



ADVISORY COMMITTEE ON RELEASES TO THE ENVIRONMENT

Response to ISIS Comments on ACRE'S Advice Concerning T25 Maize

Advice of the Advisory Committee on Releases to the Environment to the Secretary of State under section 124 of the Environmental Protection Act 1990

Advice: ACRE is satisfied at this stage on the basis of the evidence provided that the risk to human health and the environment arising from marketing T25 maize for importation and processing in the UK will be no different from that of other maize imported for processing and animal feed purposes. In coming to this conclusion ACRE have taken account their previous advice and a thorough consideration of the points raised by ISIS below.

ACRE emphasises that all new GMOs are comprehensively evaluated with regard to their safety for release into the environment and consumption and on a case-by-case basis.

The arguments here relate specifically to the case of Chardon LL T25. ACRE concludes that there is no evidence that the T25 insertion event has altered the maize line in any way that makes it less safe to human health or the environment.

Background

1. CHARDON LL is a GM maize variety containing the T25 transformation event that confers tolerance to the herbicide glufosinate ammonium. The T25 / CHARDON LL GM maize lines were developed by AgrEvo (who more recently became Bayer Cropscience).
2. Permission was sought for the maize to be cultivated (grown from seed) in Europe or imported as grain and for the material from either source to be processed into food and animal feed. Following assessment by Member States, it was agreed at European Community level that a marketing consent should be granted, and France issued this in August 1998¹.
3. Recently the decision has been challenged by those objecting to the listing of this variety on the National List of approved varieties. ACRE held an Open Meeting on the criticisms of the risk assessment for T25 GM maize on 20 February 2002. Representatives of the Advisory Committee on Animal Feedstuffs (ACAF) also attended the open meeting. A report of the open meeting, a verbatim transcript and the statements of witnesses invited to speak are available on the ACRE website at: <http://www.defra.gov.uk/environment/acre/index.htm>.

¹ CHARDON LL is a maize variety containing the transformation event T25, approved under Directive 90/220/EEC. Transformation events are regulated under the GMO regulations. The seed legislation operates at the "variety" level.

4. Following the Chardon LL hearing and ACRE's open meeting, the Committee issued detailed advice concerning the risk assessment of T25 maize on 13 December 2002².
5. In response to ACRE's advice, a report written by Dr Mae-Wan Ho, was issued by the Institute of Science in Society (ISIS) which raises further concerns in three areas: (i) horizontal gene transfer, (ii) the hazards of CaMV 35S promoter, (iii) transgene instability³.

Consideration of the points raised by ISIS

Horizontal Gene Transfer (HGT)

6. ACRE members are intimately connected with research and as such keep up with recent scientific developments.
7. ACRE analyses each case individually and the risks associated with horizontal gene transfer (HGT) are taken into account. Horizontal gene transfer is a well-documented mechanism by which bacteria exchange genetic material in the environment. One of the concerns here is that transgenic genetic material may be transferred from the T25 maize to soil bacteria with unknown consequences. ACRE have made detailed assessments of this risk previously^{2,4}.
8. ACRE summed up their advice by saying that there will always be the opportunity for DNA to be taken up by bacteria in the soil whether from transgenic or conventionally bred crops and it is not possible to say that integration will not happen at very low frequency. What was also clear however, is that selection pressure is key, i.e., unless there is very strong selection for the gene that is transferred, it will remain at a low frequency in the population at large.
9. ISIS criticise ACRE for (a) misrepresenting and ignoring publications which demonstrate that HGT can occur between GM crops to unrelated species such as bacteria and mammals and (b) not calling for more definitive investigations in this area.
10. ACRE keeps a close eye on developments concerning the possibility of transfer of genes between plants and bacteria. It has published advice recently⁴ and although there have been a number of recent publications^{5,6,7,8,9}, no evidence has emerged necessitating the committee to modify its advice.
11. For example, Kay *et al.* (2002a)⁵ demonstrated that under laboratory conditions, certain soil bacteria (*Acinetobacter* sp. strain BD413) are capable of colonising non-transgenic plants which are simultaneously infected with the bacterium *Ralstonia solanacearum* and that under these conditions *Acinetobacter* can

