Reprints

Transgenic Instability

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Transgenic Instability refers to the loss of transgene or transgene expression in genetically modified (GM) or transgenic organisms, best studied in transgenic plants. This collection contains reviews of the relevant scientific literature, focussing on the structural instability of transgenic DNA, starting from a witness statement presented recently to the UK Advisory Committee for Release to the Environment (ACRE) Open Hearing.

The Best Kept Secret of GM Crops
Dr. Mae-Wan Ho's Witness Statement to ACRE
For ACRE open hearing on the criticisms of T25 GM maize risk assessment

(T25 Chardon LL is a transgenic maize line belonging to Aventis)

I am speaking against the market approval of T25 because there is no evidence that it is a genetically stable, uniform line, the single most important criterion for approval. For unless it is genetically stable, you might as well forget about environmental or health risk assessment. And genetic instability is also a serious safety issue. The public hearing on T25 was suspended over a year ago when it was found not to have passed the required EC test for Distinctiveness, Uniformity and Stability (the DUS test), as I pointed out when giving evidence to the hearing [1].

The new EC Directive on deliberate release requires strict molecular evidence of genetic stability, which is also necessary for establishing the identity of the transgenic line and to ensure traceability. The best-kept secret of GM crops is that they are *not* stable.

There is a large literature on gene silencing, in which the transgenes remain in the genome, but are not expressed. More serious, from the safety point of view, is structural instability, the tendency for the transgenic DNA to come loose, to rearrange or become lost in part or in whole in successive generations [2,3]. This could change the transgenic line in unpredictable ways in terms of health and environmental risks. And it will increase the chance of transgenic DNA being taken up by unrelated species to make new combinations with their genetic material. That's referred to as horizontal gene transfer and recombination. Transgenic DNA can spread to every species that interact with the transgenic plant, in the soil, in the air, in the mouth and gut and the respiratory tracts of animals including human beings.

New viruses and bacteria that cause diseases could be generated, and antibiotic resistance marker genes could spread to the pathogens. Transgenic DNA may also get into human cells and insert into the human genome; and a large body of evidence from so-called gene therapy experiments have amply demonstrated this *does* occur [4]. The constructs used in gene therapy are very similar to those used in transgenic plants, and one main side-effect of transgenic DNA inserting into human genome during gene therapy is cancer.

Despite that, our regulators have not required biotech companies to provide molecular evidence of stability. ACRE's latest guidelines for industry put out for public consultation asks industry to provide molecular evidence of genetic stability over one generation only [5], which is derisory. We need data for at least five successive generations [6]. No such data have come forward from the companies. On the contrary, companies have been allowed to hide under 'commercial confidentiality'.

I am putting to you twelve reasons why **trangenic DNA is different from natural DNA, and is more likely to spread by horizontal gene transfer and recombination, both by design and otherwise. I hope you will refute these arguments, point by point.**

(In the interest of promoting critical public debate, I am presenting to ACRE, in advance, and for free, two ISIS reprint collections on transgenic instability [3] and horizontal gene transfer [7].)

- All artificial constructs tend to be unstable, so much so that this is a topic in a standard text-book on genetic engineering [8]. Transgenic DNA is more likely to break and join up again, ie, to recombine.
- Transgenic DNA typically contains DNA from widely different sources, mainly bacteria and viruses and other genetic parasites that cause diseases and spread antibiotic resistance, and hence, has the potential to recombine *homologously* with all those agents, ie, due to similarities in DNA base-sequence. Homology enhances horizontal gene transfer 10 million to 100 million-fold [9].
- Transgenic DNA is designed to cross species barriers and to invade genomes. They are flanked by recombination sequences, such as the left and right borders of T-DNA or the

terminal repeats of viral vectors, which enable them to jump into genomes. By the same token, they could jump out again. Enzymes catalysing jumping in also catalyse jumping out.

