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To the
GM Team
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Sent by email to gm-regulation@defra.gsi.gov.uk

August 2011

Dear Madam/Sir,

Re: Application for Consent to release a GMO – Reference number 11/R8/01

Concerning: APPLICATION FROM ROTHAMSTED RESEARCH FOR CONSENT TO RELEASE GENETICALLY MODIFIED WHEAT

Having studied the Part A and Part B documentation provided by DEFRA - of the application for the environmental release of GM wheat genetically engineered to repel aphids by constitutively producing aphid alarm hormone (*E*)-β-farnesene (EBF) - I have come to the conclusion that the data, information and hypothesis provided does not warrant an open environmental release.

Whilst the two GM wheat lines (Event 2803R6P1 and Event 2812R9P1) may be adequately or sufficiently characterised, analysed or designed for secure indoor greenhouse trials, this is not the case for an open release.

More greenhouse trials, data and analysis should be required before testing in the open environment (where risks as well as variables are always higher.

I thus wish to register my objection to the field trial (environmental release) planned for 2012 and 2013 and wish to provide the regulator with my points of concern, based on my knowledge and experience as a biologist and geneticist and on my expertise in risk assessment of GMOs, due to which I currently serve as a member of the ad hoc technical expert group (AHTEG) on risk assessment and risk management of LMOs under the Cartagena Protocol on Biosafety.

Main points of concerns are:

1) Underlying hypothesis not developed or robust enough and also disputed by recent findings, as to scientifically require or warrant an outdoor step (which introduces more variables and the risks).
2) Lack in molecular and phenotypic characterisation.
3) Presence of antibiotic resistance marker genes.
4) Lack of proper safety assessment concerning human and animal health - as risks cannot be estimated without data, and no guarantee can be given that no escape or mix-up by any route will take place.
5) Could the use of the GM wheat increase rather than reduce pesticide applications?
6) Risk assessment provided does not cover all potential hazards (step 1) and fails in a number of cases/points to fully elaborate steps 2 and 3, thus arriving at an underestimation of the overall risks (here step 6).
7) Lack of uncertainty analysis throughout the document, including the risk assessment.

Some of the points are further expanded below.

(1) Shortcomings of the underlying hypothesis

The idea stated behind the genetic modification and the requested field trials is to develop and test a 'novel resistance to aphids', based on the idea to mimic the alarm signal of aphids and elicit the predator-avoidance reaction of aphids, by having wheat produce the aphid alarm pheromone (*E*)-β-farnesene (EBF).

According to the hypothesis (and some initial indoor experiments the data to which is not available to me), the aphids will be repelled by the EBF and thus stay away from or largely avoid the GM wheat. Thus wheat engineered to constitutively produce EBF all the time in all the cells would no longer require spraying with chemical pesticides against aphids.

Prior to genetically modifying wheat with the EBF synthase gene, the applicant modified thale cress (*Arabidopsis thaliana*) with the EBFS gene (derived from peppermint – see point (2) below). They kindly provided other research teams with this GM *A. thaliana*, who used it in experiments with the green peach aphid (*Myzus persicae*), which are known to produce and respond to EBF.

Both these research teams **found that the plant-produced EBF was not providing the protection from aphids as intended, postulated or hoped for**.

De Vos et al. (2010) found that exposure to EBF led to habituation within only three generations – with plant-based production of EBF thus not offering an agricultural benefit. Whilst the presence of EBF is known to attract some predators (eg coccinellid beetles), "*some coccinellids, including H. convergens, and parasitoid wasps use EBF as a kairomone for locating aphid prey (5, 22, 38–41), and might be less effective hunting in a field of EBF-producing plants. It is unknown whether parasitoids and predators become habituated to EBF upon constant stimulation, or whether they would alter their behavior toward EBF as a reliable kairomone if no suitable host/prey is found*."

Kunert et al. (2010) stated "no evidence was found for the ability of EBF to directly defend the plant against aphids. EBF emission did not significantly repel winged or wingless morphs from settling on plants. Nor did EBF reduce aphid performance, measured as reproduction, or lead to an increase in the proportion of winged offspring."

