ACRE's advice remained the same as for the previous trials carried out by RR. Because of their small-scale, environmental exposure will be very low. Potential dosage levels will clearly be highest in seeds, but again, because of the size of the trial, exposure of seed feeders is likely to be very low. As ACRE's advice was predicated on this being a small scale trial, it recommended strict management measures to limit persistence of GM plants at, and dispersal of seed and transgenes from, the trial sites.

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[1] https://www.gov.uk/government/collections/genetically-modified-organisms-applications-and-consents

[2] Colombo S. M., Campbella L.G., Murphy E.J., Martin S.L., and Arts M.T. (2018). Potential for novel production of omega-3 long-chain fatty acids by genetically engineered oilseed plants to alter terrestrial ecosystem dynamics. Agricultural Systems 164: 31–37

[3] http://journals.plos.org/plosone/article?id=10.1371%2Fjournal.pone.0152264

Increased oleic acid levels

RR's application to carry out this trial described two approaches for producing *C. sativa* lines with increased oleic acid content. One involves using RNAi to disrupt the expression of two fatty acid desaturase genes (*FAD2* and *FAD3*) and a fatty acid elongase gene (*FAE1*). The other approach is to use the CRISPR-Cas9 system to edit these genes. The first step of this process is to generate transgenic plants producing the Cas9 cutter and RNA molecules that guide Cas9 to the target sequences. In future generations, plants containing the edited genes, but not the transgenes, will be selected for inclusion in the field trial. RR has carried out a risk assessment that takes into account genetic elements that may be present in transformed plants. ACRE agreed that unintended effects (for example, cuts in DNA with sequence similar to the target genes) are unlikely to convert *C. sativa* from being an annual species that requires active management to out-compete weedier plants to a problem weed in agronomic systems or to an invasive species in unmanaged ecosystems. ACRE has recommended management measures to minimise gene flow from the trial sites (please refer to the next section of ACRE's advice).

A reduction in sinapine and total sinapine ester content in seeds

RR also intends to trial GM *C. sativa* plants containing a modified bacterial sinapic acid decarboxylase transgene under the control of a seed specific promoter. The aim is to produce seed with low levels of the phenolic compound sinapine, which accumulates in *Brassica* plants and has antinutritional properties. Some of the public representations submitted to Defra raised concern that sinapine could increase the palatability of the seeds to wildlife and that RR should have conducted feeding tests with pest species prior to the trial. RR has found that GM *C. sativa* plants grown in controlled glasshouse had reduced sinapine levels in their seeds; it plans to determine whether this is the case for field-grown plants. If it is, the impact of perturbing the sinapine biosynthesis pathway would need to be analysed and risk assessed if versions of these GM plants were developed for commercial use in the future.

The information provided in the application sets out clearly how these plants were produced, including the genetic elements involved and their sources. ACRE did not require data on the copy number of inserted elements or their stability in the genome of GM *C. sativa* plants over several generations before the plants are used in the field trial. This information is required on a case by case basis depending on whether it is necessary for the risk assessment. In this particular case, taking into account that material from the trials will not enter the human food chain or the animal feed chain and that these trials are small-scale, ACRE considers that additional data of this type would not inform the risk assessment. ACRE also considered that integration of vector backbone would not confer an environmental risk in this case. ACRE concluded that sufficient information had been provided to support the environmental risk assessment.

Environmental risk assessment

The material from these trials (GM and non-GM) will not be allowed to enter the human food or animal feed chains. If in the future, GM *C. sativa* lines are developed for commercial use, a food/ feed safety assessment will be necessary; but not in this case.

ACRE was satisfied with the information provided by the applicant and its assessment of whether there would be any environmental risks posed by these trials.

ACRE considered that combining the different traits in individual plant lines does not generate additional risk hypotheses to those associated with the presence of individual traits in individual plant lines in the context of these trials.

The biology and ecology of *C. sativa*[4] indicates that it has a low baseline of invasiveness and does not compete well with surrounding vegetation. The genetic modifications are unlikely to alter this or to confer any selective advantage in the absence of glufosinate ammonium herbicides. Tolerance to glufosinate is used to identify and select transformed plants during their production; this herbicide will not be used on the trial sites.

There is some uncertainty about the baseline persistence of *C. sativa* seed in the seed bank in UK conditions. Monitoring results from previous RR trials (authorised in 2014, 2016 and 2018) showed a flush of volunteer plants germinating immediately post-harvest but no plants grew on the plots in the following two years. ACRE advised that the same management measures should be used to minimise the persistence of *C. sativa* in this trial as in previous trials. This means leaving experimental plots fallow post-harvest until the following spring and then then shallow cultivating them to a depth of no more than 5 cm. This will stimulate germination of any volunteer seed preventing it from persisting in the seed bank. Any volunteer plants must be killed before they set seed. Glufosinate herbicides should not be used as some of these plants may contain the PAT gene used to select for transformants during the development process. RR should also avoid re-using experimental plots to avoid interfering with monitoring for volunteer plants. RR should monitor for two years post-harvest before termination of monitoring can be considered.

The flora of the Rothamsted and Brooms Barn sites have been well-characterised and species that are most likely to be sexually compatible with *C. sativa* such as *C. microcarpa* and *C. alyssum* are very unlikely to be present. Non-GM *C. sativa* is not grown on these sites. Other species closely related to Camelina with the potential to cross-hybridise can be found within the *Camelineae* tribe will be present at the sites. These include *Arabidopsis, Capsella bursa-pastoris* and *Cardamine hirsuta*. If crossing does occur and if this results in viable seed being produced, studies have shown that hybrid plants will not be fertile. Therefore, introgression of the transgenes into weedy species growing on the

[4] 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

trial sites is very unlikely. However, as a precautionary measure, ACRE recommended that measures to minimise the likelihood of cross-hybridisation with non-GM *C. sativa*, *C. microcarpa*. and *C. alyssum* should be adopted.

ACRE considered 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 reduce the likelihood that pollen might 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 *C. sativa* plants

As *C.sativa* is a small-seeded crop, birds and small mammals may disperse seed. Measures to keep these out of the trial site should be adopted (e.g. humming strips). The small size of *C. sativa* seeds should also be taken into account when selecting, checking and cleaning equipment used for sowing and harvesting. Prior to harvest, the combine 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.

Public representations noted that RR proposes to conduct this new trial on the sites used for its existing trials. RR is required to adhere to the conditions that apply to each of these authorisations, including conditions that prevent experimental plots from being re-used until monitoring for volunteer plants has been completed.

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 results of monitoring for volunteer plants). If in the future, GM plants developed on the basis of this research were intended for food /feed use or if commercial cultivation were likely to result in material entering the food/ feed chain, a detailed food/ feed safety assessment would be required.

There were a number of additional issues raised in public representations that did not concern the potential risks posed by these particular trials. These queried the benefits of developing GM plants with these traits and growing crops for industrial use in the wider environment. There were also more general concerns about the development of GM crops.

Although not associated with environmental risk, ACRE recommended that the GM inspectorate[5] should establish how the integrity of this large collection of GM *C. sativa* lines will be maintained and confirmed as well as establishing the procedures that will be employed for managing field records and seed stocks.

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[5] https://www.gov.uk/government/collections/guidance-and-reports-on-gm-inspections