- Certain 'receptive hotspots' have now been identified in both the plant [10] and the human genome [11]. These may also be 'recombination hotspots', prone to breaking and rejoining. That would mean inserted transgenes are more likely to be lost, to recombine, or to invade other genomes.
- There are mechanisms in the cell that actively seek out, inactivate or eliminate foreign DNA from the genome [12].
- Cell and embryo culture methods are well-known to induce unpredictable, uncontrollable (somaclonal) variations that persist in the plants generated. There is now evidence that the transformation process for making transgenic plants induces further genetic instability [13-15] leading to chromosomal rearrangements, genome scrambling, in other words.
- Monsanto's Roundup Ready soya, commercially grown for years, was finally analysed by molecular methods. Not only is the gene order of the insert found to be scrambled, the plant genome at the site of insertion is also scrambled, and there is a 534 bp fragment of unknown origin in there as well [16]. All very different from the original data provided by Monsanto.
- Recombination hotspots within the transgenic DNA, such as that associated with the ubiquitous cauliflower mosaic virus (CaMV) 35S promoter, could enhance horizontal gene transfer and recombination. We highlighted that in 1999 [17-19], and demanded that all transgenic crops with the promoter should be immediately withdrawn for safety reasons. Two years later, the researchers who discovered the promoter's recombination hotspot also recommended that it should no longer be used [20], not because of safety, but because its instability compromises agronomic performance.
- Recently, landraces of corn growing in remote regions of Mexico were found contaminated with transgenic corn DNA by probing with the CaMV 35S promoter [21]. Molecular analysis showed that the sequences next to the promoter are very diverse, as consistent with horizontal gene transfer and recombination [22].
- CaMV 35S promoter is active in species across the entire living world, including frog eggs and human cells [19], as we uncovered in the literature more than ten years old that had apparently escaped the notice of plant geneticists who attacked us. CaMV 35S promoter, if transferred to human or animal cells, could activate cancer-associated genes as well as dormant viruses that are in all genomes. Another side effect of gene-therapy is the generation of active viruses in cell lines used to package the gene-therapy vectors [4]. Our critics are still dismissing the risks of CaMV 35S promoter, but are avoiding doing any experiments. It is a case of don't look, don't see [6].
- Transgenic DNA from GM plants was found to transfer to soil bacteria. The possibility of transfer to bacteria in the mouth and gut of animals was suggested in laboratory investigations funded by the UK government. There is also evidence suggesting that transgenic DNA from crop plants has transferred to soil bacteria in the field [23]. But ACRE has ignored that by a selective interpretation of the scientific evidence that seems to me contrary to both the precautionary principle and good science [24].

In summary, there is no reason to believe T25 is stable. Furthermore, it has especially hazardous sequences, including the CaMV 35S promoter and an ampicillin resistance gene that, though inactive, can easily be transferred into integrons that will provide it with a promoter to make it functional [1]. T25 has uncharacterised sequences that might be involved in causing diseases. Finally, it has an *origin of replication*, which enables the transgenic DNA to be replicated as a plasmid if transferred into bacteria, thereby greatly increasing horizontal gene transfer on to other species. The *origin of replication* is also a recombination hotspot, and there have been strong recommendations from a recent joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology that transgenic lines containing this sequence should not be approved on safety grounds [26].

1. Ho MW. Chardon LL Public Hearing Ocober 26 2000 on behalf of Burnham Group, also in transcript.
2. See Ho MW. *Genetic Engineering Dream or Nightmare?* Gateway, Gill & Macmillan, Bath and Dublin, 1998, 1999, Chapter on Perils amid Promises of Genetically Engineered Foods.
3. *ISIS Reprints on Transgenic Instability*, *1999-2001*, ISIS Publications, London.
4. Ho MW, Ryan A, Cummins J and Traavik T. Slipping Through the Regulatory Net: 'Naked' and 'Free' Nucleic Acids, Third World Network Biotechnology Series, Third World Network, Penang 2001.
5. See "Watering down EC Directive on Deliberate Release" ISIS Report, February 2002.

