GM Team
Department for Environment, Food and Rural Affairs
Second Floor
Seacole Building, Marsham Street
London
SW1P 4DF
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/R52/02 as published at https://www.gov.uk/government/publications/genetically-modified-organisms-john-innes-centre-19r5202

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 Bread Campaign, the Real Seed Catalogue, Banc Hadau Llambed / 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) wheat 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 Bread Campaign, part of the food and farming charity Sustain, finds and shares ways to make bread better for us, better for our communities and better for the planet. The **Real Seed Catalogue** provides open pollinated seeds for home gardens and organic growing. **Banc Hadau Llambed / 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. The applicant has not included key information; the GM plants contain antibiotic resistance genes; the applicant's containment measures do not reflect the high risk of escape; and there are significant questions about the value of the project to which the proposed trial contributes. In summary, our objection covers the following points:

1. The applicant's risk assessment is incomplete

2. The GM wheat lines contain antibiotic resistance marker genes

3. There is significant potential for escape and contamination
   - 3.1. There have been multiple escapes of GM wheat from field trials in the United States (US)
   - 3.2. The containment measures proposed by the applicant are inadequate

4. The proposed trial is unnecessary and will be of no net benefit to society
   - 4.1. Genetic engineering is not necessary or suited to obtaining elevated levels of iron in wheat
   - 4.2. There is no evidence that the GM plants will improve nutrition in target groups
   - 4.3. The planned biofortification may have directly harmful effects

1 THE APPLICANT'S RISK ASSESSMENT IS INCOMPLETE

The application does not include a full molecular characterisation of the GM wheat to be planted, or of the VIT2 protein produced by the intended genetic modifications. This means that the full risks of the proposed trial cannot be meaningfully assessed.

The three lines (Part A1, para 14) to be tested in the proposed trial will have 8, 9 and 11 copies of the transgene inserted into the wheat genome. The inclusion of so many copies of the transgenic material increases the potential for adverse effects. It is now widely established that genetic modification is an inherently unpredictable process that is associated with unintended effects at the genetic, epigenetic and cellular level. These unintended effects may go on to have negative agronomic, environmental and health implications, as demonstrated in other GM crop varieties[1,2].

The use of the vector *Agrobacterium tumefaciens* has been shown to induce genetic deletions, insertions, chromosomal rearrangements, translocations, scrambling of sequences and epigenetic perturbations[3]. Similarly, the cauliflower mosaic virus 35S promoter has been linked to increased potential for genetic rearrangements[4] and analysis of the nos 3' terminator in transgenic plants has shown that it does not reliably terminate transcription, leading to the generation of novel RNA variants[5]. The applicant does not mention any assessment for genetic rearrangements as observed in other GM varieties[6], or for the absence of novel RNA variants, highlighting a lack of characterisation of the wheat plants.

It is vital that any GM plant is fully characterised prior to field trials to assess for molecular, physiological or compositional alterations. In this case, unexpected effects have already been documented with an increased number of tillers in the GM lines and fewer grains in those with 20 or more transgene copies[7].

These could be caused by unintended genome changes or unexpected effects of the intended genetic modifications, such as the target transgene being expressed outside the endosperm.

In addition, it is vital to consider whether or not the VIT2 protein produced by the intended genetic modification is the same as that produced naturally in wheat. The wheat grown in the proposed trial is not intended for human or animal consumption but (as we detail in 3, below), there is a significant risk of escape. In addition, the research project that the proposed trial contributes to is focused on eventual human consumption. The GM field trial should not, therefore, be considered until contained use studies have identified the causes of the observed unexpected effects and the human and wildlife toxicity and allergenicity profile of the VIT2 protein.

2 THE GM WHEAT LINES CONTAIN ANTIBIOTIC RESISTANCE MARKER GENES

Part A1 Para 18 of the applications states that "*the plasmid used possesses two antibiotic resistance genes (nptI and Hyg) and we have assumed that these are integrated into the plant genomic DNA along with the genes of interest. These elements may increase the rates of horizontal gene transfer and establishment in soil bacteria because they provide a theoretical mechanism for homologous recombination and selection.*"

We request that our response[7] to a previous GM wheat trial at Rothamsted Research (Defra ref. 16/R8/02), which also contained the nptI gene is taken into account. We noted that the application for this previous field trial of GM wheat, like the current one, did "*not consider the findings by Chinese researchers that synthetic antibiotic-resistance genes were found in microorganisms in Chinese rivers, apparently from plasmids in lab-waste*[8]."

