Application for consent to release a GMO

Part A2: Data or results from any previous releases of the GMO

Give information on data or results from any previous releases of this GMO by you either inside or outside the European Community [especially the results of monitoring and the effectiveness of any risk management procedures].

One line derived from construct DHA2015.1 was previously released as part of DEFRA consent 16/R8/01. The same line was also released in Canada in 2017 under CFIA permit ICA6-46020 and also in the USA under APHSIS permit # 15-357-101r. This latter permit also covered the line ASX-A2.

Part A3: Details of previous applications for release

Give details of any previous applications to release the GMO made to the Secretary of State under the 2002 Regulations or to another Member State under the Deliberate Release Directive 2001/18/EC.

Rothamsted Research has received consents to release GM wheat (e.g. 97/R8/3, 01/R8/4 and 11/R8/01 and more recently GM *C. sativa* (14/R8/01, 16/R8/01)

Part A4: Risk assessment and a statement on risk evaluation

Summary

Based on the analyses provided below, the overall risk of harm to human health or the environmental arising from this trial is assessed as very low.

Environmental risks

The probability of *C. sativa* seeds escaping from the trial site or the transfer of inserted characteristics to sexually-compatible species outside the trial area is estimated as very low. *C. sativa* seeds are moderate in size and not normally dispersed by wind. Management measures including netting when the *C. sativa* is in flower and the use of gas guns and hawk kites will be employed to mitigate the risk of seed removal by birds. Management procedures to minimise the spread of seeds will further reduce the probability of these events occurring. There will be no compatible species grown for 1000 meters from the boundary of the site and no sexually-compatible wild relatives of *C. sativa* exist in the vicinity of the Rothamsted farm. In the unlikely event of a hybrid being generated, the presence of EPA+DHA and/or astaxanthin in the seed oil of any such progeny would not convey a selectable

advantage and most likely the omega-3 and/or ketocarotenoid trait would not be retained. This is equally true for the marker gene *bar* since no selective bialaphos-containing herbicides will be used in the management of this field trial.

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. The genes introduced in *C. sativa* have been inserted via *Agrobacterium tumefaciens*-mediated gene transfer.

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).

Human health risks

Where applicable, the gene donor organisms are not known to be pathogenic or allergenic to humans, and 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 *C. sativa* plants will not be consumed by humans.

Risk assessment

Conclusions on the Potential Environmental Impact from the Release or the Placing on the Market of GMOs

Detailed evaluation of hazards, magnitude of exposure and management strategies to minimise risk.

We adopted a classic six-step process of risk assessment. Systematic identification of all potential hazards arising from this field trial; evaluation of hazard-realisation in the specific field-trial environment; potential for harm; frequency of exposure; mitigation of risk by appropriate management and finally, an estimate of the overall risk. The table below considers all the scenarios listed below (i – ix).

i. **Likelihood of the genetically modified higher plant (GMHP) becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats.**

ii. **Any selective advantage or disadvantage conferred to the GMHP.**

iii. **Potential for gene transfer to the same or other sexually compatible plant species under conditions of planting the GMHP and any selective advantage or disadvantage conferred to those plant species.**

iv. **Potential immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GMHP and target organisms, such as predators, parasitoids and pathogens (if applicable).**

v. **Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMHP with non-target organisms, (also taking into account organisms which interact with target organisms), including impact on population levels of competitors, herbivores, symbionts (where applicable), parasites and pathogens.**

vi. **Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMHP and persons working with, coming into direct contact with, or in the vicinity of the GMHP release(s).**

vii. **Possible immediate and/or delayed effects on animal health and consequences for the food/feed chain resulting from consumption of the GMO and any products derived from it if it is intended to be used as animal feed.**

