Part B: Information about the release application to be included on the public register

B1 The name and address of the applicant

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B2 A general description of the genetically modified organisms in relation to which the application is being made

The organism to be released is the oilseed *Camelina sativa* (hereafter referred to as “Camelina”) and we have used genetic modification to introduce into this plant the capacity to produce the omega-3 long chain polyunsaturated fatty acids eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA). The synthesis of these health-beneficial fatty acids has been engineered so as to only occur in the seeds of the GM Camelina. Similarly, GM Camelina plants have been engineered to accumulate the ketocarotenoid compound, astaxanthin. Astaxanthin is considered to be an antioxidant, and also has a distinctive pink pigment which is used in fish-farming diets. Again, the synthesis and accumulation of astaxanthin has been engineered to only occur in the seeds of the GM Camelina. In a third iteration, GM Camelina plants have been engineered to accumulate wax esters, which have useful properties as biolubricants. The synthesis and accumulation of these wax esters has also been engineered to only occur in the seeds of the GM Camelina. We have also used one plant line containing the EPA and DHA trait to be crossed in the laboratory with the astaxanthin-accumulating plants, to produce progeny which contained both traits.

The GM Camelina plants have been engineered with the novel capability to accumulate the non-native omega-3 long chain polyunsaturated fatty acids EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) through the introduction of the biosynthetic genes for these fatty acids. Such genes are normally only found in marine microbes such as microalgae and diatoms and some oomycetes and lower plants. Synthetic genes (meaning that the native DNA sequences have been codon-optimized and chemically synthesized) from EPA- & DHA-accumulating organisms have been integrated into the genome of Camelina, under the control of seed-specific promoters.

Eight different constructs for the synthesis of DHA and/or EPA are described in this application . In the first example (DHA2015.1), synthetic genes derived from activities present in the picoalgae *Ostreococcus tauri*, the moss

Physcomitrella patens, the Thraustochytriaceae *Thraustochytrium*, the oomycetes *Phytophora infestans* and *Phytophora sojae* and the picoalga *Ostreococcus* RCC809 have each been linked to seed-specific regulatory sequences and introduced into the genome of Camelina to direct the synthesis of both EPA and DHA only in seeds. In a second iteration (DHA2015.2), the activity and associated synthetic gene from *Phytophora infestans* was replaced with an identical activity (but different synthetic gene), derived from another oomycete, *Hyaloperonospora parasitica*. The activity encoded by the □12-desaturase from *Phytophora sojae* was removed. In all other respects, DHA2015.1 and DHA2015.2 are identical. In a third iteration, DHA2015.3, the elongase activity encoded by a sequence derived from *Ostreococcus tauri* was replaced by a similar activity from *Ostreococcus RCC809.* In all other respects, DHA2015.2 and DHA2015.3 are identical. In a fourth iteration, DHA2015.4, the desaturase activity encoded by a sequence derived from *Ostreococcus RCC809* was replaced by a similar activity from *Thallasiosira pseudonana.* In all other respects, DHA2015.3 and DHA2015.4 are identical. In a fifth iteration, DHA2015.5, derived from DHA2015.1, a □15-desaturase activity encoded by a sequence derived from *Perilla fructans* was added to the cassette, also under the control of a seed-specific promoter. In all other respects, DHA2015.1 and DHA2015.5 are identical.

Three constructs were also built to direct the synthesis of just EPA but not DHA, thus lacking the last two activities in the omega-3 LC-PUFA biosynthetic pathway. The first of these constructs, EPA2015.4, contained desaturase activities from *Mantionella squamata*, *Emiliana huxleyi* and *Hyaloperonospora parasitica and* an elongase activity from *Physcomitrella patens.* All these sequences were under the control of seed-specific promoters. In a second iteration, EPA2015.8, the desaturase sequence from *Mantionella squamata* was replaced with a similar activity *Ostreococcus tauri, and* a D15-desaturase activity encoded by a sequence derived from *Perilla fructans* was added to the cassette. In all other respects, EPA2015.4 and EPA2015.8 are identical. In a third iteration, EPA2016.1, the desaturase sequence from *Emiliana huxleyi* was replaced with a similar activity from *Thraustochytrium.*