Further, in Part A1, para 35b, the applicant states "*All straw will be chopped and left on site".* This proposed treatment of straw is identical to that in the previous GM wheat trial. We repeat, therefore, our comments[9] on the previous trial, "*This is unacceptable as the genetic material, including the antibiotic marker resistance gene(s) will still come into contact with biota, on the surface of the soil and also at depth within the soil.*

"Although the nptI gene will be in the plant DNA, rather than a plasmid, the Chinese study shows that antibiotic resistance marker genes can persist in environments, even when no selection pressure is applied, and can be taken up into organisms. Therefore, decomposition into the soil cannot be assumed, also taking into account the persistence rate of DNA in soil.

"Kanamycin is on the United Nations World Health Organisation (WHO)'s list of essential medicines for priority diseases[10]*. The use of any antibiotic marker gene in GM plants, even if solely for field trial purposes is irresponsible given the amount of concern regarding antibiotic resistance globally* (*eg WHO's annual World Antibiotic Awareness Week*[11]*)."*

In addition to nptI, the GM wheat lines in the current application also contain an antibiotic marker resistance gene for hygromycin which is used in animals to control intestinal parasites such as worms[12]. This means that any escape of hygromycin resistance from this GM wheat could threaten the future use of an important veterinary medicine. It is worth noting that GM Golden Rice originally contained the hygromycin resistance marker gene, but the use of this gene was discontinued in the mid-2000s[13].

The signatories to this submission are far from alone in our concern about this issue. As stated in ACRE's advice on application 16/R8/02 "*the majority of public representations reflected concern that growing plants containing antibiotic resistant marker genes would compromise the use of associated antibiotics in human and veterinary medicine*"[14].

Since then, concern among learned organisations such as the European Medicines Agency (EMA)[15] has been growing about the future of therapeutic antibiotics. Globally, there is a high level of concern regarding the rise of antibiotic resistance that could render key antibiotics ineffective in treating infections in humans and animals.

The UK government recently published a 20-year vision and 5-year national action plan[16] 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 agriculture[17]. 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 escape (see 3, below), the release of GM plants with antibiotic marker genes is irresponsible and this field trial should be refused.

3 THERE IS SIGNIFICANT POTENTIAL FOR ESCAPE AND CONTAMINATION

3.1 There have been multiple escapes of GM wheat from field trials in the US

As in our response to a previous GM wheat trial application (Defra ref. 16/R8/02) [18], we would like to draw the Minister's attention to the fact that GM wheat had been discovered growing outside US field trials on three separate occasions, even though no GM wheat has been approved for commercial cultivation anywhere in the world.

Glyphosate tolerant (Roundup Ready) wheat containing the genetic insert used in experimental MON71800 was discovered in Oregon in 2013[19]. A second type of Roundup Ready wheat, also containing the MON71800 insert but genetically distinct from the first escape, was found in Montana in 2014[20]. A third distinct type of glyphosate tolerant wheat, MON71700, was discovered in Washington State in July 2016[21]. These discoveries prompted some countries to halt purchasing of US wheat[22] and led to market concerns for US farmers and traders.

Investigations by APHIS (United States Department of Agriculture – Animal and Plant Health Inspection Service)[23, 24] failed to find the route of contamination in any of these cases. Field trials of Roundup Ready wheat took place between 1998 and 2005[25] and Monsanto closed its GM Roundup Ready wheat development program in 2004[26]. This means that the rogue GM wheat was only discovered between eight and 15 years after the conclusion of the GM Roundup Ready wheat field trial from which it escaped.

Together, these incidents present a worrying picture of how easy it is for GM wheat to escape from field trials and remain a GM contamination threat for many years. The uptake and expression of trialled GM traits in other wheat varieties suggests that pollen escaped from the trials. The timelines show that GM wheat trial escapees can remain either undetected or dormant for over 10 years. The impact of these incidents on US wheat trading demonstrates a considerable risk to UK farmers and processors in the event of any escape from the proposed trial.

As the routes of contamination from the US trials are not apparent, it is not possible to suggest mitigation measures that could have prevented the escapes. As a result, open-air field trials of GM wheat should not proceed.

