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

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| Notifier: | Rothamsted Research |
| Notification reference: | 18/R8/01 |
| Product: | *Camelina sativa* that has been genetically modified to contain constructs containing genes coding for: (i) two types of omega-3 long chain polyunsaturated fatty acids (LC-PUFAs) (ii) the pigment astaxanthin (iii) wax esters (iv) subunits of the glycolate dehydrogenase complex and (v) overexpression of a microtubule associated protein. |

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 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 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 intends to grow genome-edited Camelina plants in the trial. Defra is considering whether these plants trigger the GMO legislation. If Defra's view is that they are GMOs, Rothamsted will need to carry out a risk assessment in accordance with the GMO deliberate release legislation. Irrespective of whether they are GMOs or not, ACRE considered whether their presence in the trial introduces additional, plausible risk scenarios; for example, if these plants were to cross hybridise with the GM Camelina plants described in the application. ACRE did not consider that this was the case.

The GM Camelina plant lines contain one or more of the fourteen constructs described in the application[1]. These constructs contain genes coding for:

- omega-3 long chain polyunsaturated fatty acids that are components of fish oil. These are expressed in the seeds of the GM Camelina plants.

[1] The application is available at: https://www.gov.uk/government/publications/genetically-modified-organisms-rothamsted-research-18r0801

- the pigment astaxanthin, which is present in a range of marine microorganisms and is included in the diets of farmed fish. This is also expressed in the seeds of the GM Camelina plants.

- wax esters that are usually produced on the surface of leaves to provide a barrier against disease-causing organisms. These GM Camelina plants have been developed to accumulate these wax esters in their seeds as there is potential for them to be used as natural lubricants.

- a microtubule-protein from *Arabidopsis thaliana*, which when overexpressed in GM Camelina plants resulted in larger leaves and stems (i.e. increased photosynthetic area) in glasshouse trials.

- glycolate dehydrogenase, which when expressed in the chloroplast decreases photorespiration and increases photosynthesis. This increased photosynthetic efficiency can increase plant growth and seed yield.

- visual selectable markers i.e.: (i) tolerance to glufosinate-ammonium herbicides (conferred by the *bar* gene derived from soil bacteria) and (ii) a red fluorescent protein (derived from a *DsRed* gene present in a reef coral).

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

The applicant proposes to trial GM Camelina plants expressing one or more of the fourteen constructs described in the application. The information provided in the application sets out clearly how these plants were produced, including the genetic elements involved and their sources. The applicant has checked that the genetic modifications are stably inherited. However, this is not relevant to the safety assessment of these particular trials. The applicant had not provided details on whether vector backbone had been inserted into the GM Camelina plants. ACRE 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.

Some public representations suggest that insufficient information has been provided on the inserted DNA and associated changes to the recipient plant’s genome, including data on rearrangements and deletions. 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.

## 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 Camelina lines are developed for commercial use, a food/ feed safety assessment will be necessary; but not in this case.

In general, ACRE is satisfied with the information provided by the applicant and its assessment of whether there would be any environmental risks posed by these trials. However, ACRE felt that the applicant had not reflected evidence to support its conclusion that there are 'no known toxic, allergenic or harmful effects known to be associated with the DsRed protein'. ACRE noted that this information is available.

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 *Camelina sativa*[2] indicate 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. Glufosinate ammonium was used by the researchers to select plants that had been genetically modified from those that had not. It will not be used on the trial sites.

There is some uncertainty over the baseline persistence of *C. sativa* seed in the seed bank in UK conditions. Monitoring results from previous GM Camelina trials (authorised in 2014 and 2016) showed a flush of volunteer plants germinating immediately post-harvest but no plants grew on the plots in the following two years. The public representations raise concern that altered characteristics that affect the GM plants' architecture, photosynthetic capacity and herbicide tolerance could alter the persistence of their offspring. However, they do not provide a plausible hypothesis whereby these traits could alter the biology of this species with respect to dormancy. The trial sites should be managed to minimise the persistence of Camelina on them and the experimental plots monitored for two years post-harvest before termination of monitoring can be considered. ACRE also recommends leaving the experimental plots fallow post-harvest until the following spring and 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

[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

must be killed before they set seed. The applicant should also avoid re-using experimental plots so as not to interfere with monitoring for volunteer plants.

The flora of the Rothamsted and Broom's 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 Camelina 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 thaliana, 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 trial sites is very unlikely. However, as a precautionary measure ACRE recommends that measures to minimise the likelihood of cross-hybridisation with non-GM *C. sativa*, *C. microcarpa* and *C. alyssum* should be adopted.

ACRE considers 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 Camelina.

As Camelina 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 Camelina 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.

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).

Material from these trials will not be used as human food or animal feed. 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. This would address concerns raised in some of the public representations. However, other representations raise concerns about wildlife feeding on these plants or coming into contact with them. They cite

a paper by Hixson *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." ACRE reviewed its advice on a previous application to trial GM Camelina producing these long chain fatty acids in its seed when this paper was published. Whilst ACRE agrees that the introduction of such novel compounds into the terrestrial food web on a larger scale would need to be considered in detail, its view in the case of these small-scale trials remains the same. ACRE considers that levels of exposure to phytophagous insects will be relatively low. In this case, the expression of the additional genes is under the control of a seed specific promoters, so levels of exposure for leaf-feeders will be negligible. Whilst potential dosage levels will clearly be higher in seeds, exposure of seed feeders is likely to be very low due to the size of the trials. Representations suggest that vegetative material should be collected during the trial and tested for these long chain fatty acids. ACRE does not consider that this is necessary. These data may be necessary in combination with toxicological studies to assess risks to non-target organisms in any application to cultivate these GM plants on a wider scale. The same would apply to GM plants producing wax esters in their seeds.

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.

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