Advisory Committee on Releases to the Environment

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

Applicant: Rothamsted Research

Application: This release is a research trial to determine the agronomic performance and seed oil yield of GM *C. sativa* plants that have been engineered to accumulate non-native lipids (such as omega-3 long and ultra-long chain polyunsaturated fatty acids, ketocarotenoids) in their seed oils, or variation in the accumulation of native fatty acids such as oleic and palmitic acid and short chain fatty acids.

Notification reference: 23/R8/01

Date: March 2024

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

ACRE is satisfied that the risks to human health and the environment associated with this revised, proposed release are extremely low. ACRE has not identified any reasons for the trial not to proceed. After careful consideration of the revised application, ACRE suggests similar measures are put in place to that of previous trials. These are set out below.

1. The optional planting of a non-modified *Camelina sativa* pollen barrier of 4 metres depth surrounding each plot of GM camelina (to flower synchronously with the GM camelina, and which at 4 metres is of a width consistent with that previously used for GM oilseed rape).

2. In addition to the above, a separation distance consistent with that used in previous GM camelina consents of 50 metres 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. Rothamsted Research also propose a further exclusion zone extending beyond the outer boundary of the trial field perimeter in all directions for a distance of 750 metres, within which no *Camelina sativa* will be cultivated.

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

5. 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.

6. 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.

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

8. 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.

9. 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.

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

11. 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

ACRE considered the risks to human health and the environment posed by the proposed release of camelina sativa that has been genetically modified to synthesise and accumulate seed storage compounds, including omega-3, long and ultra-long chain polyunsaturated fatty acids, Astaxanthin, and also saturated fatty acid milk fat substitutes. Rothamsted Research (RR) has described clearly and comprehensively the methods and the individual 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
- the production of omega-3 ultra-long chain polyunsaturated fatty acids in seeds.
- The production of Astaxanthin in seeds
- The accumulation of triacylglycerol molecules with higher levels of saturated fatty acids 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, and also raised a number of its own, which were addressed by RR in a revised application.. 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 either from the cassava vein mosaic virus or from *Agrobacterium tumefaciens*.

Increased oil production

RR has previously developed GM *C. sativa* plants with increased oil content in their seeds under glasshouse conditions; and has published results from the testing of these in the field. RR's ongoing approach involves introducing a combination of

genetic modifications that increase the accumulation and 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 transgenic lines developed by RR therefore express within their seeds 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. This is the first step towards the synthesis of a number of different classes of fatty acids and oils, the molecular characteristics of each class of the transgenic constructs involved in their synthesis will now be discussed.

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, 2018 and 2019[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 transgenic plants correspond to those produced by marine microbes and which are present in fish oils.

The results from previous trials have been published, which this new trial aims to build on with fine tuning of expression of the PUFA synthesis genes to maximise their seed-specific accumulation. The application was revised to more clearly indicate which of the individual genes had been used in constructs and therefore already trialled, along with a reduction in the total number of these genes to enable ACRE to see more clearly the rationale behind the development of the lines to be, or being, trialled.

In this trial, RR also proposes to include GM *C. sativa* lines containing transgenes encoding the synthesis of another class of fatty acids, the ultra-long chain omega-3 polyunsaturated fatty acids (ULC-PUFA). These are found in specific animal organs, including the retina and testis tissues, where they play essential physiological roles[2]. Although higher plants do not contain EPA or DHA in their seed oils, very many vegetable oils are rich in the simpler/shorter (C18) omega-3 α-linolenic acid (ALA). In previous field trials, as described in the previous paragraph, RR have incorporated transgenes within camelina lines that, through the seed-specific expression of these

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

[2] Nwagbo U, Bernstein PS. Understanding the Roles of Very-Long-Chain Polyunsaturated Fatty Acids (VLC-PUFAs) in Eye Health. Nutrients. 2023 Jul 10;15(14):3096. doi: 10.3390/nu15143096. PMID: 37513514; PMCID: PMC10383069.

genes involved in the biosynthesis of omega-3 LC-PUFAs, convert ALA to EPA and through subsequent reactions to DHA. However, this application is the first to include the deliberate release of transgenic *Camelina* lines in which an additional class of fatty acid elongases are included (under seed-specific expression promoters) that use EPA and DHA as substrates to elongate the fatty acid backbone of these to 28 or more carbon molecules, thus synthesising ULC-PUFAs.

The genes encoding these fatty acids are under the control of seed specific promoters. ACRE asked for further information with regards to this, mirroring those public representations received that were concerned these promoters might not be seed-specific and that transgene expression could occur in vegetative tissue as well as in seed. In response to this RR have revised the application to indicate which transgenes have previously been field trialled and included references to published results in both Camelina and other transgenic host plants, in which the seed-specific synthesis of PUFA was measured[3],[4],[5].

Milk fat substitute production in seeds

RR's previous trials described a transgenic approach for producing *C. sativa* lines with increased oleic acid content. This new trial will make use of this construct as a background to produce substitutes for animal/milk fats by either transformation, or by crossing with lines separately transformed, with two further constructs (designated HO and SN2). These constructs are designed either to increase short to long-chain saturated fatty acid (in the case of HO) or to incorporate long-chain saturated fatty acyls into the glycerol backbone of triacylglycerol (TAG), both in a seed-specific manner.