In the case of plants engineered to synthesise the ketocarotenoid astaxanthin, we produced a single construct, ASX-A2, containing synthetic genes for the astaxanthin biosynthetic pathway, derived from sequences present in the higher plants *Adonis aestivalis* and *Zea mays.* All sequences were individually been linked to seed-specific regulatory sequences and introduced into the genome of Camelina to direct the synthesis of astaxanthin only in seeds. We have also crossed the ASX-A2 line with DHA2015.1 to combine both traits in one plant.

In the case of plants engineered to synthesise wax esters, two iterations were generated. In the first iteration, THIO14, a medium chain thioesterase from the plant *Cuphea palustris* was placed under the control of a seed specific promoter, resulting in elevated levels of myristic acid in that organ. In a second iteration, in addition to the activity from Cuphea, sequences encoding wax ester biosynthetic activities from the marine bacteria *Marinobacter*

hydrocarbonoclasticus and *Marinobacter aquaeolei* were included, also under the control of seed specific promoters.

In order to improve the architecture and growth of Camelina, we have used genetic modification to enhance some natural processes. Firstly, we expressed a microtuble-associated protein (MAP22) from *Arabidopsis thaliana* to generate plants with thicker stems and more leaves. Secondly, we expressed three proteins from *E. coli* that are able to improve photosynthetic reactions in the chloroplast, resulting in plants with enhanced metabolism, either as individual proteins or as a single polypeptide. We have also used one plant line containing the EPA and DHA trait to be crossed in the laboratory with the MAP22-expressing plant, to produce progeny which contained both traits.

Collectively, this means that we wish to trail in the field sixteen different plants lines, comprising eight DHA/EPA constructs, one astaxanthin construct, two wax ester constructs, one architecture trait (MAP22) and two photosynthetic improvement traits, making a total of fourteen different constructs. In addition, we have generated two genetic crosses combining either ASX-A2 or MAP22 with DHA2015.1, to generate the lines DHASX and DHAPP22.

All constructs apart from ASX-A2 also contain the visual reporter protein DsRed, which allows for the simple identification of GM Camelina seeds. The DsRed protein is derived from the marine coral species *Discosoma* sp and has been codon-optimised for expression in plants. Two constructs (ASX-A2, EPA2016.1) contain the selectable marker *bar* which confers resistance to the compound bialaphos, which was used to select GM Camelina plants in the laboratory. Bialaphos (and related compounds which form the active ingredient of specific Class H herbicides) will not be used in the course of this field trial. The genetic cross between ASX-A2 and DHA2015.1 contains both of the genes and associated activities described above.

B3 The location at which the genetically modified organisms are proposed to be released

We propose to carry out 2 trials in three seasons and will avoid reusing the same plots. It will be sited on the experimental farm at Rothamsted Research, Harpenden, at grid reference TL120130, and also at the experimental farm at Rothamsted Research, Brooms Barn, at grid reference TL756654.

B4 The purpose for which the genetically modified organisms are proposed to be released (including any future use to which they are intended to be put).

The purpose of this experimental trial is to determine the performance of these different GM Camelina iterations in the field, with respect to oil composition and oil quantity for different lipids (omega-3 LC-PUFAs,

ketocarotenoids, wax esters). It is also to assess any additional phenotypic and agronomic variations, including those arising as a consequence of manipulation to plant architecture or photosynthesis, of the stacking of traits in the genetic cross. Specific questions to be examined are:

- Do the GM Camelina plants efficiently accumulate EPA and DHA in seed oil in the field?
- Do the GM Camelina plants efficiently accumulate astaxanthin in their seeds in the field?
- Do the GM Camelina plants efficiently accumulate both EPA/DHA and astaxanthin in their seeds the field?
- Do the GM Camelina plants still accumulate total seed oil to appropriate levels?
- Do the GM Camelina plants efficiently accumulate wax esters in seed oil in the field?
- Is there any further alteration to the lipidome of field-grown GM Camelina?
- Is there any difference between the different lines (including the genetic cross) in terms of agronomic performance?
- Is there any advantage or disadvantage to the GM Camelina plants in terms of field-based performance?
- Does different management regimes alter the level of EPA and DHA?

