



## ADVISORY COMMITTEE ON RELEASES TO THE ENVIRONMENT

### *Advice on the implications of a Defra-funded desk study: 'RNA-mediated gene silencing mechanisms and their implications for the risk assessment of GM plants'.*

January, 2007

#### 1. Background:

Within the past decade there has been a significant amount of research directed at understanding the mechanisms by which double-stranded RNA<sup>1</sup> in plants and other organisms (including humans) can alter gene expression. RNA-mediated gene silencing interrupts the transfer of information that is encoded in DNA. It therefore affects gene expression but it also has other functions such as providing a defence mechanism against viral infection.

As double-stranded RNA associated with plant transformation events has the potential to alter the expression of endogenous plant genes (unintentionally as well as by design) and of transgenes, Defra has commissioned a desk study to review the current understanding of RNA-mediated gene silencing mechanisms in plants and the implications that these may have for the risk assessment of GM plants. ACRE has been asked for advice on any environmental hazards identified in the report and the recommendations it makes for further research.

Professor David Baulcombe's group in the Sainsbury Laboratory, Norwich, was commissioned to carry out the desk study. The report of the study<sup>2</sup> concentrates on GM plants in which genes have been silenced intentionally (in particular those containing inserts that generate double stranded RNA directly as opposed to those modified to contain sense and antisense DNA constructs). Unintentional RNA-mediated silencing also can be triggered in these GMOs as it can in GM plants that have been modified to produce a novel protein. It is noteworthy that silencing can, and has, occurred as a result of non-GM breeding e.g. in mutagenesis breeding where the loss of a termination sequence resulted in RNA-mediated silencing<sup>3</sup>.

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<sup>1</sup> Like DNA, RNA is a nucleic acid (although it has some chemical differences) but unlike DNA, it can move outside of the nucleus into a cell's cytoplasm. There are many forms of RNA, including messenger RNA, transfer RNA, ribosomal RNA. All of these RNA species are single-stranded and involved in protein synthesis. This report concerns double-stranded RNA that alters gene expression through targeting short stretches of RNA or DNA with which it shares a high degree of sequence homology. The target RNA is either degraded, prevented from being translated into protein (both of which represent post-transcriptional gene silencing) or its transcription from DNA is interfered with (transcriptional gene silencing).

<sup>2</sup> Published at

[http://www2.defra.gov.uk/research/project\\_data/More.asp?l=CB02041&M=KWS&V=cb02041&SCOPE=0](http://www2.defra.gov.uk/research/project_data/More.asp?l=CB02041&M=KWS&V=cb02041&SCOPE=0).

<sup>3</sup> Kusaba M., Miyahara K., Lida S., Fukuoka H., Takano T., Sassa H., Nishimura M. *et al.* (2003). *Low glutelin content1*: a dominant mutation that suppresses the Glutelin multigene family via RNA silencing in rice. *Plant Cell* 15: 1455-1467.

Both post-transcriptional gene silencing (PTGS) and transcriptional gene silencing (TGS) are discussed in this report. It also suggests best practice in designing GM plants for gene silencing purposes to avoid instability in transgene expression and to minimise 'off-target effects'.

## **2. Advice**

ACRE considers the report to be well written and comprehensive and notes the eminence of Professor Baulcombe's group in this area of research. We concur with the report's finding that there are gaps in understanding with respect to RNA-mediated gene silencing in plants, but we consider that these gaps are not significant in the environmental risk assessment of GM plants. Consequently we do not recommend that Defra fund further work in this area (with respect to the environmental risk assessment of GM plants).

In coming to this conclusion, we have considered the environmental hazards associated with RNA-mediated gene silencing in GM crops that have been identified in this report:

**3.1 Instability/ unpredictability of a GM trait<sup>4</sup> as a result of silencing** such that there is loss/reduction in silencing, variability between genetically identical siblings or variability between generations. As part of ACRE's assessment of GMOs for which consent to market is being sought, we consider information on genotypic<sup>5</sup> and phenotypic<sup>6</sup> stability. For most GM plants, the loss of the GM trait through silencing would have an economic, rather than environmental impact e.g. loss of herbicide tolerance or loss of pest resistance (i.e. they would revert to having the same phenotype as their non-GM counterparts). However, the partial or complete loss of other GM traits could affect the environmental risks posed by the GMO e.g. if it were associated with biological containment. Consequently, the risks depend on the particular GMO in question and as such, we consider that our current case-by-case approach to environmental risk assessment is appropriate. It is also worth noting that phenotypic instability (whatever the cause) would almost certainly be detected during the breeding programme at which point, lines exhibiting this characteristic would be discarded.

**3.2 Unintentional blocking of the translation of endogenous plant genes.** Notifications for the release of GM crops must consider whether these organisms present an increased environmental risk as compared to their non-GM counterparts. As such, we assess information on whether there are differences in a range of characteristics that could conceivably alter the environmental consequences of cultivating the GMO rather than a non-GM alternative e.g. modes and/ or rate of reproduction, dissemination and survivability. We consider this to be the most effective approach in determining whether any consequence of a GM event (including gene silencing) has altered the phenotype of the plant such that it poses an unexpected environmental risk to the environment. Again, we consider that

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<sup>4</sup> Including GM traits that are the result of intentional silencing of endogenous plant genes.