Astaxanthin production in seeds

RR has previously trialled camelina lines containing the transgenes required to synthesise and accumulate Astaxanthin in a seed-specific manner. In this new trial, fine tuning of this process will be performed, involving an optimisation of which seed-specific promoters are used for each of the three genes required in this synthesis pathway. Previous trials of transgenic camelina revealed that repetition of promoter

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[3] Han, L., Silvestre, S., Sayanova, O., Haslam, R. P., & Napier, J. A. (2022). Using field evaluation and systematic iteration to rationalize the accumulation of omega-3 long-chain polyunsaturated fatty acids in transgenic Camelina sativa. *Plant biotechnology journal*, *20*(9), 1833–1852. https://doi.org/10.1111/pbi.13867

[4] https://www.aphis.usda.gov/brs/aphisdocs/17_32101p.pdf

[5] https://www.aphis.usda.gov/brs/aphisdocs/17_23601p.pdf

within the same construct can trigger silencing[6]; hence this application includes a number of alternative seed-specific promoters, in addition to those previously used, in order to optimise the synthesis of the oils and other compounds.

Improved amino acid composition in seeds

Along with Astaxanthin production, the trial will also investigate the field performance of camelina lines containing transgenic cassettes that direct the synthesis of non-proteinogenic amino sulfonic acid taurine, used in aquafeed diets and which is a metabolite of the amino acid cysteine. The Taurine synthesis construct as described in the application text allows for the use of alternative seed-specific promoters, for the reason discussed previously; to prevent silencing through promoter redundancy.

Gene-editing of transgenic lines

The application further includes details of a CRISPR-Cas9 cassette that could be introduced into one or more of the above constructs to inactivate endogenous genes through sequence-specific gene editing. The field trialling of such identified mutants was carried out under previous consents to identify gene edits that contribute positively to the introduced phenotypes, and results of this work published[7]. This new application plans to continue this work, and 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. Then, in future generations, plants containing the edited genes, but not the transgenes, are selected for inclusion in the field trial. However, RR has carried out a risk assessment that takes into account genetic elements that may be present in transformed plants. ACRE had previously 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

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[6] Han, L., Usher, S., Sandgrind, S., Hassall, K., Sayanova, O., Michaelson, L. V., Haslam, R. P., & Napier, J. A. (2020). High level accumulation of EPA and DHA in field-grown transgenic Camelina - a multi-territory evaluation of TAG accumulation and heterogeneity. *Plant biotechnology journal*, *18*(11), 2280–2291. https://doi.org/10.1111/pbi.13385

[7] Han, L., Haslam, R. P., Silvestre, S., Lu, C., & Napier, J. A. (2022). Enhancing the accumulation of eicosapentaenoic acid and docosahexaenoic acid in transgenic Camelina through the CRISPR-Cas9 inactivation of the competing FAE1 pathway. *Plant biotechnology journal*, *20*(8), 1444–1446. https://doi.org/10.1111/pbi.13876

minimise gene flow from the trial sites (please refer to the managing the trial site section of ACRE's advice).

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.

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. In public representations, there was concern that the presence of novel omega 3-fatty acids might alter terrestrial ecosystem dynamics. 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. ACRE's advice was predicated on this revised trial being a smaller scale trial than that originally proposed. The committee was minded in the light of previous trials, to recommend the same strict management measures used previously to be applied this time, to limit persistence of GM plants at, and dispersal of seed and transgenes from, the trial sites.

ACRE also noted that under the conditions of a consent, the consent holder is required to fully describe the GM lines to be sown in any growing season as part of the auditing process conducted by the GM Inspectorate. With the result that any genetic elements not implicitly described within the application would be identified in advance of sowing and this information fed back to Defra.

The 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*[8] 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.

ACRE previously considered that unintended effects arising from the interactions of some of the genetic components (such as the silencing genes of the CRISPR-Cas9 cassette) 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 section on managing the trial site of ACRE's advice).

The application in its current form has been revised from that originally received, to address concerns raised by ACRE, some of which were also seen in public representations. These revisions included identifying exactly which genetic components of the constructs had been previously trialled, along with references to the published results of such research. This, along with a reduction in the proposed area of the trial (from a total of 15 675 m^2 to 4650 m^2; slightly less than for previous trials) in turn afforded ACRE more certainty when assessing any plausible path to harm considered by the applicant's revised environmental risk assessment.

During ACRE's discussion, it was observed that insects synthesise long chain fatty acids meaning there are equivalent products within those organisms likely to predate on or consume these plants. The observation was also made that if long chain fatty acids were harmful to primary consumers, then plants may well have evolved metabolic pathways to produce these compounds as a defence against herbivory.

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[8] 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

Managing the Trial site

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, 2018 and 2019)[9] showed a flush of volunteer plants germinating immediately post-harvest but no plants grew on the plots in the following two years. ACRE were therefore content to advise 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 trial sites is very unlikely. However, as a precautionary measure, ACRE reiterated its previous recommendation 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, of 50 metres 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. The applicant requested the option of also putting in place a 'pollen barrier' to a depth of 4 metres 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.

RR have, additionally proposed to include a 750 metres distance from around the outer perimeter (defined by fence) of each of the GM trial grounds within which no

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

compatible crop species will be grown. This will enable any volunteer *C. sativa* to be readily identified and controlled.

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 consent conditions that apply to each of these authorisations, 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.