² The Advisory Committee on Releases to the Environment's response to concerns raised in written representations and submissions associated with the CHARDON LL public hearing and to statements made at ACRE's open hearing relating to the safety assessment of T25 GM maize conducted under Directive 90/220/EEC is available at <http://www.defra.gov.uk/environment/acre/advice/advice20.htm>

³ The Institute of Science in Society (ISIS) reply is available at <http://www.gmsciencedebate.org.uk/topics/forum/0030.htm>

⁴ ACRE's advice to the Secretary of State & Minister for Agriculture: Horizontal Gene Transfer: Genetically Modified Crops & Soil Bacteria. Available at <http://www.defra.gov.uk/environment/acre/advice/advice08.htm>

⁵ Kay, Bertolla, Vogel & Simonet (2002a). *Microbial Ecology* **43**(3) 291-297.

⁶ Kay, Vogel, Betolla, Nalin & Simonet (2002b). *Applied & Environmental Microbiology* **68**(7) 3345-3351.

⁷ Nielsen, Elsas & Smalla (2000). *Applied & Environmental Microbiology* **66**(3) 1237-1242.

⁸ Berghthorsson *et al.* (2003). *Nature* **424**, 197-201.

⁹ Intrieri & Buiatti (2001). *Mol. Phylogenetics & Evolution* **20** (1), 100-110.

develop a competent state, enabling DNA uptake, *in planta*. Given optimal *in vitro* conditions for gene transfer, such as a bacterial competent state (as in a plant colonising *Acinetobacter* sp. strain BD413), homologous gene sequences (as in genetically modified bacteria containing plant DNA) and high plant transgene copy number (as in transgenic chloroplasts), natural genetic transfer barriers were overcome (Kay *et al.*, 2002b)⁶. This paper reinforces our current knowledge, that given sufficient appropriate conditions, HGT can occur. However, in the case of the T25 transformation event although it contains some bacterial sequences (a partial, non-functional antibiotic resistance gene), the GM sequences are not present in high copy number, thus the likelihood of HGT remains very low, even under optimal conditions

12. Conditions *in vitro* often have little relevance to natural, highly heterogeneous and dynamic systems such as soil (Nielsen *et al.*, 2000)⁷. Their studies, also with *Acinetobacter* sp. strain BD413, suggested that natural soil conditions would rarely produce the selective pressure required for transgene transfer to the soil bacterium, and indeed even if rare transfer did occur, its environmental impact would depend on subsequent selection of the acquired characteristic (Nielsen *et al.*, 2000).
13. ACRE notes that a recent publication has presented evidence for widespread HGT of plant mitochondrial genes between species of flowering plants⁸. Bergthorsson *et al.* have found that this type of HGT has occurred relatively frequently in evolutionary timescales. They also report that HGT is known to occur from bacteria to plants⁹, however, HGT in the reverse order from plants to bacteria has not been found. Bergthorsson *et al.* conclude that their results are not relevant to concerns over potential escape of transgenes from GM plants because of the timescales involved.
14. The safety evaluation process recognises and addresses the potential hazard of transfer of transgenic DNA from GM plants following its consumption, particularly by mammals. ACRE is fully aware of the research published in this area and finds no new evidence to modify the response given on the safety of T25 maize^{10,11}. ACAF was also satisfied that there was no evidence to indicate that T25 maize grain or its products pose any more risk to animals or humans if used in animal feed than non-GM maize varieties¹². The Advisory Committee on Novel Foods and Processes (ACNFP) considered the information submitted to the Public Hearing and is also content that no new evidence was submitted that would question the safety of foods derived from Chardon LL maize¹³.
15. ISIS refer to Hohlweb & Doerfler (2000)¹⁴ and state that the paper shows that transgenic DNA can transfer through the gut and placenta into the blood stream and some cells of the foetus and newborn. However, the evidence presented in the paper demonstrated that despite feeding mice continuously with isolated plasmid DNA for 8 generations germline transfer was never observed. Intramuscular injection of plasmid DNA, showed that fragments were recovered

¹⁰ Thompson (2001). *Journal of Food Science* **66**(2) 188-193. Concludes there is no known risk associated with the rare possibility that mammalian cells could be transformed with and express plant transgenes.