6. Ho MW and Steinbrecher RA. Fatal flaws in food safety assessment: critique of the joint FAO/WHO Biotechnology and Food Safety Report. *Environmental & Nutritional Interactions* 1998, 2, 51-84.
7. *ISIS Reprints on Horizontal Gene Transfer, 1999-2001,* ISIS Publications, London.
8. *Principles of gene manipulation*, by Old and Primrose, Blackwell Science, 5th ed, 1994.
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10. Kumar S and Fladung M. 2000. Transgene repeats in aspen: molecular characterisation suggests simultaneous integration of independent T-DNAs into receptive hotspots in the host genome. *Mol Gen. Gent* 2000, 264, 20-8.
11. Miller DG, Rutledge EA and Russell DW. Chromosomal effects of adeno-associated virus vector integration. *Nature genetics* 2002, 30, 147-8.
12. Kumpatla, S.P., Chandrasekharan, M.B., Iyer, L.M., Li, G. and Hall, T.C. (1998). Genome intruder scanning and modulation systems and transgene silencing. *Trends in Plant Sciences* 3, 96-104.
13. Horvath H, Jensen L,Wong O, Kohl E, Ullrich S, Cochran J, Kannangara C, and von Wettstein D. Stability of transgene expression, field performance and recombination breeding of transformed barley lines, *Theor Appl Genet*. 2001,1-11.
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15. Tax FE and Vernon DM. T-DNA-associated duplication/transloations in Arabidopsis. Implications for mutant nanalysis and functional genomics. *Plant Physiology* 2001, 126, 1527-38.
16. Windels P, Taverniers I, Depicker A, Van Bockstaele E and De Loose M (2001). Characterisation of the Roundup Ready soybean insert. *Eur Food Res Technol* DOI 10.1007/ s002170100336, © Springer-Verlag; see also "Scrambled genome of Roundup Ready soya" by Mae-Wan Ho, *ISIS Reprints on Transgenic Instability*, *1999-2001*, ISIS Publications, London.
17. Ho MW, Ryan A and Cummins J. Cauliflower mosaic viral promoter – a recipe for Disaster? *Microbial Ecology in Health and Disease* 1999: 11: 194-197.
18. Ho MW, Ryan A and Cummins J. Hazards of transgenic plants with the cauliflower mosaic viral promoter. *Microbial Ecology in Health and Disease* 2000: 12: 6-11.
19. Ho MW, Ryan A and Cummins J. CaMV35S promoter fragmentation hotspot confirmed and it is active in animals. *Microbial Ecology in Health and Disease* 2000: 12: 189.
20. Christou P, Kohli A, Stoger E, Twyman RM, Agrawal P, Gu X. Xiong J, Wegel E, Keen D, Tuck H, Wright M, Abranches R and Shaw P. Transgenic plants: a tool for fundamental genomics research. John Innes Centre & Sainsbury Laboratory Annual Report 1999/2000, p. 29. See "Top research centre admits GM failure" *ISIS Reprints on Transgenic Instability*, *1999-2001*, ISIS Publications, London.
21. Quist D and Chapela IH. Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature* 2001, 414, 541-3, 2001.
22. "Transgenic pollution by horizontal gene transfer?" by Mae-Wan Ho, in *ISIS Reprints on Horizontal Gene Transfer, 1999-2001,* ISIS Publications, London.
23. Gebbard F and Smalla K. Monitoring field releases of genetically modified sugar beets for persistence of transgenic plant DNA and horizontal gene transfer. *FEMS Microbiology Ecology* 1999, 28, 261-72.
24. "Horizontal gene transfer happens. A practical exercise in applying the precautionary principle" by Mae Wan Ho in *ISIS Reprints on Horizontal Gene Transfer, 1999-2001,* ISIS Publications, London.
25. Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, WHO Headquarter, Geneva, September 24-28, 2001.

Structural Instability of transgenic DNA and artificial constructs
Some quotations
Artificial vectors

"Structural instability of plasmids may arise by deletion, insertion or rearrangement of DNA. Some of the earliest reports of deletions were in chimaeric plasmids which can replicate in both *E. coli* and *B. subtilis*…Spontaneous deletions have now been observed in a wide range of plasmid, virus and chromosomal DNAs. A common feature of these deletions is the involvement of homologous

recombination between short direct repeats….Artificial plasmids with multiple tandem promoters are particularly prone to deletion formation….However, deletion formation can occur between two sites with no homology…..Transcription dramatically affected deletions in an orientation-dependent way such that 95% of deletion endpoints were localized downstream from the inserted promoter when it faced the major plasmid transcripts…"

Old RW and Primrose SB. *Principles of Gene Manipulation*, 5th ed., Blackwell Science, Oxford, 1994.

"…Structural instability, which leads to problems such as the formation of multimers, is more difficult to eliminate….These genetic-stability problems of plasmids usually increase with the size of the DNA fragment inserted." p.171

Prazeres DMF, Ferreira GNM, Monteiro GA, Cooney CL and Cabral JMS. Large-scale production of pharmaceutical-grade plasmid DNA for gene therapy: problems and bottlenecks. *TIBTECH* 1999, 17, 169-74.

Transgenes

"Molecular analyses of transgenes introduced by direct gene transfer….often reveal that a high frequency of transgenic events exhibit extensive rearrangements of transgenic DNA sequences….rearrangements of transgenic sequences are either deletions or ligations [ie joining] of introduced DNA…." p.19

"….Loss of transgene sequences during plant regeneration and reproduction has been described in a number of cases. All or only a portion of the transgene sequences may be eliminated…" p.25

Pawlowski WP and Somjers DA. Transgene inheritance in plants genetically engineered by microprojectile bombardment. *Molecular Biote*chnology 1996, 6, 17-31.