B5 The intended dates of the release.

The exact timing of sowing of the trial will depend upon weather conditions at the time. The field trial start date will be in April/May 2018 and the plants will be harvested in Aug or Sept the same year. We intend to repeat the trial with the specified 17 GM lines in 2019 and 2020.

B6 The environmental risk assessment.

Of the 14 GM Camelina constructs described in this application, the majority (11) have a seed-specific expression of their transgenes and as such are indistinguishable from the non-GM equivalent except for the modified composition of their seeds, in particular by the presence of either omega-3 long chain polyunsaturated fatty acids EPA and DHA and/or astaxanthin, or wax esters. This modified composition is found only in the seeds of the GM Camelina and is absent from all other vegetative tissues (e.g. leaves, roots, stems). There are no known hazards associated with these modifications.

In the case of 3 GM Camelina lines, the transgenes are expressed throughout the whole plant, to modify either the architecture or metabolism of the plant. This has resulted in some alterations to the gross appearance of the plant (thicker stems, increased leaf number) but has not obviously altered other aspects of the life cycle including fertilization and seed-set. There are no known hazards associated with these modifications.

In all 14 cases, the gene template organisms are not known to be pathogenic or allergenic to humans. None of the genes under investigation, or the selectable or visual marker genes, are expected to result in the synthesis of products that are harmful to humans, other organisms or the environment. Any unknown hazards arising from the expression and ingestion of foreign proteins will not occur since the Camelina plants will not be consumed by humans.

The probability of GM Camelina seeds escaping from the trial sites or the transfer of inserted characteristics to sexually-compatible species outside the trial area is estimated as very low. Camelina seeds are moderate in size and not normally dispersed by wind. Management measures including the use of gas guns and hawk kites will be employed to mitigate the risk of seed removal by birds. There will be no compatible species grown for 1000 meters from the boundary of the sites and no sexually-compatible wild relatives of *C. sativa* exist in the vicinity of the Rothamsted farms.

The risk of non-sexual, horizontal gene transfer to other species is extremely low. In the event of horizontal gene transfer to bacteria, neither the trait genes nor the marker genes would be expected to confer a selective advantage in the field environment under consideration. We estimate the likelihood of horizontal gene transfer as low and the consequences, were it to occur, as negligible. The area proposed to be planted with GMOs is small and temporary (lasting between 4 and 5 months).

Bearing in mind its limited scope, overall risk of harm to human health or the environmental arising from this trial is assessed as very low.

B7 The methods and plans for monitoring the genetically modified organisms and for responding to an emergency.

The release sites will be visited by trained laboratory personnel who are working on the project at no less than weekly intervals (and at some periods, daily) during the growing season of each year of the trial. Any unexpected occurrences that could potentially result in adverse environmental effects or the possibility of adverse effects on human health will be notified to the Defra immediately. Should the need arise to terminate the release at any point the emergency plans detailed below

will be followed.

At the end of each season, the plot will remain in stubble and monitored for volunteers during the remainder of the year and the following season. Any volunteers identified will be destroyed by appropriate herbicide treatment (e.g. glyphosate) or removed by hand and destroyed.

Following completion of the two-year trial the release site will remain fallow for a further season to enable easy identification of volunteers. The site will be inspected regularly and any volunteers identified will be immediately destroyed either by application of a systematic broad leaf herbicide.

Emergency procedures: In the unlikely event that the integrity of the site is seriously compromised, the trial will be terminated and all plants, (including GM and control Camelina plots, and pollen barrier) will be destroyed using a suitable herbicide or harvesting as deemed appropriate. All harvested material will be removed from the site and disposed of by incineration or deep burial at a local authority-approved landfill site using an approved contractor. Transportation of waste materials will be in secure containers. The phone numbers of all key staff will be available to site security and farm.