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].

Not applicable.

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.

Not applicable.

Part A4: Risk assessment and a statement on risk evaluation

Summary

Environmental risks

The probability of *B. oleracea* seeds or pollen escaping from the trial site or the transfer of inserted characteristics to sexually-compatible species outside the trial area is estimated as very low. Plants grown in the trial will have their inflorescences removed at the early stages, prior to pollen exposure and therefore seed set. Primary inflorescences produced will be harvested for analysis, along with leaf material. Following this, all plants will be uprooted and destroyed in their entirety by autoclaving at the John Innes Centre to prevent further flowering. Less than ten individuals will be allowed to flower with their inflorescences contained within a pollen proof bag, preventing pollen or subsequent seed release. *As B. oleracea* is unable to clonally propagate and reproduces exclusively through sexual reproduction, it is unlikely any residual plant material will lead to further emergence of plants. With the exception of a barrier of *Brassica napus* plants surrounding the plants in the trial, no *Brassica* plants will be grown within 20 metres of the trial site and surrounding areas will be monitored for the presence of species capable of crossing with *B. oleracea*. If any species are found which may cross pollinate within

20m of the *B. oleracea* plants in this study, they will be treated with a herbicide (glyphosate). The potential removal of defence compounds, glucosinolates, in trial plants suggests that they would not posess a selective advantage over any existing Brassica plants and will be very unlikely to outcompete any wild or ruderal plants.

The risk of non-sexual, horizontal gene transfer to other species is extremely low. Current data suggests an absence of the transgene used to generate the mutation in this line and therefore an absence of the plasmid (further analysis will be conducted to confirm this), further reducing the risk of any potential gene transfer. 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 *B. oleracea* 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.

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 marker genes, are expected to result in the synthesis of products that are harmful to humans, other organisms or the environment. The breeding line used to generate these plants is AG DH1012, a doubled haploid genotype from the *Brassica oleracea* ssp *alboglabra* (A12DHd) and *Brassica oleracea* ssp *italica* (Green Duke GDDH33) mapping population (Bohuon et al., 1996; Bohuon, 1995). Both parental lines are already widely consumed as Chinese kale (ssp *alboglabra*) and broccoli (ssp *italica*) and have no currently recognised negative effects upon human consumption. Moreover these lines have been edited to potentially reduce the production of existing compounds in the plant (glucosinolates) and are not proposed to produce any novel metabolites which may pose as threat to human health upon consumption. Any unknown hazards arising from the expression and ingestion of foreign proteins will not occur since the *B. oleracea* 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.

It is highly unlikely that the genetic modification introduced to the GMHP could result in major changes in invasiveness or persistence, as the proposed modification would potentially reduce the ability of the plant to persist or outcompete. 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 highly unlikely as the GMHPs will have their inflorescences removed prior to flowering, with the exception of those that will be kept in pollen-proof bags, preventing the spread of pollen or seed to surrounding plants. Moreover the *B. oleracea* pollen is large and unlikely to come into contant with other compatible species.

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

Selective disadvantage may result from the intended traits (reduced defence compounds) or as a result of unintended effects of the genetic modification. However the risk of this is very low as GMHP plants with the intended mutation do not have a differing phenotype when grown in the glasshouse. 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 highly unlikely as the the low number of plants allowed to flower will be contained in pollen-proof bags and therefore not spread pollen or seed outside of the trial area.

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.

Gene transfer would only be accomplished through spread of seed or cross-pollenation with compatible species, which is unlikely due to the low number of plants that will be left to flower, which will be contained within pollen-proof bags. Moreover these plants will be surrounded by a barrier of *Brassica napus* plants, with any other compatible species within 20 metres of the trial site being removed through herbicide treatment. Should the highly unlikely event of cross-pollenation arise, there should be no predicted advantage conferred to those plants 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).

Due to the proposed reduction in secondary defence compounds introduced by the modification, it is possible that the GMHPs may undergo more damage by pathogens and pests than the parental lines whilst out in the field, though this is effect is likely to be minor and will only affect the GMHP. Management practices will be put into place to minimise pest and pathogen contact with the GMHPs, including pesticide/herbicide practices, as the plants will be treated in a practice that best reflects commercial *Brassica* cultivation. In addition, this scenario remains

hypothetical as low defence levels are not guaranteed in these GMHPs nor is the effect this will have on interactions.

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.

Due to the proposed reduction in secondary defence compounds introduced by the modification, it is possible that the GMHPs may undergo more damage by pathogens and pests than the parental lines whilst out in the field, though this is effect is likely to be minor and will only affect the GMHP. Management practices will be put into place to minimise pest and pathogen contact with the GMHPs, including pesticide/herbicide practices, as the plants will be treated in a practice that best reflects commercial *Brassica* cultivation. In addition, this scenario remains hypothetical as low defence compound levels are not guaranteed in these GMHPs nor is the effect this will have on interactions.

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).

The nature of the trial relies on some human contact between the trained scientists and the GMHP and appropriate advice and SOPs will be used to minimise exposure to the GMHP, despite the risk of effects on human health being negligible. The compounds being potentially reduced in the GMHPs are already very widely represented in the human food chain, without any reported negative effects. Moreover, no plant material from the trial will enter the food chain.

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.

It is not intended to use the GMHP for direct animal feeding studies, nor will the GMHP enter the food chain.

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).

Biogeochemical processes are not expected to be affected by the cultivation of the genetically modified plants and the area in which the GMHP will be grown will be small and temporary.