viii. **Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).**

ix. **Possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHPs.**

Step1: ***Potential hazards which may be caused by the characteristics of the novel plant***	**Step 2:** ***Evaluation of how above hazards could be realised in the receiving environments***	**Step 3:** ***Evaluation the magnitude of harm caused by each hazard if realised***	**Step 4:** ***Estimation of how likely/often each hazard will be realised as harm***	**Step 5:** ***Modification of management strategies to obtain lowest possible risks from the deliberate release***	**Step 6:** ***Overall estimate of risk caused by the release***
(i) Likelihood of the genetically modified higher plant (GMHP) becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats	Increased invasiveness may arise from intended or unintended effects of the genetic modification that resulted in *C. sativa* plants with a more 'weedy' habit that are better able to establish and thrive in uncultivated environments or to persist in agricultural habitats.	*C. sativa* is an annual species that requires active management to out-compete more weedy plants. Left unmanaged, it does not establish well in nature and thus has a low base line of invasiveness and persistence. Even if intended or unintended effects of the genetic modification resulted in major changes in invasiveness or persistence, it is considered that this would not result in significant environmental harm for agricultural or unmanaged ecosystems. *C. sativa* is a benign plant that can be easily managed by cultivation or specific herbicides.	It is highly unlikely that intended or unintended effects of the genetic modification will result in major changes in invasiveness or persistence. If it were to occur, this hazard would be realised only if seeds or pollen possessing genes encoding these traits were to spread from the trial site and successfully become established elsewhere. This is very unlikely as there are no wild or cultivated relatives of *C. sativa* that can cross-hybridise and produce viable seeds. Seed removal	Harvested seeds will be transported from the trial sites in sealed containers. Machinery will be cleaned thoroughly prior to removal from the site. No *C. sativa* will be cultivated for at least 1000m surrounding the trial so it will be easy to see any *C. sativa* plants in the surrounding area. Appropriate physical barriers and/or deterrents will be employed to minimise access by large mammals and birds.	Overall risk is negligible .

		The magnitude of harm if the hazard was realised is considered to be very small.	from the site will be rigorously managed (see step 5). The chances of modified *C. sativa* plants establishing themselves outside the trial site are negligible.		
(ia) Increased invasiveness in natural habitats or persistence in agricultural habitats due presence of the selectable marker gene (*bar*)	Increased invasiveness may arise from the genetic modification in the specific cases where the selectable marker gene (*bar*) is included in the transgene cassette (present in Construct ASX-A2, the derived cross CASX, and EPA2016.1), resulting in *C. sativa* plants that are better able to resist specific herbicides	The *bar* marker gene present in three *C. sativa* lines described in this application provides tolerance of the broad spectrum herbicide bialaphos (also known as glufosinate). The presence of this transgene could provide a selectable advantage to the GMO	The selectable advantage provided by the *bar* gene is only realised when the plant is exposed to the specific Class H herbicide. In the absence of this selection pressure, there is no obvious advantage conferred by the presence of this gene. Monitoring and management measures will be carried out to ensure that no plants or seeds are removed from the trial site in anything other than controlled conditions. We estimate that the potential hazard associated with the presence of the *bar* gene is low.	No positive selection for the *bar* gene will be applied to the trial site, unless in the form of a closely controlled plot-size experiment to evaluate the efficacy of this trait. No Class H herbicides (to which bar confers resistance) will be used in the general management of the trial	Overall risk is very low.

(ii) Any selective advantage or disadvantage conferred to the GMHP	Selective advantage or disadvantage may result from the intended traits (improved oil composition) or as a result of unintended effects of the genetic modification.	We anticipate that the conferred trait of improved seed composition will provide little or no change in selective advantage compared to other factors determining a plants ability to survive in unmanaged ecosystems. This is equally true for the visual marker protein DsRed, and also for the *bar* resistance marker, since in the latter case, no herbicides targeting the inhibition of glutamine synthetase will be used. In the case of the constitutively-expressed MAP and GDH genes, it is conceivable that these provide a slight metabolic advantage compared to wildtype Camelina. However, this is unlikely to be realised in the natural environment given the general performance of this crop in unmanaged systems (see (i) above).	This potential hazard would be realised only if seeds or pollen possessing genes encoding these traits were to spread from the trial site and successfully become established in environments were the appropriate selection pressures were present. This is very unlikely as there are no sexually compatible species for out-crossing for at least 1000m from the trial site. Seed removal from the site will be rigorously managed. The frequency of this potential hazard resulting in environmental harm is very low.	Harvested seeds will be transported from the site in sealed containers. Machinery will be cleaned thoroughly prior to removal from the site. There is a buffer zone to minimize the spread of pollen. Surrounding the trial site is an 1000 metre area in which no *C. sativa* will be grown Appropriate physical barriers and/or deterrents will be employed to minimise access by large mammals and birds. No herbicides of the H class will be used	Overall risk is very low.
(iii) Selective advantage or disadvantag	These hazards could be realised in the receiving environment via	This would be dependent on cross-pollenation between the GMHP and compatible species, of which there are no examples on the Rothamsted farms	It is highly unlikely that pollen from the GMHP will successfully fertilise a compatible species (see ii)	There is a pollen barrier “buffer zone” surrounding the GMHP plots to minimize the spread of pollen.	Overall risk is very low.

