

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].

Although none of the lines envisaged in this current proposal have previously been released as a GMO, many 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 18/R8/01 and 16/R8/01) and outside - release in Canada in 2017&18 under CFIA permit ICA6-46020 and also in the USA under APHIS permit # 15-357-101r.

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 and 16/R8/02 and more relevant to this application, GM *C. sativa* (14/R8/01, 16/R8/01, 18/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 environment 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 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 gas guns 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 unlikely event of a hybrid being generated, the presence of EPA+DHA or NMI-PUFAs in the seed oil of any such progeny would not convey a selectable advantage and most likely the omega-3 or NMI-PUFA trait would not be retained. This is equally true for any marker genes (visible markers or herbicide tolerance) since no selective bialaphos-containing herbicides will be used in the management of this field trial, negating any selective pressure arising from any optional use of the *bar* gene.

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 small 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. 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 negligible.

Step 1: <i>Potential hazards which may be caused by the characteristics of the novel plant</i>	Step 2: <i>Evaluation of how above hazards could be realised in the receiving environments</i>	Step 3: <i>Evaluation the magnitude of harm caused by each hazard if realised</i>	Step 4: <i>Estimation of how likely/often each hazard will be realised as harm</i>	Step 5: <i>Modification of management strategies to obtain lowest possible risks from the deliberate release</i>	Step 6: <i>Overall estimate of risk caused by the release</i>
(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 <i>C. sativa</i> plants with a more 'weedy' habit that are better able to establish and thrive in uncultivated environments or to persist in agricultural habitats.	<i>C. sativa</i> 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. <i>C. sativa</i> 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 <i>C. sativa</i> that can cross-hybridise and produce viable seeds. Seed removal from the site will be rigorously managed (see step 5). The chances of modified <i>C. sativa</i> 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 <i>C. sativa</i> will be cultivated for at least 750m surrounding the trial so it will be straight-forward to detect any <i>C. sativa</i> 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 <i>bar</i> marker gene present in any <i>C. sativa</i> lines envisaged in this application provides tolerance of the broad spectrum herbicide bialaphos (also known as	The selectable advantage provided by the <i>bar</i> gene is only realised when the plant is exposed to the specific Class H	No positive selection for the <i>bar</i> gene will be applied to the trial site, unless in the form of a closely controlled	Overall risk is very low.

<p>persistence in agricultural habitats due presence of the selectable marker gene (<i>bar</i>)</p>	<p>in the specific cases where the selectable marker gene (<i>bar</i>) is included in the transgene cassette (optionally present in some configurations), resulting in <i>C. sativa</i> plants that are better able to resist specific herbicides</p>	<p>glufosinate). The presence of this transgene could provide a selectable advantage to the GMO</p>	<p>herbicide. In the absence of this selection pressure, there is no obvious advantage conferred by the presence of this gene. In the event of the release of GMHPs containing the optional <i>bar</i> 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 <i>bar</i> gene is low.</p>	<p>plot-size experiment to evaluate the efficacy of this trait. No Class H herbicides (to which <i>bar</i> confers resistance) will be used in the general management of the trial</p>	
<p>(ii) Any selective advantage or disadvantage conferred to the GMHP</p>	<p>Selective advantage or disadvantage may result from the intended traits (improved oil composition) or as a result of unintended effects of the genetic modification.</p>	<p>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 <i>bar</i> resistance marker (see above), since in the latter case, no herbicides targeting the inhibition of glutamine synthetase will be used.</p> <p>Equally, plants that have undergone CRISPR-Cas9 targeted gene-editing of FAD2 or FAE1, resulting in elevated levels of oleic acid or reduced C20+ fatty acids, respectively, would not be expected to provide any selective advantage. In the case of the <i>fad2</i> mutant A7, where oleic acid levels are maximally increased, plants perform poorly in the field.</p>	<p>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 where 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.</p>	<p>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 <i>C. sativa</i> 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</p>	<p>Overall risk is very low.</p>

<p>(iii) Selective advantage or disadvantage conferred to other sexually compatible plant species</p>	<p>These hazards could be realised in the receiving environment via dispersal of GM seeds from trial site to the surrounding environment or via out-crossing to sexually-compatible species outside trial site.</p>	<p>This would be dependent on cross-pollination between the GMHP and compatible species, of which there are no examples on the Rothamsted farms</p>	<p>It is highly unlikely that pollen from the GMHP will successfully fertilise a compatible species (see ii)</p>	<p>There is a pollen barrier “buffer zone” surrounding the GMHP plots to minimize (i.e. trap) the spread of pollen. Surrounding the trial site is an 1000 metre area in which no <i>C. sativa</i> will be grown. Normal agricultural practice will be used to control weeds in the area beyond the trial site.</p>	<p>Overall risk is very low.</p>
<p>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).</p>	<p>Omega-3 long chain polyunsaturated fatty acids or NMI-PUFAs may illicit a change in behaviour of other organisms.</p> <p>Two <i>fad2</i> CRISPR lines have an increased level of oleic acid in all tissues; Two transgenic lines (HO, LPAT) have elevated oleic acid</p> <p>The SAD lines might be more heavily predated due to loss of synapine</p>	<p>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, NMI-PUFAs or seed altered fatty acid profile retained and compartmentalised in the seeds of the GMHP.</p> <p>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. Seed-specific accumulation in HO & LPAT lines is unlikely to have any detectable impact</p> <p>Sinapine accumulation is normally restricted to seeds</p>	<p>Many organisms will encounter the modified <i>C. sativa</i> plants in the field trial, though most of the plants to be evaluated have restricted expression (seed-specific) of the traits.</p>	<p>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.</p>	<p>Overall risk is very low.</p>

