



GM Team
 Department for Environment, Food and Rural Affairs
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 Submitted by email to gm-regulation@defra.gsi.gov.uk

26 February 2019

Dear Madam/Sir

Re: Application from John Innes Centre to release a genetically modified organism, reference 19/R52/01 as published at <https://www.gov.uk/government/publications/genetically-modified-organisms-john-innes-centre-19r5201>

We are writing on behalf of GM Freeze, EcoNexus, GeneWatch UK, the Sustainable Food Trust, OF&G, the Soil Association, the Organic Research Centre, Garden Organic, the Landworkers Alliance, WWOOF UK, the Kindling Trust, Sheepdrove Organic Farm, Shepton Farm, the Real Seed Catalogue, Banc Hadau Llambled / Lampeter Seed Library, Unicorn Grocery, ACE Energy, the Springhead Trust, GM Watch, Beyond GM, Mums Say No to GMO, GM Free Dorset, GM Free Somerset, GM Free Cymru, Genetic Engineering Network, Agri-Activism UK, Pro-Natural Food Scotland, South East Essex Organic Gardeners, Cardiff Friends of the Earth, East Dorset Friends of the Earth, Sustainable Dorset/Dorset Agenda 21 and Resurgence Dorset to request that the above application to release genetically modified (GM) Brassica is refused.

GM Freeze is the umbrella campaign for a moratorium on GM in food and farming in the UK.

EcoNexus analyses developments in science and technology and their impacts on environment and society. **GeneWatch UK** monitors developments in genetic technologies from a public interest, human rights, environmental protection and animal welfare perspective. The **Sustainable Food Trust** is a registered charity with a goal of promoting food and farming systems that nourish the health of the planet and its people.

OF&G (Organic Farmers and Growers) was the first body to be approved by the government to inspect and certify organic food and farming and is now the largest certifier of organic land in the UK. The **Soil Association** is the UK's leading membership charity campaigning for healthy, humane and sustainable food, farming and land use. **The Organic Research Centre** is the UK's leading independent research, development and advisory institution for organic agriculture. **Garden Organic** (formerly known as the Henry Doubleday Research Association) is the UK's leading organic growing charity with over 20,000 members throughout the UK and abroad.

The Landworkers Alliance is a grassroots union representing farmers, growers and land-based workers. **WWOOF UK (World Wide Opportunities on Organic Farms)** is a membership charity which connects people wanting to learn about ecological growing and low impact lifestyles with sites across the country living ethically and needing practical help on the land. **The Kindling Trust** is working to create a more sustainable local food system through a number of practical initiatives in Greater Manchester. **Sheepdrove Organic Farm** and award-winning eco-conference centre are committed to sustainability, conservation and education. **Shepton Farm** in Somerset grows grass/clover, arable crops and apples.

The **Real Seed Catalogue** provides open pollinated seeds for home gardens and organic growing. **Banc Hadau Llambled / Lampeter Seed Library** offers free locally adapted and produced open pollinated seeds to its members. **Unicorn Grocery** in Manchester has pioneered a cooperative approach to sustainable urban food supply. **ACE Energy** helps farmers to use less energy intensive methods of farming. The **Springhead Trust** promotes environmental education, sustainability, organic agriculture and local performing arts.

GMWatch is a news and information service that aims to keep the public up to date on issues around GM crops and foods and associated pesticides. **Beyond GM** is a creative initiative to educate and engage the public and raise the level of debate around the issues of GMOs and sustainable food production in the UK. **Mums Say No to GMOs** is a coalition of mothers and their families using consumer pressure to stop GM crops being grown and sold in the UK. **GM Free Dorset** and **GM Free Somerset** are grass roots campaigns supported by individuals, groups, local businesses and charities that exist to promote rural sustainability. **GM Free Cymru** is the community pressure group campaigning to keep Wales free of genetically-modified crops. **Genetic Engineering Network** facilitates the exchange of information between groups and campaigners. **Agri-Activism UK** is a network of people who campaign for cleaner, healthier and more sustainable agricultural and food systems. **Pro-Natural Food Scotland** is a long-established Scottish GM-concern group aiming to empower the public by raising awareness.

South East Essex Organic Gardeners promotes the principles of organic gardening. **Cardiff Friends of the Earth** and **East Dorset Friends of the Earth** work on a local level to create a just world where people and nature thrive. **Sustainable Dorset/Dorset Agenda 21** is the online and outreach interface of Dorset Agenda 21, a central hub for sustainable and resilient activity across the county, with the aim of raising awareness and increasing interest and involvement in sustainability. **Resurgence Dorset** is a monthly community group of environmentalists and nature-lovers set up to discuss Resurgence & Ecologist articles and host talks to raise public awareness of environmental issues.