3.2 The containment measures proposed by the applicant are inadequate

Given the US experience detailed above (3.1), the containment measures proposed by the applicant for this GM wheat field trial are inadequate. The applicant states in Part A1, para 35 that "*We have excluded the use of a pollen barrier given that its inclusion would more than quadruple the area under the release; a 2 m pollen barrier surrounding the release area would translate to a total area of 106.9 m^2 compared to the 23.1 m^2 of transgenic material. We are prepared to include a 2 m pollen barrier if requested (e.g the designated area is large enough, etc) but we propose that the separation distance of 20 m should minimise dispersal of any modified material.*"

ACRE has recommended a 2 m pollen barrier in previous GM wheat field trials[27] in addition to maintaining a separation distance of 20 m between GM wheat plants used in the trial and other wheat plants. We are extremely concerned that the applicant for this trial does not consider the pollen barrier an essential minimal measure to prevent the kind of contamination that has been shown to be particularly prevalent with GM wheat.

Considering the possibility of the GM wheat outcrossing to compatible weeds, the applicant states (Part A1, para 35) "*E. repens [common couch grass] will be controlled along with other weeds in and around the trial site using standard farm practices.*" Outcrossing of the GM wheat to couch grass is a possibility so this trial should not be considered until the applicant details what the planned "standard farm practices" involve and how they will be monitored.

For a previous GM wheat trial, ACRE recommended[28] "*as a precautionary measure, couch grass growing within the trial site and in the surrounding 20 m area should be destroyed before it flowers (June – August). ACRE recommends that this area should be monitored for the presence of this weed until mid-October following harvest and in the subsequent 2 years following the final harvest of GM material. It may be appropriate to use mechanical or hand-weeding in addition to the use of herbicides*". We are concerned that the applicant has chosen not to replicate couch grass control measures previously recommended by ACRE or any related monitoring after the end of the proposed trial.

The majority of representations made to ACRE for a previous GM wheat trial (16/R8/02) raised concerns about the possibility of escape of GM wheat, with reference to the US contamination incidents described above (3.1)[29]. In response "*ACRE noted that in the USA the development of GM wheat had progressed to large-scale trials where a large amount of GM wheat seed was used.*" [30] However, as the route of GM wheat contamination in the US is still unknown, it is not possible to conclude that it was only made possible by the scale of the trial. It may be equally likely that GM contamination will happen from a small field trial.

In addition, we reject ACRE's previous side-lining of contamination as "*an economic issue*"[31]. The uncontrolled, and for years unnoticed, escape of unlicensed GM plants into the wild is an issue that encompasses food safety, the protection of wildlife and potential harm to the wider ecosystem. As highlighted above (1), the plants in this trial exhibit unexpected effects yet have not been subject to molecular characterisation. Also, antibiotic resistance is an issue of global significance. Any escape from this trial is of particular concern.

We also reject the suggestion that socio-economic issues have no place in the consideration of applications to deliberately release GMOs. There is growing awareness that a solely safety-based risk assessment for GMOs is too limited in scope[32]. Expanding the scope of the regulation and governance of GMOs beyond purely scientific concerns includes proposals for: respecting ethical, societal and cultural values; recognizing the underlying values and assumptions shaping science and innovation; ensuring the sustainability of agricultural systems and consideration of a range of alternatives to genetically engineered food[33]. We urge the Minister to broaden the remit of this and future deliberate GM release evaluations to include social science evidence, economic analysis, alternatives and societal values.

4 THE PROPOSED TRIAL IS UNNECESSARY AND WILL BE OF NO NET BENEFIT TO SOCIETY

4.1 Genetic engineering is not necessary or suited to obtaining elevated levels of iron in wheat

Whilst a healthy balanced diet is the optimum solution to nutrient deficiencies (see 4.2, below), conventional breeding can also elevate nutrient levels in wheat. Modern varieties of wheat contain low levels of iron (and zinc), with narrow genetic variation for micronutrient concentrations[34]. In contrast many wild species and traditional varieties (landraces) of wheat contain appreciable levels of iron (and other micronutrients)[35]. This suggests that the current low level of iron in much of the wheat consumed is the result of breeding programs that have focussed on other factors, such as yield, at the expense of the quality of the resulting grain. Indeed, the loss of mineral nutrients from wheat appears to be associated with breeding for high yield during the Green Revolution in the last half of the 20th century[36].