"The widespread occurrence of transgene inactivation in plants…suggest that all genomes contain defence systems that are capable of monitoring and manipulating instrusive DNA. Such DNA might be recognized by its structure, its sequence composition relative to that of its genomic environment and possibly by its disruption of normal biochemical function. Although methylation, especially of repeated sequences is widely associated with gene inactivation, other attributes, including chromatin modification, may be involved. Elimination of inactivated intrusive sequences…..may also contribute to genomic defence mechanisms…" p. 97

Kumpatla SP, Chandrasekharan MB, Iyer LM, Li G and Hall TC. Genome intruder scanning and modulation systems and transgene silencing. *Trends in Plant Sciences* 1998, 3, 97-104.

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'Recombination hotspot' in CaMV 35S promoter

A recent study on transgenic rice carried out at the John Innes Institute supports previous evidence that there is a 'recombination hotspot' in the CaMV 35S promoter. In other words, there is a weak point where the DNA is prone to break and join up with other bits of DNA. Furthermore, most of the recombination events analyzed were 'illegitimate' or nonhomologous and do not require substantial similarity in nucleic acid base sequence. The recombination events were also found to occur independently, in the absence of other viral genes.

Our comment: Transgenic lines containing the CaMV promoter, which includes practically all that have been released, are therefore prone to instability due to rearrangements, and also have the potential to create new viruses or other invasive genetic elements. The continued release of such transgenic lines is unwarranted in light of the new findings.

Reference; Kohli, A. et al 1999. Molecular characterization of transforming plasmid rearrangement in transgenic rice reveals a recombination hotspot in the CaMV promoter and confirms the predominance of microhomology mediated recombination. *The Plant Journal* 17(6), pp 591-601.

AR&MWH

Chaotic gene silencing in GM plants

New study reveals that each transformed plant expressed a different and specific instability profile. Both transcriptional and post-transcriptional gene silencing mechanisms were operating in a chaotic manner and demonstrates that epigenetic (position) effects are responsible for transgene instability in GM plants. These results indicate that transgene silencing and instability will continue to hinder the economic exploitation of GM plants.

Reference; Dr Neve M et al. (1999) Gene Silencing results in instability of antibody production in transgenic plants. *Molecular and General Genetics* 260:580-592.

AR

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Viral Gene Switch – A Recipe for Disaster?

This story highlights the hazardous nature of the genetic engineering process as well as the new gene constructs created and released into the environment.

A scientific paper on the cauliflower mosaic viral promoter (CaMV promoter) has attracted at least nine attacks, including one from Monsanto, before it is actually published. The attacks and rebuttals have been ricocheting around the web, but what is it all about? (Please visit ISIS website for the paper, Ho, M.W., Ryan, A. and Cummins, J. (1999). The cauliflower mosaic viral promoter – a recipe for disaster? *Microbial Ecology in Health and Disease* (in press), and the official author's reply to critiques.)

Prof. Joe Cummins of the University of Western Ontario was the first scientist to question the safety of the cauliflower mosaic viral (CaMV) promoter, which is in practically all GM crops currently grown commercially or undergoing field trials. He pointed out that the promoter could recombine with other viruses to generate new disease-causing viruses. In our joint paper, we review some recent findings which give further grounds for concern, and recommend the immediate withdrawal of all crops and products containing the CaMV promoter, which effectively means all commercial and field tested GM crops, and products with incompletely degraded DNA.

The story begins with the 'promoter'. A 'promoter' is a stretch of genetic material that acts as a switch for turning genes on. Every gene needs a promoter in order to work, or to become expressed. But the promoter is not a simple switch like that for an electric light, for example, which has only two positions, either fully on or fully off. Instead, the gene promoter has many different parts or modules that act as sensors, to enable it to respond, in ways we do not yet fully understand, to signals from other genes and from the environment. These signals tell it when and where to switch on, by how much and for how long. And under certain circumstances, the promoter may be silenced, so that it is off all the time.

The role of the promoter of a normal gene in an organism is to enable the gene to work appropriately in the extremely complex regulatory circuits of the organism as a whole. The promoter associated with each of the organism's own genes is adapted to its gene, while the totality of all the genes of the organism have been adapted to stay and work together for millions, if not hundreds of millions of years. The genome of each organism is organized in a particular way that is more or less constant across the species, so individuals within a species can freely interbreed. Each species protects its integrity and remains genetically stable because there are biological barriers that prevent distant species from interbreeding or otherwise exchanging genetic material. Foreign DNA is generally broken down or put out of action. Genetic engineering attempts to overcome these biological barriers so genes can be arbitrarily transferred between species that would never interbreed in nature. In order to do so, special tricks are needed.