We do not believe that this trial should go ahead. Key information is missing from the application; any escape from the trial could cause significant harm; the proposed containment measures are inadequate; and the research could be undertaken in a modern contained-use facility. In summary, our objection covers the following points:

1. The applicant's risk assessment is incomplete
 - 1.1. The GM lines have not been characterized
 - 1.2. The application does not consider genomic errors caused by genome editing
2. The genetic modifications could cause harm
 - 2.1. Antibiotic resistance marker genes represent a risk
 - 2.2. Increased herbivory is a potentially damaging trait
3. There is potential for GM escape from the trial
 - 3.1. GM contamination from *Brassica oleracea* is possible
 - 3.2. The containment measures proposed by the applicant are insufficient
4. There is no need for a field trial

1 THE APPLICANT'S RISK ASSESSMENT IS INCOMPLETE

This application for deliberate release of a genetically modified organism should be refused on the grounds that the risk assessment included in the application is incomplete.

1.1 The GM lines have not been characterised

The application includes a description of the transgenic insert that was originally introduced (Part A1, para 12) but no molecular information on the GM *Brassica oleracea* lines included in the trial.

The applicant states (Part A1, para 14) that *"PCR analysis and copy number analysis, in the progeny, of the NPTII promoter sequence described in Part III Section 12 has found no presence of the transgene in these plants, suggesting that the insert is no longer present in these plants and has segregated out in the subsequent generations. However, there is the possibility of the backbone of the vector being integrated into the plants therefore absence of the vector components described in Part III Section 12, including the Cas9 sequences, will be confirmed using PCR techniques which are currently being developed."*

Similarly, in Part A1, para 15 *"Current data suggests the absence of the insert from the plants intended for the trial. However, should the presence of the Cas9 used for gene editing remain, it would likely be driven by the Cassava vein mosaic virus promoter and therefore be expressed in all tissue-types of the plant to varying degrees. Q-PCR results confirming the presence or absence of the Cas9 will be provided prior to planting, to the satisfaction of the regulator."*

The applicant is making significant assumptions here and is not providing enough information to determine whether elements of the inserted CRISPR transgene (including backbone sequences) remain in the GM plant. Without knowing the nature of the insert that remains in the plant, it is not possible to perform a meaningful risk assessment. For example, it is not known whether the GM plant contains antibiotic resistance genes or not (see 2.1 below). Full characterisation of the GM plant, including whether the CRISPR cassette has been fully removed should have been completed prior to application so that a full risk assessment could be included in the documents published for both public and Departmental scrutiny.

1.2 The application does not consider genomic errors caused by genome editing

There is now a wealth of evidence showing that genome-editing, including CRISPR/Cas techniques, gives rise to genetic errors, including off-target effects and interference with regulation of gene expression¹. Indeed, the published paper² which the applicant cites to detail their methodology describes off-target effects in *B. oleracea* caused by the genome-editing process.

The applicant has not shared any evaluation of the GM lines for genomic errors or their implications for plant chemistry and physiology (and hence for food and environmental safety). Without details of such studies the risk assessment is incomplete.

2 THE GENETIC MODIFICATIONS COULD CAUSE HARM

2.1 Antibiotic resistance marker genes represent a risk

As noted above (1.1), the application does not include a molecular characterisation of the GM *B. oleracea* lines to be planted in the proposed trial. This makes it impossible to know whether or not the GM plants contain antibiotic resistance marker genes. For example, either one or both of the antibiotic resistance marker genes nptII and Spec (aadA³) used in the development of the GM plants. As a result, consideration of the application must proceed on the basis that these genes are included and, as they represent a potential risk to both people and animals, the application should be rejected.

The marker gene nptII confers resistance to the kanamycin family of antibiotics, whilst aadA confers resistance to streptomycin/spectinomycin. All these antibiotics are used in human medicine and both kanamycin and spectinomycin are on the current World Health Organisation (WHO) list of essential medicines⁴. Therefore, there is an urgent need to prevent resistance to these antibiotics from spreading in the environment.

In its influential 2004 opinion on the use of antibiotic resistance marker genes, EFSA⁵ placed aadA in Group II, indicating it should not be used in cultivation. In the 15 years since, concern has been growing at a European (e.g. European Medicines Agency (EMA)⁶) and global (e.g. WHO's annual World Antibiotic Awareness Week⁷) level that the misuse and overuse of antibiotics will result in increased resistance to antibiotics, making them ineffective in treating infections in both humans and animals.