The three lines (Part A1, para 14) to be tested in the proposed trial will have 8, 9 and 11 copies of the transgene inserted into the wheat genome. The inclusion of so many copies of the transgenic material is testament to the inefficiency of the technique in manipulating iron content and exemplifies a clumsy approach to tackling dietary needs as well as compounding the potential for adverse unintended effects.

Meanwhile, there is intense research[37] ongoing, using advanced conventional breeding to raise levels of iron and other micronutrients in modern, high yielding varieties of wheat. Hundreds of wild or landrace varieties of wheat have been scanned for genomic markers (regions of DNA) associated with high levels of micronutrients[38]. The identified genomic regions can be incorporated into modern varieties of wheat using methods such as marker assisted selection and genomic selection. These advanced breeding methods rely on conventional breeding processes but track the DNA region of interest to ensure it is present in the offspring[39]. Such advanced conventional breeding methods have already been successful in raising zinc levels in high yielding wheat varieties by 30-40% and there have been promising results for iron[40].

The uptake of micronutrients such as zinc and iron tend to be controlled by multiple genes[41]. Such traits are far more suited to advanced conventional breeding methods, which can introduce several genes at once (via conventional breeding), rather than genetic engineering, which tends to focus more on the overexpression of a single gene (for example, the TaVIT2 gene in this application). In addition, it is possible to target the endosperm (the inner wheat grain used for white flour) using such approaches[42]. Hence, conventional breeding approaches are likely to be far more successful in elevating iron levels in wheat than the GM approach employed in this application, without giving rise to food and environmental safety concerns.

4.2 There is no evidence that the GM plants will improve nutrition in target groups

The applicant states (Part A1, para IV) that "*Overexpression of TaVIT2 under the control of a wheat endosperm-specific promoter increases iron in white flour fractions by greater than 2-fold, in controlled environment grown plants.*" However, as acknowledged in the applicant's published study, the bioavailability of iron in a cooked, final product remains unknown[43]. Furthermore, the 2-fold increase seen in white flour for wheat lines for a single copy translated to a lower increase in iron bioavailability when tested in cell culture assays. Some lines showed no increase in bioavailability. Unfortunately, no data are presented for wheat lines carrying the 8, 9 and 11 copies of the transgenes that would be grown in the proposed trial, so we are unable to begin to estimate iron bioavailability in white flour produced from the proposed lines.

This project focuses on increasing iron in the endosperm but the promotion of white flour to tackle iron deficiency would undermine community understanding and acceptance of core health messages. Rather than creating iron-rich white sliced bread and doughnuts, efforts should focus on continued promotion of the importance of consuming wholegrains as well as research to understand barriers to maintaining a healthy diet. Consuming a varied diet that combines micronutrients with fibre, protein and a healthy balance of complex carbohydrates and fats is key to good health as it not only achieves optimum nutrition but also supports a healthy gut microbiome[44].

It is also important to consider that iron requirements differ between populations[45]. Employing GM crops to address global iron deficiency is arguably a reductionist and simplistic approach to a problem that should be tackled holistically. Addressing micronutrient deficiency by identifying barriers to a culturally relevant healthy diet and gaining a deeper understanding of local needs will bring significantly broader and more long-lasting benefits than introducing a GM biofortified crop.

4.3 The planned biofortification may have directly harmful effects

Biofortification carries significant risks, particularly when applied to a staple foodstuff such as wheat. Toxic elements including cadmium and lead enter plant cells largely via the same pathways that are responsible for the uptake of essential metals such as iron. It is appropriate, therefore, to be concerned about the possibility that biofortification by genetic modification could lead to increased levels of toxic heavy metals and associated adverse health effects. The applicant's study[7] observed a significant increase in cadmium in lines that contain over 20 copies of the transgene. Unfortunately, the study did not provide data on the lines to be grown in the proposed trial carrying 8, 9 and 11 copies.

Even the intended increase in iron uptake could cause harm as excessive intake of iron can lead to adverse health effects including abdominal problems, nausea and intestinal damage. This is a concern for anyone consuming large quantities of wheat-based products but is potentially much more significant for vulnerable groups. For example, NHS guidance for people with haemochromatosis, a hereditary condition where the body absorbs too much iron from the diet, includes avoiding iron-fortified foods such as certain breakfast cereals[46]. This condition has a low diagnosis rate but potentially affects hundreds of thousands of people in the UK who could also suffer dangerous consequences should the use of iron-biofortified wheat become widespread, through either commercial success or contamination and outcrossing.