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

	Step1: ***Potential hazards which may be caused by the characteristics of the novel plant***	**Step 2:** ***Evaluation of how each hazard could be realised in the receiving environments***	**Step 3:** ***Evaluation of 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 of harm caused by the release for each hazard***
a	**Increased invasiveness in natural habitats or persistence in agricultural habitats.**	Potential off-target effects of the Cas9 on similar genes may provide plants with traits making them better able to establish and thrive in uncultivated environments or to persist in agricultural habitats.	Left unmanaged, *B. oleracea* does not establish 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	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	Following inflorescence harvest, plants will be uprooted and destroyed by autoclave to prevent any pollen or subsequent seed release. Less than ten individuals will be allowed to flower with their inflorescences contained within a pollen proof bag, preventing pollen or subsequent seed release.	Overall risk is negligible

			for agricultural or unmanaged ecosystems. *B. oleracea* is a benign plant that can be easily managed by cultivation or specific herbicides.	elsewhere. This is highly unlikely as the GMHPs will not flower on the trial site and therefore not spread pollen or seed to surrounding plants. Moreover the *B. oleracea* pollen is large and unlikely to come into contant with other compatible species.		
b	**Selective advantage: improved resistance to *P. infestans.***	n/a	n/a	n/a	n/a	n/a

c	**Selective advantage: improved resistance to potato cyst nematodes**	n/a	n/a	n/a	n/a	n/a
d	**Selective advantage: resistance to sulfonylureas and imidazolinones provided by the selectable marker gene (*CSR*)**	n/a	n/a	n/a	n/a	n/a
e	**Selective advantage or disadvantage conferred to sexually compatible plant species**	These hazards could be realised in the receiving environment via dispersal of GM seeds from trial site to the surrounding environment or via	The modification would potentially lead to a reduction in defence compunds, which may provide a selective disadvantage if it were to be	It is highly unlikely that pollen from the GMHP will successfully fertilise a compatible species. It is also not guaranteed that the intended	Following inflorescence harvest, plants will be uprooted and destroyed by autoclave to prevent any pollen or subsequent seed	Overall risk is low

		out-crossing to sexuallycompatible species outside.	transferred to a sexually compatable species. , This would be dependent on crosspollenation between the GMHP and compatible species which will not be present within at least 20 metres of the site, with the exception of a surrounding barrier of *Brassica napus* which will act as an additional hindrance to pollen release	modification would provide any selective disadvantage as these GMHPs display no phenotype when grown in the glasshouse.	release. Less than ten individuals will be allowed to flower with their inflorescences contained within a pollen proof bag, preventing pollen or subsequent seed release. *Brassica napus* barrier plants will also be surrounding the GMHPs to act as a physical barrier to pests and a pollen barrier.	
f	**Potential environmental impact due to interactions between the novel plant and target organisms**	Reduced glucosinolate content may reduce ability of the plant to deter herbivory and defend against pest or pathogen attack. This could lead to increased feeding by insects or	GMHPs may undergo more damage by pathogens and pests than the parental lines whilst out in the field, though this is effect is likely to be minor.	It is unlikely that this hazard will be realised as a harm to any organism other than the GMHP	Management practices will be put into place to minimise pest and pathogen contact with the GMHPs, including pesticide/herbicide practices (as the plants will be treated in a practice that	Overall risk is low

		susceptibility to disease.			best reflects commercial *Brassica* cultivation). In addition, this scenario remains hypothetical as low glucosinolate levels are not guaranteed in these GMHPs nor is the effect this will have on interactions.	
g	**Potential environmental impact due to interactions between the novel plant and non-target organisms**	Reduced glucosinolate content may reduce ability of the plant to deter herbivory and defend against pest or pathogen attack. This could lead to increased feeding by insects or susceptibility to disease.	GMHPs may undergo more damage by pathogens and pests than the parental lines whilst out in the field, though this is effect is likely to be minor.	It is unlikely that this hazard will be realised as a harm to any organism other than the GMHP	Management practices will be put into place to minimise pest and pathogen contact with the GMHPs, including pesticide/herbicide practices (as the plants will be treated in a practice that best reflects commercial *Brassica* cultivation). In addition, this scenario remains hypothetical as low	Overall risk is low

					glucosinolate levels are not guaranteed in these GMHPs nor is the effect this will have on interactions.	
h	**Potential effect on human or animal health due to the introduced genes**	By contact or ingestion of GM plant material.	The compounds being potentially reduced in the GMHPs are already very widely represented in the human food chain, without any reported negative effects.	Some contact between the GMHP and humans is expected. Field Experimentation staff working in the field site and scientists working in the trial site will come into physical contact with the plants. It is not intended to use the GMHP for direct animal feeding studies	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 negligible.	Overall risk is very low
i	**Potential effects on biogeochemical processes (changes in soil decomposition of organic material)**	Changes in biogeochemical processes may result from unintended changes in the modified plants or from	The magnitude of harm is estimated to be extremely low. Biogeochemical processes are not expected to be affected by the	The frequency of changes to biogeochemical processes is considered to be very low. The maximum area	None	Overall risk is negligible

		unintended changes in soil microbes due to horizontal transfer of DNA.	cultivation of the genetically modified plants.	proposed to be planted with GMOs is small and temporary.		
j	**Possible environmental impact due to changes in cultivation practice**		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

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.

Detailed information on the description of this GMO have yet to be published

References

Bohuon, E., Keith, D., Parkin, I., Sharpe, A., and Lydiate, D. (1996). Alignment of the conserved C genomes of Brassica oleracea and Brassica napus. Theoretical and Applied Genetics 93, 833-839.

Bohuon, E.R.J. (1995). A genetic analysis of Brassica oleracea (University of Birmingham).