<p>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.</p>	<p>Omega-3 long chain polyunsaturated fatty acids or NMI-PUFAs may illicit a change in behaviour of other organisms.</p> <p>Two <i>fad2</i> CRISPR lines have an increased level of oleic acid in all tissues;</p> <p>Two transgenic lines (HO, LPAT) have elevated oleic acid</p> <p>The SAD lines might be more heavily predated due to loss of synapine</p>	<p>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 or NMI-PUFAs retained and compartmentalised in the seeds of the GMHP.</p> <p>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.</p> <p>Seed-specific accumulation in HO & LPAT lines is unlikely to have any detectable impact</p> <p>Sinapine accumulation is normally restricted to seeds</p>	<p>Many organisms will encounter the modified <i>C. sativa</i> plants in the field trial.</p>	<p>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.</p>	<p>Overall risk is very low.</p>
<p>vi. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of</p>	<p>By contact or ingestion of GM plant material.</p>	<p>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. This is equally true of NMI-PUFAs, although these fatty acids are present at</p>	<p>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.</p>	<p>No plant material from the trial will enter the food chain.</p> <p>Appropriate advice and SOPs will be used to minimise exposure to the GMHP, despite the risk being negligible</p>	<p>Overall risk is very low.</p>

<p>the GMHP and persons working with, coming into direct contact with, or in the vicinity of the GMHP release(s).</p>		<p>lower levels. Both these compounds are present in natural food webs and do not appear to interact in a synergistic fashion. Similarly, oleic acid is a ubiquitous and normal component of the human diet</p>			
<p>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.</p>	<p>By contact or ingestion of GM plant material.</p>	<p>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 NMI-PUFAs, present for example in pine nuts and juniper berries, and whilst not so abundant in terrestrial foodwebs, still likely to be highly represented. Similarly, oleic acid is a ubiquitous and normal component of both animal and human diet</p>	<p>It is not intended to use the GMHP for direct animal feeding studies</p>	<p>No GMHP will enter the feed chain.</p>	<p>Overall risk is very low.</p>
<p>viii. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and</p>	<p>Changes in biogeochemical processes may result from unintended changes in the modified plants or from unintended changes in soil microbes due to</p>	<p>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.</p>	<p>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).</p>	<p>None</p>	<p>It is very unlikely that changes in biogeochemical processes would occur - Overall risk is negligible.</p>

target and non-target organisms in the vicinity of the GMO release(s).	horizontal transfer of DNA.				
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	No plant material from the trial will enter the food or animal feed chain.	Overall risk is very low.

			and temporary (lasting <5 months/year for several years).		
<p>Consideration of the risk of horizontal gene transfer into wild-type <i>Agrobacterium</i> species in the soil that could infect and transfer DNA to other plant species including risks associated with expression of the genes.</p>	<p>By DNA released from decomposing plant material being taken up into the T-DNA of wild-type <i>Agrobacterium</i> and the subsequent expression of functional cassettes in other plants after natural transformation by <i>Agrobacterium</i>.</p>	<p>In the very unlikely event that functional expression cassettes were horizontally transferred into soil <i>Agrobacterium</i> 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.</p>	<p>Horizontal gene transfer between plants and wild-type <i>Agrobacterium</i> species, and the subsequent infection of other plant species with recombinant DNA is considered an exceedingly small risk. Although transformation of wild type <i>Agrobacterium tumefaciens</i> 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 <i>Agrobacterium</i> 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 <i>Agrobacterium</i> and capable of autonomous replication. This could theoretically occur if the transgene insert liberated by decomposing roots was taken up by wild type <i>Agrobacterium</i> either</p>	<p>This risk will be managed by minimising the seeds and other above-ground plant biomass left in the soil.</p>	<p>The risk of this is extremely low</p>

			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 <i>Agrobacterium</i> 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 Câmara 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.

None of the iterations described in this application have previously been described or published.

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.

Ruiz-Lopez N, Haslam RP, Usher S, Napier JA, Sayanova O. (2015) An alternative pathway for the effective production of the omega-3 long-chain polyunsaturates EPA and ETA in transgenic oilseeds. *Plant Biotechnol J.* 13(9):1264-75. doi: 10.1111/pbi.12328.

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