The UK government recently published a 20-year vision and 5-year national action plan⁸ to prevent further antimicrobial resistance (AMR), which includes antibiotic resistance. The vision calls tackling antimicrobial resistance a "global priority", while the 5-year plan includes the reduction of antimicrobials in agriculture⁹. Agricultural use of antimicrobials is currently restricted to livestock so antibiotic resistance marker genes in GM plants represent a new and additional pathway for pathogenic acquisition of antibiotic resistance. This pathway adds a new threat to the continued efficacy of antibiotics in human and animal medicine.

Given the possibility of pollen or seed escape (see 3, below), the release of GM plants with antibiotic resistance marker genes is irresponsible and this trial should be refused on the basis that the GM plants may include antibiotic resistance marker genes.

2.2 Increased herbivory is a potentially damaging trait

The applicant states (Part A1 para 22) that *"The genetic modification potentially leads to a reduction in glucosinolates, sulphur metabolites used by plants of this group to deter herbivory. It is possible that this genetic modification could lead to increased herbivory, as studies in the model plant Arabidopsis thaliana have found decreased larval weight gain of a generalist herbivore when over-expressing the gene edited in this study (Gigolashvili et al., 2007). However it is still unknown as to whether these plants will have low glucosinolate content and little data is available predicting herbivore interactions with these compounds in Brassica oleracea in the field."*

The applicant further states (Part A1, para 16) that *"As glucosinolates are used primarily for defence against insects and pathogens, it is possible that the reduction of these compounds would reduce the survivability of the genetically modified plant when compared to the parental/wild-type plant when placed in the field."*

Should GM contamination occur from this field trial (see 3 below), it is possible it could have a marked effect on related wild and commercial species of Brassica. For example, outcrossing to oilseed rape could increase herbivory of commercial oil seed rape crops while outcrossing to wild relatives could affect their survivability. In addition, any unexpected change in plant chemistry (e.g. a novel or altered plant protein), could be toxic to wildlife. Given the lack of molecular characterisation of the GM plants, and lack of consideration of potential genomic errors (1.1 and 1.2, above), it is not possible to meaningfully assess the potential harm to local farmers, wildlife of the wider ecosystem and the trial should be rejected on this basis.

3 THERE IS POTENTIAL FOR GM ESCAPE FROM THE TRIAL

As we have described above, any escape of the GM plants (via seeds or pollen) could result in potentially considerable adverse effects on local farms, wildlife and the ecosystem. It is therefore vital that such escape does not occur. The applicant has not proposed adequate measures to prevent escape and the trial application should be rejected on these grounds.

3.1 GM contamination from *B. oleracea* is possible

In Part A1, paras 27 and 28, the applicant states *"With the exception of the surrounding Brassica napus guard crop, no Brassica plants will be grown within the accepted distance of 20 metres from the release site."* and that *"the plants in this trial will be removed prior to flowering, apart from a small number of plants for which their inflorescences will be secured within a pollen proof bag, therefore pollen release is highly unlikely."* However, the applicant also acknowledges (para 4) that there is *"potential for pollination to be carried out by insects"* and that some seed might escape (para 5): *"It is possible some mature seeds of flowers produced from axillary branches may remain and be lost into the soil. If not managed, these seeds could potentially over-winter in the soil and germinate the following spring as 'volunteers'."*

The Brassica family is promiscuous, as acknowledged by the applicant (para 4): *"successful hybridisation has been reported between B. oleracea and several members of the B. oleracea cytodeme: B. bourgeauii, B. cretica, B. incana, B. insularis, B. macrocarpa, B. montana, B. rupestris, and B. villosa. Outside of the cytodeme B. oleracea is also capable of successful hybridisation with the crop species, B. juncea, B. napus and B. rapa and with B. maurorum. Successful intergeneric hybridisation has been reported for crosses*

between B. oleracea and the following species: Eruca vesicaria, Erucastrum abyssinicum, Hirschfeldia incana, Moricandia arvensis, Raphanus sativus and S. arvensis”.

The area surrounding the John Innes Centre has a high concentration of Brassica crops, notably oil seed rape *B. napus*, which was grown on over 30,000 hectares of Norfolk in 2016¹⁰. Whilst outcrossing to *B. napus* oilseed rape is considered “unlikely”, it is still possible as hybrids have been obtained¹¹. Wild *B. oleracea* is found in southern England¹². In addition, plants in the Brassica family are pernicious weeds with the ability to spread across terrain. Indeed, BASF’s website Agricultural Services UK dedicates a section to eradicating volunteer oil seed rape as one of only eight common weeds¹³. Thus, there is potential for the GM *B. oleracea* from the field trial to escape into similar crops and wild relatives in the surrounding area.