Without comprehensive data on iron bioavailability in final products (e.g. bread) made from the lines to be trialled, it is impossible to predict whether these plants will address deficiency or lead to excessive intake. Such data should be gathered through contained use studies before a field trial is considered.

The proposed trial represents an unacceptable risk to farmers, wildlife and the wider environment. Any future cultivation of the crops in the proposed trial is unlikely to achieve the desired improvements in nutrition and risks causing significant harm. We request, therefore, that the Minister denies consent and prevents this open-air field trial going ahead.

Yours faithfully

Liz O'Neill
Director
GM Freeze

Dr Ricarda Steinbrecher
Co-Director
Econexus

Dr Helen Wallace
Director
GeneWatch UK

Patrick Holden
Executive Director
Sustainable Food Trust

Roger Kerr
Chief Executive
OF&G

Helen Browning CEO **The Soil Association**	Dr Bruce Pearce Director of Research & Innovation **Organic Research Centre**	James Campbell Chief Executive **Garden Organic**	Dee Butterly Project Development and Outreach Coordinator **Landworkers Alliance**	Scarlett Penn Chief Executive **WWOOF UK**
Helen Woodcock Director **The Kindling Trust**	Juliet and Peter Kindersley Farmers **Sheepdrove Organic Farm**	Oliver Dowding Farmer **Shepton Farms Limited**	Chris Young Coordinator **The Real Bread Campaign**	Kate McEvoy and Ben Gabel Founders **The Real Seed Catalogue**
Cathy Streeter Founder **Banc Hadau Llambed / Lampeter Seed Library**	Debbie Clarke Co-Operative Member **Unicorn Grocery Ltd**	Lee Smith Managing Director **ACE Energy Ltd**	Edward Parker Trust Manager **The Springhead Trust**	Claire Robinson Editor **GMWatch**
Pat Thomas Director **Beyond GM**	Sally Beare Campaigner **Mums Say No to GMOs**	George Moore Spokesperson **GM Free Dorset**	Jane O'Meara Spokesperson **GM Free Somerset**	Brian John Co-founder **GM Free Cymru**
Jim McNulty Co-founder **Genetic Engineering Network**	Gerald Miles Co-founder **Agri Activism UK**	Joanna Clarke Chair **Pro-Natural Food Scotland**	Carole Shorney Secretary **South East Essex Organic Gardeners**	Bryony Haynes Coordinator **Cardiff Friends of the Earth**
Angela Pooley Chair **East Dorset Friends of the Earth**	Pam Rosling Trustee **Sustainable Dorset/Dorset Agenda 21**	Ken Huggins Organiser **Resurgence Dorset**		

References

[1] Mesnage, R., Agapito-Tenfen, S.Z., Vilperte, V., Renney, G., Ward, M., Séralini, G.E., Nodari, R.O. & Antoniou, M.N. 2016. An integrated multi-omics analysis of the NK603 Roundup-tolerant GM maize reveals metabolism disturbances caused by the transformation process. Scientific Reports 19: 37855.

[2] Bollinedi, H., S, G.K., Prabhu, K.V., Singh, N.K., Mishra, S., Khurana, J.P. & Singh, A.K. 2017. Molecular and functional characterization of GR2-R1 event based backcross derived lines of golden rice in the genetic background of a mega rice variety Swarna. PLoS One 12: e0169600.

[3] Jupe, F., Rivkin, A.C., Michael, T.P., Zander, M., Motley, S.T., Sandoval, J.P., Slotkin, R.K., Chen, H., Castanon, R., Nery, J.R. & Ecker, J.R. 2019. The complex architecture and epigenomic impact of plant T-DNA insertions. PLoS Genetics 15: e1007819..

[4] Ho, M.W., Ryan, A. & Cummins, J. 1999. Cauliflower mosaic viral promoter – a recipe for disaster? Microbial Health and Disease. 11: 194–197.