e conferred to other sexually compatible plant species	dispersal of GM seeds from trial site to the surrounding environment or via out-crossing to sexually-compatible species outside trial site.			Surrounding the trial site is an 1000 metre area in which no *C. sativa* will be grown. Normal agricultural practice will be used to control weeds in the area beyond the trial site.	
iv. Potential immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GMHP and target organisms, such as predators, parasitoids and	Omega-3 long chain polyunsaturated fatty acids and/or ketocarotenoids, or wax esters may illicit a change in behaviour of other organisms. In the case of GMHP MAP22 and GHD, altered metabolism as a result of changes to architecture or	There are no obvious mechanisms that could result in a change in behaviour of organisms as a result of exposure to omega-3 long chain polyunsaturated fatty acids and/or ketocarotenoids, or wax esters and/or myristic acid, retained and compartmentalised in the seeds of the GMHP. Alterations to vegetative tissue chemical composition might increase or decrease attractivity to organisms such as insects, though such changes are likely to very minor and indirect. Increased vegetative levels of oleic acid are unlikely to modify interactions between GMHP	Many organisms will encounter the modified *C. sativa* plants in the field trial.	Management practices will be put into place to minimise the contact of birds and mammals (eg bird kites etc). However the hazard is purely hypothetical and highly unlikely ever to be realised.	Overall risk is very low.

pathogens (if applicable).	photosynthesis could change the chemical composition of vegetative tissues . Two reference lines in which the FAD2 □12-desaturase has been mutated by genome editing have an increased level of oleic acid in all tissues Morineau et al. (2017)	plants, comparators and non-plant organisms such as insects. Oleic acid is ubiquitous in all niches and ecosystems			
v. Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions	Omega-3 long chain polyunsaturated fatty acids and/or ketocarotenoids, or wax esters may illicit a change in behaviour of other organisms.	There are no obvious mechanisms that could result in a change in behaviour of non-target organisms as a result of exposure to omega-3 long chain polyunsaturated fatty acids and/or ketocarotenoids, or wax esters, retained and compartmentalised in the seeds of the GMHP. Alterations to vegetative tissue chemical composition might	Many organisms will encounter the modified C. sativa plants in the field trial.	Management practices will be put into place to minimise the contact of birds and mammals (eg bird kites etc). However the hazard is purely hypothetical and highly unlikely ever to be realised.	Overall risk is very low.

of the GMHP with non-target organisms, (also taking into account organisms which interact with target organisms), including impact on population levels of competitors, herbivores, symbionts (where applicable), parasites and pathogens.	In the case of GMHP MAP22 and GHD, altered metabolism as a result of changes to architecture or photosynthesis could change the chemical composition of vegetative tissues. Two reference lines in which the FAD2 □12-desaturase has been mutated by genome editing have an increased level of oleic acid in all tissues Described in Morineau et al. (2017)	increase or decrease attractivity to organisms such as insects, though such changes are likely to very minor and indirect. Increased vegetative levels of oleic acid are unlikely to modify interactions between GMHP plants, comparators and non-plant organisms such as insects. Oleic acid is ubiquitous in all niches and ecosystems			
vi. Possible immediate and/or	By contact or ingestion of GM plant material.	Omega-3 long chain polyunsaturated fatty acids are essential components of most	Some contact between the GMHP and humans is expected. People operating	No plant material from the trial will enter the food chain.	Overall risk is very low.