¹¹ Jonas *et al.* (2001). *Annals of Nutrition and Metabolism* **45**(6) 235-254. The body handles DNA in the same way irrespective of its origin. Breakdown of DNA during passage through the gastrointestinal tract reduces the possibility of transfer and functional integration of DNA into gut microflora &/ or human cells through food ingestion.

¹² ACAF (2003). ACAF advice available at:

<http://www.foodstandards.gov.uk/science/ouradvisors/animalfeedingstuffs/papers/104920>

¹³ ACNFP (2002). Response to the food safety issues raised in the evidence submitted to the Chardon LL hearing. Available at: <http://www.foodstandards.gov.uk/science/ouradvisors/novelfood/acnfpapers/103481>

¹⁴ Hohlweb & Doerfler (2000). *Molecular Genetics & Genomics* **265** 225-233.

from intestinal contents up to six hours post-injection, but as the authors acknowledge, this artificial intake either orally or by injection, of isolated DNA is not comparable to the consumption of DNA in food.

16. ISIS draw attention to “transgenic DNA transfers to gut bacteria after only a single meal”. This comment refers to Netherwood *et al.* (2002)¹⁵. This study recruited humans with intact gastrointestinal tracts and ileostomists. Both GM and non-GM DNA was recovered at the same rate in the digesta from the colostomy bags of ileostomists (see also footnote 12). Microbes in samples from ileal digesta were cultured and despite exhaustive attempts, no bacteria harbouring the transgene were isolated. In people with intact tracts, they found that although transgene DNA from GM soya survived passage through the human small bowel, it was completely degraded in the colon and was not detected in faeces. These authors determined that there were no significant risks to human health through gene transfer to either the microflora within the intestinal lumen or to the intestinal epithelium. The Food Standards Agency concluded from this human volunteer research study that it is extremely unlikely that functional DNA from GM food can be taken up by bacteria in the human gut¹⁶.
17. ISIS claim that “the *Agrobacterium* vector system used in making most GM plants can be a vehicle for escape in the soil and to transfers genes into human cells”. The risk of gene escape *via Agrobacterium* is actually minimal due the fact that transgenic explant tissue cultures are not only selected for stable expression of the transgene but are also treated to remove *Agrobacterium*. Barrett *et al.*¹⁷ showed that in those explants where *Agrobacterium* did persist, the cultures eventually senesced and were thus no longer useful. Certainly by the time any transgenic line is released into the environment, there will be minimal, if any, presence of *Agrobacterium*. Higher rates of gene transfer are known to be associated with plant roots¹⁸ and thus ISIS propose that *Agrobacterium* could multiply and transfer transgenic DNA to other bacteria and to the next crop plant. However, these arguments apply equally to naturally occurring *Agrobacterium* and gene transfer activity in the rhizosphere around non-GM plants. Kunik *et al.*¹⁹ show that *Agrobacterium* could transform HeLa cells *in vitro* by a mechanism similar to that for transformation of plant cells. However, since *Agrobacterium* is a common soil microbe, even in the absence of transgenic crops the potential already exists for human cell transformation by *Agrobacterium*, but this has never been documented.
18. Biologically active DNA (whether naturally occurring or transgenic) would be a prerequisite for there to be a HGT risk issue, however, all DNA is subject to the same degradation during the processing of GM plants for food (which is the intended use of T25) and thereby the risks are reduced even further. Every case is scrutinised thoroughly and ACRE's expert scientific opinion is that a series of extremely rare biological events have to occur systematically and simultaneously in order to overcome the combination of physical, biological and genetic barriers which exist in nature to limit interkingdom gene transfer and even if these events did occur, the resulting risks to human health and the environment are minimal.

¹⁵ Netherwood, Martin-Orue, O'Donnell, Gockling, Gilbert & Mathers (2002):

<http://www.food.gov.uk/science/research/NovelFoodsResearch/g01programme/G01genetransfer/g01008/>

¹⁶ Food Standard Agency's advice on the human volunteer study (see reference in footnote 15):

http://www.food.gov.uk/science/sciencetopics/gmfoods/gm_archive/gm_reports

¹⁷ Barrett *et al.* (1996). *Plant Cell Tissue & Organ Culture* **47**(2), 135-144.