[5] EFSA 2009. Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMO-NL-2005-22 and EFSA-GMO-RX-NK603) for the placing on the market of the genetically modified glyphosate tolerant maize NK603 for cultivation, food and feed uses and import and processing, and for renewal of the authorisation of maize NK603 as existing product. The EFSA Journal 1137: 1-50.

[6] Ho MW. 2008. Transgenic lines unstable hence illegal and ineligible for protection. Science in Society 38: 28-29.

[7] GM Freeze et al. 2016. Defra: Multi-agency response to wheat trial 16/R8/02. https://www.gmfreeze.org/wp-content/uploads/2017/04/Multi_agency_response_to_GM_wheat_trial_application_16_R8_02.pdf

[8] Chen, J., Jin, M., Qiu, Z-G., Guo, C., Chen, Z-L., Shen, Z-Q., Wang, X-W. & Li, J-W. 2012. A survey of drug resistance *bla* genes originating from synthetic plasmid vectors in six Chinese rivers. Environmental Science and Technology 46: 13448-13454.

[9] GM Freeze et al. 2016. Defra: Multi-agency response to wheat trial 16/R8/02. https://www.gmfreeze.org/wp-content/uploads/2017/04/Multi_agency_response_to_GM_wheat_trial_application_16_R8_02.pdf

[10] WHO 2015. 19th WHO Model List of Essential Medicines. Annex 1. http://www.who.int/medicines/publications/essentialmedicines/EML2015_8-May-15.pdf?ua=1 (also listed in the updated WHO 2017. 20th WHO Model List of Essential Medicines. https://www.who.int/medicines/publications/essentialmedicines/20th_EML2017.pdf?ua=1)

[11] WHO 2019. World antibiotic awareness week. https://www.who.int/campaigns/world-antibiotic-awareness-week

[12] See, e.g. Drugs.com 2019. Hygromix 8. https://www.drugs.com/vet/hygromix-8.html

[13] Golden Rice Project 2018. Why was an antibiotic resistance marker (Hygromycin) used? FAQ. http://www.goldenrice.org/Content3-Why/why3_FAQ.php#Antibiotic_R; Baisakh, N., Rehana, S., Rai, M., Oliva, N., Tan, J., Mackill, D.J., Khush, G.S., Datta, K. & Datta, S.K. 2006. Marker-free transgenic (MFT) near-isogenic introgression lines (NIILs) of 'golden' indica rice (cv. IR64) with accumulation of provitamin A in the endosperm tissue. Plant Biotechnology Journal 4: 467-475.

[14] ACRE 2017. ACRE advice on application 16/R8/02 for deliberate release of a GMO for research and development purposes. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/588947/acre-advice-gm-wheat-16r802.pdf

[15] See, e.g. EMA 2018. Antimicrobial resistance. https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/antimicrobial-resistance

[16] UK Department for Environment, Food & Rural Affairs, Department of Health and Social Care, Public Health England, and Veterinary Medicines Directorate 2019. Antimicrobial resistance (AMR). Policy papers 24th January https://www.gov.uk/government/collections/antimicrobial-resistance-amr-information-and-resources

[17] UK Department of Health and Social Care 2019. Tackling antimicrobial resistance 2019–2024 The UK's five-year national action plan https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/773130/uk-amr-5-year-national-action-plan.pdf

[18] GM Freeze et al. 2016. Defra: Multi-agency response to wheat trial 16/R8/02. https://www.gmfreeze.org/wp-content/uploads/2017/04/Multi_agency_response_to_GM_wheat_trial_application_16_R8_02.pdf

[19] USDA APHIS 2014a. USDA announces close and findings of investigation into the detection of genetically engineered wheat in Oregon in 2013. https://www.aphis.usda.gov/newsroom/2014/09/pdf/ge_wheat.pdf
USDA APHIS 2014b. Questions and answers: genetically engineered wheat investigation. September 2014. https://www.aphis.usda.gov/publications/biotechnology/2014/faq_ge_wheat.pdf

[20] USDA APHIS 2014a. USDA announces close and findings of investigation into the detection of genetically engineered wheat in Oregon in 2013. https://www.aphis.usda.gov/newsroom/2014/09/pdf/ge_wheat.pdf
USDA APHIS 2014b. Questions and answers: genetically engineered wheat investigation. September 2014. https://www.aphis.usda.gov/publications/biotechnology/2014/faq_ge_wheat.pdf

[21] USDA APHIS 2016. December 1, 2016 Update regarding detection of GE wheat volunteer plants in Washington state (incorporating August 5, 2016 and July 29, 2016 Statements on detection of GE wheat volunteer plants in Washington state) https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/brs-news-and-information/2016_brs_news/wheat_fact_finding_closed

[22] Fox, J.L. 2013. Volunteer GM wheat, mischief or carelessness? Nature Biotechnology 31: 669-670.