delayed effects on human health resulting from potential direct and indirect interactions of the GMHP and persons working with, coming into direct contact with, or in the vicinity of the GMHP release(s).		vertebrates' diet, with these fatty acids widely recognised as being health-beneficial. They are very widely represented in the human food chain, without any reported negative effects. This is equally true of ketocarotenoids and wax esters. These compounds are present in natural food webs and do not appear to interact in a synergistic fashion.	farm machinery and scientists working in the trial site will come into physical contact with the plants.	Appropriate advice and SOPs will be used to minimise exposure to the GMHP, despite the risk being negligble	
vii. Possible immediate and/or delayed effects on animal health and consequences for the food/feed chain	By contact or ingestion of GM plant material.	Omega-3 long chain polyunsaturated fatty acids are essential components of most vertebrates' diet, with these fatty acids widely recognised as being health-beneficial. They are very widely represented in both terrestrial and aquatic foodwebs, without any reported negative effects. This is equally true of ketocarotenoids and wax esters.	It is not intended to use the GMHP for direct animal feeding studies	No GMHP will enter the feed chain.	Overall risk is very low.

resulting from consumption of the GMO and any products derived from it if it is intended to be used as animal feed.					
viii. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms	Changes in biogeochemical processes may result from unintended changes in the modified plants or from unintended changes in soil microbes due to horizontal transfer of DNA.	The magnitude of harm is estimated to be extremely low. Biogeochemical processes are not expected to be affected by the cultivation of the genetically modified plants.	The frequency of changes to biogeochemical processes is considered to be very low. The maximum area proposed to be planted with GMOs is small and temporary (lasting <5 months/year for two years).	None	It is very unlikely that changes in biogeochemical processes would occur - Overall risk is negligible.

in the vicinity of the GMO release(s).					
ix. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHPs.		No differences in the cultivation and management of the GMHP compared with the non-GMHP will occur		No differences in the cultivation and management of the GMHP compared with the non-GMHP will occur	Overall risk is negligible.
		Additional Considerations & Risk Evaluation			
Unexpected interactions between	Toxic or unpredicted interactions	The magnitude of harm resulting from such an interaction is low. This would occur only in the seeds	If such an interaction was to generate a hazardous or toxic outcome, it is unlikely	No plat material from the trial will enter the feed or food chain.	Overall risk is very low.

omega-3 long chain polyunsaturated fatty acids and ketocarotenoids	between the omega-3 fatty acids and ketocarotenoids as a result of their co-expression in the seeds of the GMO	of the stacked lines, and if generating a toxic or deleterious outcome, might be expected to not result in viable seed. Both compounds (omega-3s, ketocarotenoids) already co-exist in both natural and artificial (i.e. farming) foodwebs, and no known examples of such hazardous interactions are known	viable seeds (and hence plants) would have been recovered. However, it is possible such a hazard would only be realised through exposure or ingestion to the two compounds.	Human and/or animal exposure will minimized and monitored via the appropriate management practices	
Potential effects on human or animal health due to horizontal gene transfer of recombinant DNA	By contact, ingestion or infection with bacteria that had received recombinant DNA via horizontal gene transfer.	The magnitude of harm caused by contact, ingestion or infection with bacteria that had received the recombinant DNA via horizontal gene transfer is low. The introduced genes are not expected to be expressed in bacteria and would have no safety concern if they were.	The rate of horizontal gene transfer from genetically modified plants to other species is accepted to be extremely low. The absence of plasmid backbone sequence and origins of replication which are derived from E. coli and *Agrobacterium tumefaciens*, decrease the chances of homologous recombination between plant and microbial DNA in the soil. If recombinant DNA were to move by horizontal transfer to soil bacteria, it is extremely unlikely to alter their survivability or pathogenicity. The area proposed to be planted with	No plant material from the trial will enter the food or animal feed chain.	Overall risk is very low.

			GMOs is small and temporary (lasting <5 months/year for two years).		
Consideration of the risk of horizontal gene transfer into wild-type Agrobacterium species in the soil that could infect and transfer DNA to other plant species including risks associated with expression of the genes.	By DNA released from decomposing plant material being taken up into the T-DNA of wild-type Agrobacterium and the subsequent expression of functional cassettes in other plants after natural transformation by *Agrobacterium*.	In the very unlikely event that functional expression cassettes were horizontally transferred into soil *Agrobacterium* cells and then somehow expressed in newly transformed plant cells, it is possible that this may alter the FA profile of the transformed cells in these plants.	Horizontal gene transfer between plants and wild-type *Agrobacterium* species, and the subsequent infection of other plant species with recombinant DNA is considered an exceedingly small risk. Although transformation of wild type *Agrobacterium tumefaciens* has been reported in laboratory experiments using pre-inoculated sterile soil and high concentrations of circular Ti plasmid with appropriate antibiotic selection (Demaneche et al 2001), no such demonstration has been reported in the field or with linearised plant DNA with or without selection. Even in optimised laboratory conditions, electroporation or freeze-thaw methods are required to effectively	This risk will be managed by minimising the seeds and other above-ground plant biomass left in the soil.	The risk of this is extremely low