When genetic engineers transfer foreign genes into an organism to make a GMO, they also have to put a promoter in front of each of the genes transferred, otherwise it would not work. The promoter plus the gene it switches on make up a 'gene-expression cassette'. Many of the genes are from bacteria and viruses, and the most commonly used promoter is from the caulifower mosaic virus. Several gene-expression cassettes are usually stacked, or linked in series, one or more of them will be genes that code for antibiotic resistance, which will enable those cells that have taken up the foreign genes to be selected with antibiotics. The stacked cassettes are then spliced in turn into an artificial gene carrier or 'vector'. The vector itself is generally made by joining together parts of viruses and other infectious genetic parasites (plasmids and transposons) that cause diseases or spread antibiotic and drug resistance genes. In the case of plants, the most widely used vector is the 'T-DNA' which is part of the tumour-inducing plasmid ('Ti plasmid') of *Agrobacterium*, a soil bacterium that infects plants and give rise to plant tumours or galls.

The role of the vector is to smuggle genes into cells that would otherwise exclude them. And more importantly, the vector can jump into the cell's genome and so enable the gene-expression cassettes it carries to become incorporated into the genetic material of the cell. The genetic engineer cannot control where and in what form the vector jumps into the genetic material of the cell, however. And this is where the first unpredictable effects can arise. Each transgenic line or GMO is unique, and gives rise to different unintended effects. In the case of food, this can mean unexpected toxins and allergens (see GM Soya & Increased Soya Allergy in Science Notes, this issue).

The foreign genetic material in the GMO – referred to as the 'transgenic DNA' or the 'construct' – is quite complicated. It consists of new genes and new combinations of genes - from diverse species and their genetic parasites - that have never existed in nature. Such chimaeric

constructs are already known to be structurally unstable, that is, they have a tendency to break and join up and rearrange. It is to be expected that such structural instability can only increase when the construct is introduced, by a hit or miss process, into a new genome. The instability of GMOs is a big problem for the industry. GMOs often do not breed true (Terminator in New Guises, this issue).

Why use a promoter from a virus such as the CaMV? Like all viruses, CaMV is a genetic parasite that has the capability to infect cells and hi-jack the cell to make many copies of itself in a short period of time. Its promoter is therefore very aggressive, and is also found to be active in *all* plants, monocots, dicots, algae, and the *E. coli* bacteria that live in the gut of all mammals. Hence, the CaMV promoter is very popular with genetic engineers. It effectively makes the gene placed next to it turn on full blast in any plant genome, at perhaps a thousand times the volume of any of the organism's own gene.

Having it in the genome is rather like having the loudest phrase of a heavy-metal piece, played with the most powerful amplifier, over and over again, throughout a live performance of a Mozart concerto. What the CaMVpromoter does is to place the foreign gene outside the normal regulatory circuits of the host organism, subjecting the host organism to unremitting metabolic stress. This will multiply the unintended, unpredictable effects in the GMO. It may also be another reason why GMOs are notoriously unstable (Finnegan, J. & McElroy, D. 1994, Bio/Technology 12, 883). The organism generally reacts to the presence of foreign genetic material by breaking it down or putting it out of action in other ways. Even after the genetic material is incorporated into the genome, it can silence the foreign genes so they are no longer expressed (see Terminator in New Guises, this issue).

The key recent finding, which provoked us to write our paper, was the report by Kohli *et al*, (1999) *The Plant Journal* 17, 591, that the CaMV promoter contains a 'recombination hotspot' – a site where the DNA tends to break and join up with other DNA, thus changing the combination and arrangement of genes. Around the hotspot are several short stretches, or modules, for binding various enzymes, all of which are also involved in recombination , ie, breaking and joining DNA. Furthermore, the CaMV promoter recombination hotspot strongly resembles the borders of the T-DNA vector carrying the transgenes, which are also known to be prone to recombination. It is that which enables the vector to invade the cell's genome in the first place.