[23] USDA APHIS 2014a. USDA announces close and findings of investigation into the detection of genetically engineered wheat in Oregon in 2013. https://www.aphis.usda.gov/newsroom/2014/09/pdf/ge_wheat.pdf
USDA APHIS 2014b. Questions and answers: genetically engineered wheat investigation. September 2014. https://www.aphis.usda.gov/publications/biotechnology/2014/faq_ge_wheat.pdf

[24] USDA APHIS 2016. December 1, 2016 Update regarding detection of GE wheat volunteer plants in Washington state (incorporating August 5, 2016 and July 29, 2016 Statements on detection of GE wheat volunteer plants in Washington state) https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/brs-news-and-information/2016_brs_news/wheat_fact_finding_closed

[25] Fox, J.L. 2013. Volunteer GM wheat, mischief or carelessness? Nature Biotechnology 31: 669-670.

[26] http://www.monsanto.com/gmwheat/pages/gm-wheat-history.aspx

[27] ACRE 2017. ACRE advice on application 16/R8/02 for deliberate release of a GMO for research and development purposes.

https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/588947/acre-advice-gm-wheat-16r802.pdf
[28] ACRE 2017. ACRE advice on application 16/R8/02 for deliberate release of a GMO for research and development purposes.
https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/588947/acre-advice-gm-wheat-16r802.pdf
[29] ACRE 2017. ACRE advice on application 16/R8/02 for deliberate release of a GMO for research and development purposes.
https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/588947/acre-advice-gm-wheat-16r802.pdf
[30] ACRE 2017. ACRE advice on application 16/R8/02 for deliberate release of a GMO for research and development purposes.
https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/588947/acre-advice-gm-wheat-16r802.pdf
[31] ACRE 2017. ACRE advice on application 16/R8/02 for deliberate release of a GMO for research and development purposes.
https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/588947/acre-advice-gm-wheat-16r802.pdf
[32] Jasanoff, S. & Hurlbut, B.J. 2018. A global observatory for gene editing. Nature 555: 435-437; Jordan, N.R., Dorn, K.M., Smith, T.M., Wolf, K.E., Ewing, P.M., Fernandez, A.L., Runck, B.C., Williams, A., Lu, Y. & Kuzma J. 2017. A cooperative governance network for crop genome editing. EMBO Reports 18: 1683-1687; Helliwell, R., Hartley, S., Pearce, W. & O'Neill, L. 2017. Why are NGOs sceptical of genome editing? EMBO Reports 18: 2090-2093; Hartley, S., Gillund, F., van Hove, L. & Wickson, F. 2016. Essential features of responsible governance of agricultural biotechnology. PLoS Biology 14: e1002453; Sarewitz, D. 2015. Science can't solve it. Nature 522: 412–413.
[33] Jordan, N.R., Dorn, K.M., Smith, T.M., Wolf, K.E., Ewing, P.M., Fernandez, A.L., Runck, B.C., Williams, A., Lu, Y. & Kuzma J. 2017. A cooperative governance network for crop genome editing. EMBO Reports 18: 1683-1687; Hartley, S., Gillund, F., van Hove, L. & Wickson, F. 2016. Essential features of responsible governance of agricultural biotechnology. PLoS Biology 14: e1002453.
[34] Velu, G., Tutus, Y, Gomez-Becerra, H.F. et al. 2017. QTL mapping for grain zinc and iron concentrations and zinc efficiency in a tetraploid and hexaploid wheat mapping populations. Plant and Soil 411: 81–99.