			transform *Agrobacterium* spp (Holsters 1975, Mattanovich et al 1989). It is considered highly unlikely that free DNA liberated by degradation of GM plant roots in the soil would become stabilised in wild-type *Agrobacterium* and capable of autonomous replication. This could theoretically occur if the transgene insert liberated by decomposing roots was taken up by wild type *Agrobacterium* either as an intact plasmid or as a DNA fragment and subsequently incorporated into the resident Ti plasmid by for instance, homologous recombination. The former would stabilise only if the host *Agrobacterium* cell shared the same IncR compatibility group as the pSa origin of the transgene vector used in this trial.		

References

Demanèche, S., Kay, E., Gourbière, F., & Simonet, P. (2001). Natural transformation of Pseudomonas fluorescens and Agrobacterium tumefaciens in soil. *Applied and environmental microbiology*, *67*(6), 2617-2621.

Holsters, M., De Waele, D., Depicker, A., Messens, E., Van Montagu, M., & Schell, J. (1978). Transfection and transformation of Agrobacterium tumefaciens. *Molecular and General Genetics MGG*, *163*(2), 181-187.

Mattanovich, D., Rüker, F., da Cämara Machado, A., Laimer, M., Regner, F., Steinkeliner, H., ... & Katinger, H. (1989). Efficient transformation of Agrobacterium spp. by eletroporation. *Nucleic acids research*, *17*(16), 6747-6747.

Morineau C, Bellec Y, Tellier F, Gissot L, Kelemen Z, Nogué F, Faure JD. 2017. Selective gene dosage by CRISPR-Cas9 genome editing in hexaploid Camelina sativa. Plant Biotechnol J. 15(6):729-739

Part A5: Assessment of commercial or confidentiality of information contained in this application.

Identify clearly any information that is considered to be commercially confidential. A clear justification for keeping information confidential must be given.

This is publicly-funded research and has no associated commercial confidentiality considerations.

Part A6: Statement on whether detailed information on the description of the GMO and the purpose of release has been published

Make a clear statement on whether a detailed description of the GMO and the purpose of the release have been published, and the bibliographic reference for any information so published.
This is intended to assist with the protection of the applicant's intellectual property rights, which may be affected by the prior publication of certain detailed information, e.g. by its inclusion on the public register.

Previous iterations of the omega-3 constructs have previously been published in peer-reviewed journals:

Ruiz-Lopez N, Haslam RP, Napier JA, Sayanova O. (2014) Successful high-level accumulation of fish oil omega-3 long-chain polyunsaturated fatty acids in a transgenic oilseed crop. *Plant J*. 77(2): 198-208. doi: 10.1111/tpj.12378.

Results from Yr1 (2014) and Yr2 (2015) of the field trial covered under R8/14/01 consent have been published:

Usher S, Haslam RP, Ruiz-Lopez N, Sayanova O, Napier JA (2015) Field trial evaluation of the accumulation of omega-3 long chain polyunsaturated fatty acids in transgenic *Camelina sativa*: Making fish oil substitutes in plants." *Metabolic Engineering Communications* 2: 93-98. doi:10.1016/j.meteno.2015.04.002

Usher S, Han L, Haslam RP, Michaelson LV, Sturtevant D, Aziz M, Chapman KD, Sayanova O, Napier JA.(2017) Tailoring seed oil composition in the real world: optimising omega-3 long chain polyunsaturated fatty acid accumulation in transgenic Camelina sativa. *Sci Rep*. 7(1):6570. doi: 10.1038/s41598-017-06838-0

Camelina plants accumulation wax esters have been described by us:

Ruiz-Lopez N, Broughton R, Usher S, Salas JJ, Haslam RP, Napier JA, Beaudoin F. (2017). Tailoring the composition of novel wax esters in the seeds of transgenic

Camelina sativa through systematic metabolic engineering. Plant Biotechnol J. 2017 Jul;15(7):837-849. doi: 10.1111/pbi.12679.

All other iterations are currently unpublished.