¹⁸ Sengelov *et al.* (2001). *Current Microbiology* **42** 160-167.

¹⁹ Kunik *et al.* (2001). *Proc.Natl Acad. Sci USA* **98**(4) 1871-1876.

The hazards of CaMV 35S promoter

19. CaMV has two promoters 19S and 35S, of these two the 35S promoter is most frequently used in biotechnology because it is most powerful and is not greatly influenced by environmental conditions or tissue types. The 35S promoter is a DNA sequence about 400 base pairs in length. The DNA in the virus particles is transcribed in the nucleus (using the 35S promoter) to give RNA, which is replicated by reverse transcription in the cytoplasm to form the DNA molecules, which are encapsidated in the virus particles.
20. The major concerns from ISIS are that the 35S promoter might accidentally activate plant genes or endogenous viruses, or might recombine with mammalian disease viruses with unexpected consequences. This is one of the reasons why ACRE requires applications for marketing consents to describe host DNA that flanks the site into which the transgene has inserted. ACRE is not aware of any incidences of a dormant virus being unintentionally activated by the insertion of transgene with a 35S promoter².
21. ISIS criticise ACRE for focussing on a paper written by Ho *et al.* (1999)²⁰ and for ignoring their more recent papers they have published on the hazards of the 35S promoter^{21,22}. ACRE have considered these review articles and conclude that they do not provide any new *experimental* evidence which could support the claims by ISIS. It is also important to note that the Ho *et al.* (1999) paper was accepted for publication without peer review in order to stimulate debate²³.
22. ISIS claim that eating GM plants containing the 35S promoter could cause ill effects. However, intact and unencapsidated plant viruses are ingested safely, and at much higher doses, than in a GM plant, by eating plants naturally infected with viruses^{24, 25}.
23. ISIS criticise ACRE for ignoring information relating to the promiscuity of the CaMV 35S promoter and its tendency to recombine with other DNA with potential harmful consequences. The arguments by ISIS relate to a paper by Kohli *et al.* (1999)²⁶ from which ISIS conclude that the 35S promoter has the tendency to recombine with other DNA due to a recombination hot spot. The reported recombination event within the 35S promoter (a) occurred before the integration of the transgene into the plant genome and (b), rendered the promoter incapable of driving the expression of any gene. Thus the assertion by ISIS that the promoter will recombine due to a recombination hotspot thereby inadvertently activating non-target genes or give rise to new harmful viruses is both speculative and misleading²⁷.
24. ISIS argue that insertion of foreign genes into genomes is known to be associated with cancer and that there have been two cancer victims of gene therapy. ISIS link these facts to plant genetic modification and thus to their concerns about eating anything that has transgenic DNA, particularly if containing the 35S promoter. However, as explained in paragraph 23, the assertion that the

²⁰ Ho *et al.* (1999). *Microbial Ecology in Health & Disease* **11**, 194-197.

²¹ Ho *et al.* (2000a). *Microbial Ecology in Health & Disease* **12**, 6-11.

²² Ho *et al.* (2000b). *Microbial Ecology in Health & Disease* **12**, 189.

²³ Hodgson (2000). *Nature Biotechnology* **18**, 13.

²⁴ Hull, Covey & Dale (2000a). *Microbial Ecology in Health & Disease* **12**, 1-5.

²⁵ Murphy (2002). CaMV is not a novel cancer risk: <http://www.gmsciencedebate.org.uk/topics/forum/0011.htm>

²⁶ Kohli *et al.* (1999). *The Plant Journal* **17** (6) 591-601.

²⁷ Christou (2001). <http://www.gene.ch/gentech/2001/Feb/msg00016.html>

35S promoter will activate non-target genes and therefore inadvertently cause cancer is speculative and misleading. ISIS again ignore the fact that DNA is DNA whatever its origin and that there is nothing inherently unsafe about the 35S promoter DNA sequence. ACRE believes that analogies drawn between eating GM food and infecting humans with genetically modified viruses which have been specifically engineered to insert therapeutic DNA into human cells in order to cure a genetic disease are spurious and that conclusions drawn from such comparisons are invalid.

