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].

Several of the lines described in this current proposal have previously been released as a GMO and the results are described in Han et al., 2020 (https://onlinelibrary.wiley.com/doi/10.1111/pbi.13385) and Han et al 2022 (https://onlinelibrary.wiley.com/doi/10.1111/pbi.13867).

Similar lines with the same omega-3 LC-PUFA seed oil trait have undergone evaluation, approval and release, both in the UK (under DEFRA consent **19/R8/01**, **18/R8/01** and **16/R8/01**) and environmental release in Canada in 2017 and 2018 under CFIA permit ICA6-46020 and also in the USA under APHIS permit # 15-357-101r. No concerns or issues with risk management or post-trial monitoring were identified by APHA-GMI, CFIA or APHIS.

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, 11/R8/01, 16/R8/02 and 21/R8/01 and more relevant to this application, GM *C. sativa* (**14/R8/01**, **16/R8/01**, **18/R8/01** and **19/R8/01**). Rothamsted has run continuous field trials of GM camelina since 2014 and the full implementation of the Ministerial Consents has been annually assessed by the GM Inspectorate (APHA).

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 environment arising from this trial is assessed as very low. This conclusion is supported by previous Consents for similar or identical traits in Camelina which

agreed that the risks of harm to human health or the environment arising from such trials was 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 areas is estimated as very low. *C. sativa* seeds are small-moderate in size (~1mm in length) and not normally dispersed by wind. Management measures including the use of humming tape and hawk kites will be employed to mitigate the risk of seed removal by birds. Practical farm 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 a minimum of 750 meters from the boundary of the sites and no sexually compatible wild relatives of *C. sativa* exist in the vicinity of the Rothamsted farm. In the (highly unlikely) event of a hybrid being generated, the presence of EPA+DHA or ketocarotenoids in the seed oil of any such progeny would not convey a selectable advantage and most likely the novel seed oil trait would not be retained.

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 very low and the consequences were it to occur, as negligible. The area proposed to be planted with GMOs is consistently small (<5,000m2 in total for two sites in two different counties) and temporary (lasting between 4 and 5 months/year).

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. All the compounds synthesised are already present in both aquatic and terrestrial foodwebs. 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

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.**

Overall risk is negligible.

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

Overall risk is very low.

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.**

Overall risk is very low.

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).**

Overall risk is very low.

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.**

Overall risk is very low.

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).**

Overall risk is very low.

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.

Overall risk is very low.

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).

Overall risk is negligible

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.

Overall risk is very low.

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. Trial size is small. The magnitude of harm if the hazard was realised is considered to be very small.	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 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.	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 750m surrounding the trial so it will be straight-forward to detect 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.
(ia) Increased invasiveness in natural habitats or	Increased invasiveness may arise from the genetic modification	The *bar* marker gene present in any *C. sativa* lines envisaged in this application provides tolerance of the broad spectrum herbicide glufosinate. The presence of	The selectable advantage provided by the *bar* gene is only realised when the plant is exposed to the specific Class	No positive selection for the *bar* gene will be applied to the trial site, unless in the form of a closely controlled	Overall risk is very low.

persistence in agricultural habitats due presence of the selectable marker gene (*bar*)	in the specific cases where the selectable marker gene (*bar*) is included in the transgene cassette (optionally present in some configurations), resulting in *C. sativa* plants that are better able to resist specific herbicides	this transgene could provide a selectable advantage to the GMO	H/Group10 herbicide. In the absence of this selection pressure, there is no advantage conferred by the presence of this gene. In the event of the release of GMHPs containing the optional *bar* 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.	plot-size experiment to evaluate the efficacy of this trait. No Class H/Group 10 herbicides (to which the *bar* genes confers resistance) will be used in the general management of the trial.	
(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 presence of the optional visual marker protein DsRed, and also for the optional *bar* resistance marker (see above), since in the latter case, no herbicides targeting the inhibition of glutamine synthetase will be used.	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 750m from the boundary of 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 750 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.	Overall risk is very low.
(iii) Selective advantage or disadvantage	These hazards could be realised in the receiving	This would be dependent on cross-pollenation between the GMHP and	It is highly unlikely that pollen from the GMHP will successfully	Surrounding the trial site is an 750 metre area in which no *C. sativa* will be grown.	Overall risk is very low.