Whilst aphids emit EBF alarm pheromone in a pulsed fashion, the EBF produced by plants will be present constantly due to the constitutive expression of the EBFS gene. Kunert et al. postulates that "*aphids react to EBF only if it is emitted in pulses, which would mimic the*

release caused by attack on individual members of an aphid colony. This could explain why the green peach aphid reacted to Solanum berthaultii [potato] where the EBF was only released as individual EBF-containing trichomes were destroyed [Avé et al., 1987]. The mode of EBF release, whether pulsed or continuous, might therefore be an important cue in informing aphids whether the EBF is coming from attacked conspecifics (so it is necessary to take evasive action) or from a plant (so there is no immediate predation risk)."

Additional open questions:

It is not clear whether predators will become habituated to EBF in the constant present of this pheromone.

It is not known if and when there is a metabolic cost associated with EBF production in GM plants. Kunert et al. could not find evidence for this for the particular thale cress lines, but state: "The lack of metabolic costs may simply be due to the low rate of production. The amount emitted in 24 hours corresponds to less than 0.1 ‰ of the fresh weight of the above ground biomass. However, metabolic costs of EBF emission might conceivably be observed under other stress conditions, such as greater nutrient limitation, low light, drought or various biotic stresses."

Concluding:

The underlying hypothesis is neither developed enough nor supported enough by data to justify environmental releases. Findings as well as number of open questions strongly suggest to revise the hypothesis as well as to undertake long-term indoor greenhouse trials, where variables can be minimised and assumptions safely tested.

(2) Lack in molecular and phenotypic characterisation.

Data, information and risk assessment deliberation is missing on a number of levels concerning molecular and phenotypic characterisation of the two GM wheat events:

a) Absence of data concerning transformation induced mutations.

Transformation procedures such as micro-projectile bombardment (ie particle bombardment) and tissue culture – both of which were used in the development of the two GM wheat events – are known to cause a large number of mutations (Wilson et al. 2006). These can be

- genome-wide mutations as well as
- insertion-site mutations, affecting the flanking region, the insert itself and potentially the gene the transgene has inserted into (if the latter is the case).

Any such mutations can lead to unintended and/or unpredicted effects and consequences and their assessment should be part to any risk assessment of GMOs intended for environmental release, whether for field trials or commercial release.

The applicants clearly state in Part A1/IV, that none of the above assessments have been performed:

> "*We have not analysed the position or the structure of the insertion nor sequenced the flanking genomic DNA. Apart from the expected phenotype of EBF emission, these plants are indistinguishable from untransformed controls. No other changes to the plant morphology or development are apparent."*

The assertion that the GM plants are "indistinguishable" from the non-GM parental lines is not based on any experimental or analytical data or information obtained, but seems to be rather an assumption and general opinion. This will need to be addressed.

Concluding a:

The applicant's have not analysed or determined the:

- Position and/or structure of the insertion
- Sequence and identity of the flanking genomic DNA
- Compositional analysis to test for unintended and unpredicted changes and deliberation and assessment of potential negative effects.

These should be the minimal requirements of information & data needed and made available for any environmental release, as they are basic to any environmental risk assessment (as well as to human and animal health risk assessment). Risk assessment is intended to protect the environment as well as human and animal health from negative impacts and potential harm. Without data, such assessment becomes guesswork, which cannot provide the degree of certainty required for decision making. Lack of relevant data requires the application of the precautionary principle or precautionary approach until such data is made available and assessed.

b) Lack of sequence data and identity of inserted genes labelled 'synthetic'.

It has been common practice for some time now to synthesise (or have synthesised) parts of genes according to specifications and to fuse them together to a whole gene. This has not made the gene nor the synthesised component a 'synthetic' gene as their sequences were either directly taken from or based in their sequence on an actual gene known to the researcher. Even if the whole gene has been synthesised in one go or assembled from different synthesised pieces, it will be done so on the basis of a sequence (or sequences) known to occur in nature. Even if the sequence has been altered to fit the new host or the changed conditions, it has been and is commonly named according to its origin. Synthesising a gene does not make it a synthetic gene (unless perhaps it has been completely invented – though the definition of 'synthetic gene' has not been made yet and would at present mean different things to different people).