25. ISIS refer to a paper by Hull *et al.* (2000b)²⁸ in which they describe findings of viral sequences integrated into plant genomes which are implicated in causing episomal viral disease under particular environmental conditions. CaMV is a pararetrovirus, a plant DNA virus. The infection cycle does not normally involve integration of viral sequences into the host genome. However, the risk to humans, if integration of viral sequences occurred in a GM plant genome, would be very low, since the process occurs naturally at a low frequency.
26. ISIS state that "there is a close relationship between CaMV and human disease viruses (such as hepatitis B and HIV) and that the CaMV 35S promoter DNA inserted into transgenic plants will recombine with DNA from human viruses". This has been addressed by ACRE before², and again, they have no new scientific evidence to modify their previous statements on the low similarity of DNA sequences and only a small possibility of recombination. Hull *et al.* (2000a)²² further illustrate this point of minimal risk by giving the example of banana. It is the world's fourth most important agricultural commodity and has copies of banana streak badnavirus (another plant pararetrovirus) integrated into their genomes. Bananas are the staple diet and HIV is prevalent in countries such as Uganda. Despite high exposure to these plant derived virus sequences, there is no evidence of adverse effects from the consumption of bananas²⁴.

Transgenic DNA and transgene instability

27. ISIS criticise ACRE for stating that transgenic DNA is not inherently less stable than native DNA. ISIS say that the instability of transgenic DNA is well known and is a textbook topic. However, ACRE has not found any additional reference specific to the instability of transgenic DNA. All DNA molecules, whether natural or transgenic, are unstable if they have repeat sequence regions. Since all DNA is the same, this argument by ISIS is untenable. New alleles are constantly accumulated during plant breeding²⁹ and, in the early phases of either conventional or genetic modification procedures, DNA instability is a known factor. However, unstable genomes do not persist and are bred out during a crop breeding programme, irrespective of the method used for their generation. The plant rejection rate due to instability is usually lower in lines generated through genetic modification and this is well illustrated in a recent study which showed that out of six agronomically important transgenes all were stably expressed over four generations in eleven different rice cultivars³⁰. Retrotransposons (transposable elements related to animal retroviruses) are found in all eukaryotes investigated. Insertion has been shown to represent an on-going process occurring over a period of tens of millions of years. They make up the majority of many plant genomes and maize is a good example of a stable crop with

²⁸ Hull *et al.* (2000b). *Trends in Plant Science* 5 (9) 362-365.

²⁹ Morgante & Salamini (2003). *Current Opinion in Biotechnology* 14(2), 214-219.

³⁰ Gahakwa *et al.* (2000). *Theoretical and Applied Genetics* 101(3), 388-399.

potentially unstable genetic elements³¹. There is no evidence, as far as ACRE is aware, that the variation in nutritive quality between maize cultivars is any greater than in crops without a large proportion of retroelements in their genome.

28. ISIS criticises ACRE for using transgene expression as the only criterion for the stability of transgenic lines. For consideration of risks to human health and the environment, it is the trait, not the insert which is relevant. ACRE maintain that monitoring for stable *expression* of transgenes rather than any inherent instability (which can occur in natural DNA also) is sufficient and suitable. Event specific characterisation of successive generations would offer no further information regarding the safety of T25 (and any other future GM crops) for humans and the environment.

Conclusion

29. **The arguments here relate specifically to the case of Chardon LL T25. ACRE re states "there is no evidence that the T25 insertion event has altered the maize line in any way that makes it less safe to human health or the environment".**
30. **We emphasise again that all new GMOs are thoroughly evaluated with regard to their safety for release into the environment and consumption and on a case-by-case basis. The issues raised by ISIS in their recent article² are not new. ACRE consider that it is not instructive to continually cite a restricted range of review papers, in which old arguments are recycled and which do not provide any new scientific experimental evidence to substantiate any of the claims. ACRE also considers that much of the additional research cited to support the criticisms by ISIS appears out of context. We remain committed to continuing review of the science relevant to ACRE terms of reference, but ACRE must prioritise its responses to those cases where substantive new information, appropriately evaluated, is provided.**

³¹ SanMiguel *et al.* (1998). *Nature Genetics* **20**(1), 43-45.