<sup>5</sup> The genetic constitution of an organism

<sup>6</sup> The physical characteristics of an organism

abnormalities in the characteristics of a GM crop line (e.g. as a result of gene silencing) would be detectable during its breeding programme.

**3.3 Transfer of silencing from GM plants.** As PTGS is a highly conserved mechanism in plants and animals, we have considered whether plant-generated short interfering (si)RNAs could trigger silencing in animals that feed on plant material. This is not a hazard to humans as in order for silencing to work, a concentrated extract containing RNA from the GM plant would have to be applied to, and be taken up by, human cells or cell cultures. However, the report suggests that there is potential for siRNAs from GM plants to transfer to nematodes that feed on them, and the nematodes could potentially act as vectors of the siRNAs to other plants.

Recently, Huang *et al.*, (2006)<sup>7</sup> reported that they had modified *Arabidopsis* plants to express siRNAs targeted against a parasitism gene present in root-knot nematodes (RKN). These siRNAs, when transferred from the GM plants to four common species of RKN, resulted in silencing of the parasitism gene and reduced nematode infectivity. This demonstrates that siRNAs can be transferred from plants (GM and non-GM) to nematodes. However, for silencing to occur, the genes encoding these siRNAs must be present in the nematode as well in the plant. Consequently, it is unlikely that silencing will occur other than by design (as in this case). It is also worth noting that the nematodes will not become genetically modified as their genome will not have been altered. Consequently, the effects of the transferred siRNAs will be transient. Bacteria do not employ the same gene silencing mechanism as plants and as such they are highly unlikely to be affected by the transfer of siRNAs from crops.

**3.4 Viruses acquiring virulence by evolving to evade gene silencing.** The possibility was considered of the emergence of novel viruses and/or the build up of a reservoir of virus in a supposedly resistant crop. However, gene silencing is a strategy that plants (both non-GM and GM) employ to resist virus infections and that viruses, in their turn, may evolve to avoid this resistance mechanism. Consequently, we do not consider that the potential for viruses to mutate and overcome silencing acquired through GM is a novel risk to the environment. We also note that if a virus did mutate to overcome gene silencing (that had been introduced by GM), then the GM plant would return to its wild type phenotype i.e. sensitive to infection by that particular virus. This would have economic consequences but the environmental impact would be no greater than those associated with growing its non-GM (virus-sensitive) counterpart.

**3.5 Saturation of the silencing machinery in GM plants.** As yet, such saturation effects in plants have not been researched in any detail. However, as this report points out, we do know that the silencing mechanism can become saturated by high levels of double-stranded RNAs, causing the silencing to fail. As such, certain GM constructs and events could result in a loss of endogenous silencing in the modified plant. Most of the consequences of these saturation effects would impact on the economic value of the GM crop (rather than on its environmental safety). However, the report suggests that there are associated hazards such as increased

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<sup>7</sup> Huang G., Allen R., Davis E. L., Baum T. J. and Hussey R. S. (2006). Engineering broad root-knot resistance in transgenic plants by RNAi silencing of a conserved and essential root-knot nematode parasitism gene. *PNAS* **103** (39): 14302–14306.

mutation rates (due to increased transposon<sup>8</sup> activity) and changes in metabolite composition (as a result of defects in micro (mi)RNA-mediated gene regulation). Analysing compositional data submitted in notifications under GM Food and Feed legislation (i.e. Regulation (EC)1829/2003) is not our responsibility. However, as described above, we do consider information on whether there are phenotypic differences between a GM plant and its non-GM counterpart that would alter its impact on the environment. Almost certainly, GM plant lines with increased mutation rates would be detected during the breeding programme and these would be discarded. A plant line with increased mutation rates would not be economically viable (not least because it would fail Distinctness, Uniformity and Stability tests that are required to market a new crop variety, irrespective of whether it is GM or not). We also note that the report makes suggestions on how saturation might be avoided.

### **3.6 Activation of genes by double-stranded RNA**

A recent paper by Li *et al.* (2006)<sup>9</sup> reports that, as well as silencing, double-stranded RNA can directly activate the expression of target genes (at least in human cells). This research is controversial as some argue that it is possible that activation of these genes is an indirect result of silencing of regulatory genes (that would otherwise suppress the expression of the target genes). We will continue to monitor this line of research with interest.

If this phenomenon does occur in plants, irrespective of the underlying mechanism, the implications for environmental risk assessment are the same. As discussed in paragraph 3.2, we assess information on whether there are differences in a range of characteristics that could conceivably alter the environmental consequences of cultivating the GMO rather than a non-GM alternative e.g. modes and/ or rate of reproduction, dissemination and survivability. We consider this to be the most effective approach in determining whether any consequence of a GM event (including gene up-regulation) has altered the phenotype of the plant such that it poses an unexpected environmental risk to the environment. Again, we consider that abnormalities in the characteristics of a GM crop line (e.g. as a result of gene silencing) would be detectable during its breeding programme.

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<sup>8</sup> Also referred to as 'jumping genes' as these short sections of DNA can move around the genome. They can disrupt gene expression depending where they insert.

<sup>9</sup> Li L-C., Okino S. T., Zhao H., Pookot D., Place R. f., Urakami S., Enokida H. and Dahiya R. Small dsRNAs induce transcriptional activation in human cells (2006). PNAS. **103** (46): 17337 – 17342.