The aim of our original paper, restated explicitly in our official rebuttal, was to review the relevant findings and, in particular, to point out the *implications* which the researchers themselves are unwilling or unable to draw. The findings that transgenic DNA has the tendency to break and join in several places imply that parts or all of it may be more likely than the plant's own DNA to jump out of the genome and successfully transfer horizontally to unrelated species. Horizontal gene transfer, in this context, means the transfer of the genetic material directly by infection to the genetic material of unrelated species, in principle to all species interacting with the GMO: bacteria, fungi, earthworms, nematodes, protozoa, insects, small mammals and human beings. This process is uncontrollable and cannot be recalled. Transgenic DNA has been designed to be invasive and to overcome species barriers; once released, it will invade different organisms especially bacteria which are in all environments, where it will multiply, mutate and recombine.

There are additional findings that suggest an increased potential for transgenic DNA to spread horizontally. For example, the enzymes in the cell that insert the transgenic DNA into the genome can also make it jump out again. DNA released from both dead or live cells can survive without being degraded in all environments, including the mouth and gut of mammals. DNA can be readily taken up into cells. And *all* cells can take up naked or free DNA. A recent finding suggests that integrated viral sequences are preferentially taken up and incorporated into the cell's genome (see Reusable DNA Alert, this issue). The instability of transgenic DNA may also be enhanced as the result of the metabolic stress inflicted on the organism by the CaMV promoter, which gives rise to continuous over-expression of transgenes.

The major consequences of the horizontal transfer of transgenic DNA are the spread of antibiotic resistance marker genes among bacteria and the generation of new bacteria and new viruses that cause diseases from the many bacterial and viral genes used. The generation of new viruses could occur by recombination with live or dormant viruses that we now know to be present in all genomes, plants and animals included. Recombination with defective, dormant animal viral promoters may also occur, as we know that there are modules that are interchangeable between plant and animal promoters. Recombination of CaMV promoter modules with defective promoters of animal viruses may result in recombinant promoters that are active in animal cells. This may reactivate the virus, generate new viruses or give functional viral promoters causing over-

expression of one or another of dozens of cellular genes that are now believed to be associated with cancer.

In conclusion, there is sufficient scientific evidence to support well-founded suspicion of serious, irreversible harm to justify the immediate withdrawal of all GM crops and products containing the CaMV promoter from environmental release. This is fully in accord with the precautionary principle.

New Ways to Silence Transgenes

Researchers at the John Innes Center in Norwich finds a new species of small antisense RNA molecule involved in post transcriptional gene silencing (PTGS) in plants. PTGS represents a natural antiviral defence mechanism, which works against any invasive foreign genetic element that finds expression.

RNA in GM plants are frequently targeted by PTGS mechanisms and this strongly suggests that transgenes are perceived as viruses by their host cell. This study has detected antisense RNA that is uniform in length - approx. 25 nt (nucleotides) and complementary to the targeted mRNA. It has been term 'spoiler RNA' for it forms a duplex with the target RNA and promotes degradation as well as interfering with translation. It has been shown to accumulate in cells, either when transgene transcription or RNA virus replication is taking place. The size of the spoiler RNA is also significant - it is small enough to move through the plasmodesmata (pores between cells) and has been shown to spread into nearby cells and activate PTGS elsewhere in the plant. The precise role of the 25nt RNAs in PTGS is yet to be elucidated, but it is suggested these are components of a systemic signal and specificity determinant in PTGS.

Reference: Hamilton A.J, Baulcombe D.C (1999). *Science* 286, 950-952 also see "Silent Saboteurs" New Scientist, 6.11.99 p 25.

Our comments: This new discovery shows that GM plants respond to transgenes in the same way they do viruses. Transgenic RNA transcripts are produced at a very high copy number in GM plants and are under the control of powerful promoters like the CaMV 35S promoter. Viral infection causes metabolic stress in cells and can lead to PTGS. The same can now be said of transgenic constructs in GM plants. Furthermore, the size and migratory nature of the RNAs reveal how small nucleotides have very important biological functions in cells. This calls for regulation of all naked or free nucleic acids used in genetic engineering biotechnology, a point made forcefully by Traavik (1999). *Too Early May be Too Late: Ecological Risks of Naked DNA*, Report to Directorate of Nature Management, Trondheim, Norway.

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The CaMV Promoter Saga Continues..

Nature Biotechnology makes a habit of losing e-mails and submissions

To recapitulate on the story so far, a scientific paper, "Cauliflower mosaic viral promoter – A recipe for disaster?", co-authored by Mae-Wan Ho, Angela Ryan and Joe Cummins was submitted to the Journal, *Microbial Ecology in Health and Disease* last October (now published: vol.11, 194-197, 1999). The Journal's Editor, promptly posted it on the Journal's website before publication and put out a press release. Within two days, someone managed to solicit at least nine critiques, including one from Monsanto, which were posted on a website funded by the biotech industry and widely circulated on the internet. The critiques varied in tone from moderately polite to outright rude. We wrote a detailed rebuttal, which was likewise circulated and posted to the same website, and have not received any replies from our critics since. In January, *Nature Biotechnology* published a distorted, one-sided and offensive account of our paper, concentrating on the criticisms and ignoring our rebuttal completely.