conferred to other sexually compatible plant species	environment via dispersal of GM seeds from trial site to the surrounding environment or via out-crossing to sexually-compatible species outside trial site.	compatible species, of which there are no examples on the Rothamsted farms	fertilise a compatible species (see ii)	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 pathogens (if applicable).	Altered seed lipid composition may illicit a change in behaviour of other organisms.	There are no obvious mechanisms that could result in a change in behaviour of organisms as a result of exposure to e.g. omega-3 long chain polyunsaturated fatty acids, ketocarotenoids or seed altered fatty acid profile retained and compartmentalised in the seeds of the GMHP.	Many organisms will encounter the modified *C. sativa* plants in the field trial, though most of the plants to be evaluated have restricted expression (seed-specific) of the traits. The seed-specific expression of these transgenes has been confirmed for at least one line described in this application. (Han et al., 2020). The seed-specific nature of the seed storage protein promoters described in A1 are well-established and have been routinely used in multiple transgenic crops including those which have undergone full regulatory approval in the USA.	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.
v. Possible immediate and/or delayed environmental impact resulting from	Altered seed lipid composition 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 e.g. omega-3 long chain polyunsaturated fatty acids, ketocarotenoids or other variations to the seed lipidome retained and	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	Overall risk is very low.

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.		compartmentalised in the seeds of the GMHP. Dietary lipids are metabolised by beta-oxidation to universal constituents.		hypothetical and highly unlikely ever to be realised.	
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	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 the human food chain, without any reported negative effects. Similarly, carotenoids and saturated fatty acids are a ubiquitous and normal component of the human diet. Dietary lipids are metabolised by beta-oxidation to universal constituents.	Some contact between the GMHP and humans is expected. People operating farm machinery and scientists working in the trial site will come into physical contact with the plants.	No plant material from the trial will enter the food chain. Appropriate advice and SOPs will be used to minimise exposure to the GMHP, despite the risk being negligble	Overall risk is very low.

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.	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. Similarly, carotenoids and saturated fatty acids are a ubiquitous and normal component of both animal and human diet. Dietary lipids are metabolised by beta-oxidation to universal constituents.	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.
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).	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 several years).	None	It is very unlikely that changes in biogeochemical processes would occur - Overall risk is negligible.

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			
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.. 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 GMOs is small and temporary (lasting <5 months/year for several years).	No plant material from the trial will enter the food or animal feed chain.	Overall risk is very low.
Consideration of the risk of	By DNA released from decomposing	In the very unlikely event that functional expression cassettes were horizontally	Horizontal gene transfer between plants and wild-type	This risk will be managed by minimising the seeds	The risk of this is

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.	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*.	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.	*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 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	and other above-ground plant biomass left in the soil.	extremely low

			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.

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.

Not applicable

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.

As described in Part A1, several studies describing the omega-3 trait have been published. Below are listed the most recent examples.

Han L, Haslam RP, Silvestre S, Lu C, Napier JA. Enhancing the accumulation of eicosapentaenoic acid and docosahexaenoic acid in transgenic Camelina through the CRISPR-Cas9 inactivation of the competing FAE1 pathway. Plant Biotechnol J. 2022;20(8):1444-1446. doi:10.1111/pbi.13876

Han L, Silvestre S, Sayanova O, Haslam RP, Napier JA. Using field evaluation and systematic iteration to rationalize the accumulation of omega-3 long-chain polyunsaturated fatty acids in transgenic Camelina sativa. Plant Biotechnol J. 2022;20(9):1833-1852. doi:10.1111/pbi.13867

Han L, Usher S, Sandgrind S, et al. High level accumulation of EPA and DHA in field-grown transgenic Camelina - a multi-territory evaluation of TAG accumulation and heterogeneity. Plant Biotechnol J. 2020;18(11):2280-2291. doi:10.1111/pbi.13385