Consequently, it is unusual to simply label the two main genes used as "synthetic" and state elsewhere what they most closely resemble (ie peppermint and cow). This procedure in fact deems to be little helpful and misleading.

- In the B2 part of the current application it is stated: "*The two new genes are synthetic i.e. they were not taken from another organism but chemically synthesized to function like wheat genes*."

This statement is not in line with:

- information given by Rothamsted Research to the BBSRC for their grant (Ref BB/G004781/1) entitled 'A new generation of insect resistant GM crops: transgenic wheat synthesizing the aphid alarm signal'. In this the authors state: "*we have isolated the gene responsible for the production of pure aphid alarm pheromone in peppermint plants. By inserting this gene into other plants we can make them produce the pheromone and we have recently performed this transformation with a simple plant called thale cress […] … we now need to carry out a similar transformation with wheat ..*"

Comment:

There are scientific reasons as well as patent right (IPRs) and ethical/public issues for asking an applicant to clearly indicate the origin of the gene sequences used and often to provide the sequence itself. Such information is for example relevant concerning homologies (with

potential for gene silencing and horizontal gene transfer) and viral insertion sites (eg common insertion sites (CISs), to mention two.

Open questions:

In which ways does the EBFS gene used differ from the original peppermint sequence, and in which ways does the 'cow' gene (presumably the FPPS gene) differ from the original cow sequence?

Why are the genes called 'synthetic' and what does that entail?

Have relevant aspects such as homologies to host genes and to genes of potential consuming species, or sequences of common viral insertion sites been investigated? And if so to which results?

What other aspects are important in this context?

(3) Presence of antibiotic resistance marker genes.

Developments in molecular biotechnology techniques are advanced enough to either remove any **antibiotic resistance marker** gene prior to any release or not to use it in the first place. A release of plants containing such marker genes should no longer be regarded as acceptable, esp. with antibiotics such as kanamycin becoming more and more crucial again in the fight against serious bacterial pathogens resistant to other antibiotics (as acknowledged in the application).

As the nptI gene has been used in the wheat plants, their release into the environment should not take place until the nptI genes have been removed.

The fact that the nptI gene can also be found in the natural environment is no excuse for its presence in the GM wheat, as every step needs to be undertaken to prevent further spread of the gene.

(4) Lack of proper safety assessment concerning human and animal health

Risks cannot be estimated or assessed without data. As potential escape by any route cannot be ruled out (and has taken place at numerous occasions for other GMOs and their propagules in different countries), no release into the environment should take place without data and their analysis re human and animal health.

Risks may and can arise from:

- the gene products themselves, from
- unintended and unpredicted effects due to transformation-induced mutations (see point 2a),
- or due to the interaction of the inserted DNA sequences (including promoters) with the host plants own genes
- or due to interactions and interference with the plants metabolic pathways.

It is known for example, that the constitutive production of EBF will deplete the FPP pool, and will likely deplete the supply of sterols and other isoprenoid metabolites. Kunert et al (2010) for example state:

> "*Additionally, the diversion of farnesyl diphosphate (FPP) to EBF synthesis may directly reduce the supply of sterols (a major group of membrane components) and other isoprenoid metabolites produced from FPP. Beale et al. [29] noted that, when flowering, EBF producing A. thaliana plants emit lower amounts of other sesquiterpenes than wild-type plants.*