Our paper reviews and synthesizes existing scientific findings on the cauliflower mosaic viral (CaMV) promoter that is in practically all GM crops already commercialized or undergoing field trials. The findings suggest to us that artificial gene-constructs containing the CaMV promoter may be especially prone to breaking and joining up with other genetic material, thereby increasing the chance that it can be transferred horizontally to unrelated species. The potential hazards are harmful mutations, cancers, reactivation of dormant viruses and generation of new viruses. These considerations are especially relevant in the light of recent findings by Arpad Pusztai and his collaborator Stanley Ewen (*The Lancet* 354, p.1353, 1999), that transgenic potatoes - containing the CaMV 35S promoter - may be unsafe for young rats, part of the effects being attributed to the construct or the genetic engineering process, and hence common to all GM crops.

Secret documents belonging to the US Food and Drug Administration, which came to light as the result of a civil lawsuit against the agency (see Special House of Commons Briefings, this

issue, and www.biointegrity.org) reveal that the first GM crop to be commercialized, the Flavr Savr tomato – which also had the CaMV promoter - actually failed to pass the standard safety tests. Since then, no comprehensive safety testing has been done on any GM foods. In line with the precautionary principle, we recommend the immediate withdrawal of all GM crops and products containing the CaMV promoter, until and unless they can be proven safe.

Nature Biotechnology had agreed in principle to our right to reply. But their editorial office has somehow managed to lose our e-mails and submission more than once over the past three months, and each time after a long delay. We have finally got an acknowledgement from them that they have received our corrected galleys. It is now posted on our website.

Meanwhile, we have written a more detailed reply for *Microbial Ecology in Health and Disease*, Hazards of Transgenic Plants with Cauliflower Mosaic Viral Promoter, with new references and arguments. This shall be posted on our website when it is accepted for publication.

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Corporate Science on the Offensive

ISIS targeted

Dr. C. S. Prakash, Director of the Center for Plant Biotechnology Research at Tuskegee University (USA), is the latest corporate recruit to counter the worldwide rejection of GM crops. I first came across him in a pro-GM 'documentary' I was tricked into taking part by Equinox, the science series of Channel 4 TV in the UK (see ISIS NEWs #4). I met Prakash again at the multi-stakeholders dialogue at the 8th session of the United Nation's Commission on Sustainable Development (April 24-May 4, New York), where he sat with, and spoke for the biotech industry. On June 1, I encountered him for the third time in a debate in London, organized by the US Embassy. I was told Prakash has been sent over by the US State Department. Unlike his predecessor Val Giddings, Prakash oozes charm and bonhomie. He said he has already been touring Europe "to prevent other Mae-Wan Hos from springing up" and London, UK, was his last stop.

The debate, held in The School of Oriental and African Studies, was on the motion, "Agricultural biotechnology is vital for the developing world", with Prakash and Matt Ridley, speaking for, and myself and John Vidal speaking against. Ridley and Vidal are both well-known journalists on opposite ends of the political spectrum. To my surprise and dismay, it was not an open debate as only 'stakeholders' were invited. Judging by comments from the floor, the majority were from industry or pro-biotech pressure groups. The Monsanto 'science outreach' representative came out smelling like roses compared to two molecular geneticists associated with Cropgen, a new pressure-group of scientists funded by industry, members of which have been very prominent in the media recently, and appearing to be targeting ISIS in particular.

A few days later, one of the Cropgen scientists, Conrad Lichtenstein, wrote a pompous article in *The Guardian* newspaper ("A misguided media swarm" June 6) where he dismissed all the scientific studies that cast any doubt on the safety of GM crops, especially those that have been given a lot of press coverage: Arpad Pusztai's work that GM potatoes adversely affecting young rats and John Losey's finding that GM pollen is lethal to Monarch butterflies. In anticipation of the as yet unpublished report from Jena University in Germany - that GM genes have transferred from GM pollen to the bacteria and yeasts of baby bees - he argued that, if so, it must be occurring all the time. (Not so long ago, these scientists have denied that such horizontal gene transfer can occur.) And, he claims, it doesn't matter, because neo-Darwinian natural selection will select them out: the organisms to which the foreign genes have transferred will die out either immediately or in the long run, by the principle of the survival of the fittest. He failed to notice that neo-Darwinian natural selection operating on human beings to which GM genes and constructs have spread won't be very good for health. The article ended with an attack on me.