The establishment of feral populations of GM oilseed rape as a result of escape from cultivated fields or transport of GM material has occurred in several countries, notably the United States (US), Canada, Japan and Switzerland¹⁴. In Canada and the US, feral oilseed rape populations occur in provinces where they are widely grown, or through which the grain is transported. In Japan, cultivation of GM oilseed rape is minor, but much oilseed rape is imported from Canada and feral populations have been reported in and around Japanese ports. In Switzerland, although GM oilseed rape is neither cultivated nor imported, feral populations of GM oilseed rape occur at ports and along railways, where seed cargo either is, or has been transported in the past¹⁵.

Recently, oilseed rape seed imported from Argentina for cultivation in Europe was found to be contaminated with an unauthorised GM variety¹⁶. It is not known how the contamination occurred as the seed was produced in a GMO-free area.

Although the incidents described above relate to oilseed rape (*B. rapa* or *B. napus*), rather than *B. oleracea*, they show how easily contamination occurs within the Brassica family. Therefore, the potential for GM contamination must be taken very seriously. GM contamination could have serious consequences for farmers (e.g. economic losses) and wildlife (e.g. if the genetic modification produced a novel toxin or altered the attractiveness to herbivory, disturbing the ecological balance).

3.2 The containment measures proposed by the applicant are insufficient

The applicant proposes a separation distance of only 20m from other Brassica plants (Part A1, para 27): *“With the exception of the surrounding Brassica napus guard crop, no Brassica plants will be grown within the accepted distance of 20 metres from the release site.”* Although very little information is available concerning pollen distances from *B. oleracea*, there is good information on the close relative *B. napus* (oilseed rape). Whilst pollen levels decrease with distance, oilseed rape is known to pollinate over large distances. Pollen has been found up to 4,000 m from the source¹⁷. Thus, a separation distance of the order of kilometres would be much more appropriate.

Similarly, although little information on the seed dormancy of *B. oleracea* is available, oilseed rape seeds persist for five to ten years¹⁸. Despite this, the proposed GM field trial does not include any plans for the long-term monitoring of volunteers. Rather, the applicant states (Part A1, para 36) *“Following harvest, the plot will be left fallow, monitored for remaining Brassica oleracea material during the remainder of the year and sprayed with a systemic broadleaf herbicide. Any Brassica oleracea identified will be destroyed by herbicide treatment (e.g. glyphosate) or removed by hand and destroyed by autoclaving as described below. The monitoring of the plot will be continued at monthly intervals by walking the trial site, in accordance with DEFRA guidance.”* and (Part A1, para 38) *“Post-trial the release site will remain fallow to enable easy identification of volunteers. The site will be inspected monthly and any Brassica oleracea identified will be*

immediately destroyed either by application of a systemic broadleaf herbicide or by hand pulling plants and digging out of the root systems. These will then be autoclaved within the John Innes Centre."

The trial application should be refused on the basis that the applicant has not arranged sufficient containment measures.

4 THERE IS NO NEED FOR A FIELD TRIAL

While we recognise the value of research aiming to determine the role of Myb28 in *B. oleracea* in regulating sulphur metabolism and accumulation of glucosinolates, there is no need for an open field trial.

The applicant states (Part A1, para 30) that the field trial is required because the Brassica being studied does not accumulate glucosinolates under glasshouse conditions. *"This trial is required in order to better imitate the commercial interaction between these compounds and their environment and ultimately how this transcription factor Myb28 may mediate this interaction."* However, facilities are available that would combine the safety of contained use with more realistic field conditions, for example, the controlled environmental facility, 'Ecotron'¹⁹.

We have demonstrated above that the proposed trial represents a risk through the potential escape of novel genetic material that has not been characterised, may contain genomic errors, may contain antibiotic resistance markers and is expected to induce increased herbivory. It is, therefore, unacceptable to conduct early research of this kind in an open field when investment in more suitable contained facilities would allow research to continue without risking harm to local farmers, wildlife and the wider ecosystem.

The proposed trial represents an unacceptable risk to farmers, wildlife and the wider environment. The application has significant gaps and the research it is intended to pursue should be conducted in a modern contained environment. We request, therefore, that the Minister denies consent and prevents this open-air field trial going ahead.

Yours faithfully

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