[35] Manickavelu, A., Hattori, T., Yamaoka, S., Yoshimura, K., Kondou, Y., Onogi, A., Matsui, M., Iwata, H. & Ban, T. 2017. Genetic nature of elemental contents in wheat grains and its genomic prediction: toward the effective use of wheat landraces from Afghanistan. PLoS ONE 12: e0169416.; Velu, G., Crossa, J., Singh, R.P et al. 2016. Genomic prediction for grain zinc and iron concentrations in spring wheat. Theoretical and Applied Genetics 129: 1595–1605; Kumar, U., Mathpal, P., Malik, S. & Kumar, N. 2016. Evaluation of iron and zinc in grain and grain fractions of hexaploid wheat and its related species for possible utilization in wheat biofortification. Plant Genetic Resources 14: 101-111.
[36] Fan, M.S., Zhao, F.J., Fairweather-Tait, S.J., Poulton, P.R., Dunham, S.J. & McGrath, S.P. 2008. Evidence of decreasing mineral density in wheat grain over the last 160 years. Journal of Trace Elements in Medicine and Biology 22: 315–324.
[37] See, e.g. Velu, G., Singh, R.P. & Crespo-Herrera, L. 2018. Genetic dissection of grain zinc concentration in spring wheat for mainstreaming biofortification in CIMMYT wheat breeding. Nature Scientific Reports 8: 13526.; Velu, G., Tutus, Y, Gomez-Becerra, H.F. et al. 2017. QTL mapping for grain zinc and iron concentrations and zinc efficiency in a tetraploid and hexaploid wheat mapping populations. Plant and Soil 411: 81–99; Velu, G., Crossa, J., Singh, R.P et al. 2016. Genomic prediction for grain zinc and iron concentrations in spring wheat. Theoretical and Applied Genetics 129: 1595–1605.
[38] Manickavelu, A., Hattori, T., Yamaoka, S., Yoshimura, K., Kondou, Y., Onogi, A., Matsui, M., Iwata, H. & Ban, T. 2017. Genetic nature of elemental contents in wheat grains and its genomic prediction: toward the effective use of wheat landraces from Afghanistan. PLoS ONE 12: e0169416.; Velu, G., Crossa, J., Singh, R.P et al. 2016. Genomic prediction for grain zinc and iron concentrations in spring wheat. Theoretical and Applied Genetics 129: 1595–1605.
[39] See, e.g. Bevan, M.W., Uauy, C., Wulff, B.B.H., Zhou, J., Krasileva, K. & Clark, M.D. 2017. Genomic innovation for crop improvement. Nature 543: 346–354.
[40] Velu, G., Singh, R.P., Crespo-Herrera, L. 2018. Genetic dissection of grain zinc concentration in spring wheat for mainstreaming biofortification in CIMMYT wheat breeding. Nature Scientific Reports 8: 13526.
[41] Velu, G., Singh, R.P., Crespo-Herrera, L. 2018. Genetic dissection of grain zinc concentration in spring wheat for mainstreaming biofortification in CIMMYT wheat breeding. Nature Scientific Reports 8: 13526.z
[42] Kumar, U., Mathpal, P., Malik, S. & Kumar, N. 2016. Evaluation of iron and zinc in grain and grain fractions of hexaploid wheat and its related species for possible utilization in wheat biofortification. Plant Genetic Resources 14: 101-111.
[43] Connorton, J.M., Jones, E.R., Rodríguez-Ramiro, I., Fairweather-Tait, S., Uauy, C. & Balk, J. 2017. Wheat vacuolar iron transporter TaVIT2 transports Fe and Mn and is effective for biofortification. Plant Physiology 174: 2434-2444.

[44] For reviews, see Valdes, A.M., Walter, J., Segal, E. & Spector, T.D. 2018. Role of the gut microbiota in nutrition and health. The BMJ 361: k2179; Kau, A.L., Ahern, P.P., Griffin, N.W., Goodman, A.L. & Gordon, J.I. 2011. Human nutrition, the gut microbiome and the immune system. Nature 474: 327–336.
[45] Schümann, K., Ettle, T., Szegner, B., Elsenhans, B. & Solomons, N.W. 2007. On risks and benefits of iron supplementation recommendations for iron intake revisited. Journal of Trace Elements in Medicine and Biology 21: 147-68.
[46] NHS 2016. Treatment. Haemachromatosis. https://www.nhs.uk/conditions/haemochromatosis/treatment/

GM Freeze, Open Space Cooperative, 41 Old Birley Street, Hulme, Manchester M15 5RF
Tel: 0845 217 8992 **Email:** liz@gmfreeze.org **Web:** www.gmfreeze.org **Twitter:** @GMFreeze