> *The transgenic, EBF-producing A. thaliana lines expressed the EBF synthase gene under the control of a constitutive promoter. Hence the enzyme should be present in almost every cell and produce EBF whenever and wherever FPP is available. From this perspective, the level of EBF produced is an indicator of the size of the FPP pools. Given the increase in emission during the day as compared to the night period (additional file 3), these pools appear to be larger during the light phase, possibly due to the action of photosynthesis. Of the two basic isoprenoid pathways operating in plant cells, the MEP pathway is strongly stimulated by light [38]. A direct relationship between the rate of photosynthesis and the rate of EBF formation is consistent with the trend observed for greater emission from larger plants (Figure 1), which presumably have more active photosynthetic leaf area. Fertilization also promoted EBF formation (Figure 3), probably by increasing plant size (Figure 4)*." (Kunert et al., 2010)

Furthermore, Beale et al. (2006) state:

> "*the production of Eβf [EBF] appears to be at the expense of other sesquiterpenes, presumably by competing for the common substrate, farnesyl diphosphate*." (Beale et al., 2006)

Open questions:

- Could a compositional comparison between the two events show up differences in the presence of metabolites linked to the EBS (and FPP) pathways?
- Has a compositional analysis been performed to assess the changes in nutritional value of the plants?
- What other metabolic consequences (elsewhere in the biochemical metabolic pathways) or behavioural or fitness consequences could arise due to the constitutive production of EBS and FPP?

Concluding:

Giving approval to the environmental release of the GM wheat without any health relevant data would not be in line with the precautionary approach and would unduly place risk to health.

(5) Could the use of the GM wheat increase rather than reduce pesticide applications?

In addition to issues raised under point (1), questions arise as to the behaviour of pests as well as predators (and pest pathogens).

Open questions:

- Will the presence of EBF in soil (due to emission by the roots of the GM wheat) attract pests or pathogens that will injure or feed on the roots of the GM wheat? Such combined pest attacks above and below ground have frequently be observed.
- Will the population of predators in an area be reduced due to predators being attracted to the GM plant due to the presence of EBF, yet without any prey present to feed on or without being able to locate the prey due to the overall presence of EBF? How will this effect the survival of predators? And how will this (as well as the constant EBF presence in wheat fields) affect the predator density in the surrounding area and ecosystems, will there be a reduction – and to which consequences?
- If aphids get habituated and not sufficient predators are available, may this increase the aphid burden on the wheat and thus potentially increase the need for pesticides?

There are many more open questions that need addressing as to prevent negative consequences to the farmer as well as to the environment.

In Conclusion:

I have wanted to highlight and list a few points that elucidate the shortcomings of the data provided and the risk assessment carried out and provided. These, in my view, give evidence to the necessity to do further indoor trials, reassess the hypothesis and test for health consequences before any open environmental release should or could take place.

It should further be deliberated whether the approach taken to address the aphid problem does not in itself cause new problems.

I can supply further points of concern, further scientific literature or provide further detail to points 6 & 7 if so required.

I kindly thank you for the opportunity to comment on the application by Rothamsted Research and wish to convey my objection to the environmental release.

With kind regards,

Dr. Ricarda A Steinbrecher

References:

Avé DA, Gregory P, Tingey WM (1987). Aphid repellent sesquiterpenes in glandular trichomes of *Solanum berthaultii* and *S. tuberosum*. *Ent Exp & Appl* 44(2):131-138. doi: 10.1111/j.1570-7458.1987.tb01057.x

de Vos M, Cheng WY, Summers HE, Raguso RA and Jandera G (2010). Alarm pheromone habituation in Myzus persicae has fitness consequences and causes extensive gene expression changes. *PNAS* 107(33): 14673-14678. See www.pnas.org/content/107/33/14673.full.pdf

Kunert G, Reinhold C, and Gershenzon J (2010). Constitutive emission of the aphid alarm pheromone, (E)-β-farnesene, from plants does not serve as a direct defense against aphids. *BMC Ecology* 10:23. See http://www.biomedcentral.com/1472-6785/10/23

Wilson AK, Latham JR and Steinbrecher RA (2006). Transformation-induced Mutations in Transgenic Plants - Analysis and Biosafety Implications. *Biotechnol Genet Eng Rev* 23: 209-234. See http://www.econexus.info/sites/econexus/files/ENx-BGER_vol23_2006.pdf