He was "alarmed to hear an anti-GM university biologist state that GM genes are more resistant to the natural processes by which enzymes break down other DNA and that GM genes, as they are designed to "invade" genomes, are also more unstable and can more easily move around, dangerously spreading". He claimed that when he asked for direct experimental evidence, he was given "the techno-babble which puts fear into the hearts of the scientifically uneducated".

I wrote a letter to *The Guardian* (June 8) answering his attacks, and inviting him yet again to visit the ISIS website where all the evidence has been presented with detailed citations of the scientific papers. *The Guardian* then published another attack from him in the same tone (June 12), demanding actual references to the scientific literature. I again submitted my reply.

But *The Guardian* did not publish my letter the next day, nor the next after I made a polite enquiry. Finally, when I threatened to complain to the Independent Press Commission, they agreed

to publish a much shorter version without the references because their spokesperson said they simply cannot engage in detailed scientific debates of that kind. Why did they allow Lichtenstein to demand the references knowing that they won't allow me to supply them?

Lichtenstein and others like him are the reason why the public continue to perceive scientists as "arrogant and dysfunctional", as UK Member of Parliament Dr. Ian Gibson wrote (See "Scientists, Don't Forget the Social context!", this issue). They are also guilty of abuse of scientific evidence (as well as abuse of scientists) and acting against the precautionary principle.

The text of my talk, "GM Crops – How Corporations Rule and Ruin the World" can be found on the ISIS website. My first reply to Lichtenstein was published in the letters section of *The Guardian* (8 June) with the last two sentences omitted. The second reply published (16 June) was much shorter than what I had originally submitted, but makes the key point that the best kept secret of the biotech industry is that there is no evidence for the long term stability of the GM inserts in both structure and location in the plant genome for any GM line already commercialized or undergoing field trials.

Both original letters are reproduced at the end of this report. Lichtenstein's comments on horizontal gene transfer and natural selection are typical of GM proponents adhering to the discredited, reductionist neo-Darwinian paradigm (see "An End to Bad Science and Beginning with Life Again" www.i-sis.org on how the new genetics makes neo-Darwinian theory untenable).

Ho Replies to Lichtenstein 1

I am the "anti-GM university biologist" that Conrad Lichtenstein referred to in his article on the GM controversy (6 June). The debate he described was arranged by the US Embassy for biotechnologist, Dr. C.S. Prakash, sent by the US State Department to promote GM agriculture in Europe. I agreed to participate because I believe in promoting critical public understanding of science and to draw attention to well-known and relevant scientific knowledge that is being ignored.

Almost by definition, genetic engineering organisms involves designing GM-constructs which invade genomes and overcome natural processes that break down foreign genetic material. Due to their highly mixed origins, however, GM-constructs are more unstable than natural genetic material as well as more invasive; and may therefore be more likely to spread to unrelated species. Those points were not challenged by Prakash because these basic principles and observations of genetic engineering are covered in text books and are also areas of active research. I answered Lichtenstein's questions in full and referred him to our website <www.i-sis.org> where the relevant scientific papers are cited and where more than 300 scientists from 39 countries, including many molecular geneticists who share my concerns, are demanding a moratorium on releases of GM organisms.

There is genuine scientific dissent among scientists and the public are not served by those who continue to misrepresent the GM debate as science *versus* anti-science. In demanding a moratorium, we are not trying to stop research into molecular genetics. On the contrary, we are arguing for more basic research that can tell us how and if GM technology can be safely used. More than that, we need open, wide-ranging and inclusive debates on the kind of science and technology that can best serve society.

Ho replies to Lichtenstein 2

Conrad Lichtenstein (Letters, 12 June) demands references on the invasiveness and instability of GM constructs in genetic engineering. There are many; here are just a few.

For designing GM constructs to overcome being broken down, and to increase invasiveness and stability, read Kumpatla *et al*, *Trends in Plant Sciences* 3, 96, 1998.

A major class of GM constructs are artificial vectors for transferring genes, made from the most invasive natural viruses and genetic parasites; their instability is highlighted in a text book, *Principles of gene manipulation*, by Old and Primrose, Blackwell Science, 5th ed, 1994.

There are many articles on the instability of GM plants, a recognised problem area. The most actively investigated are mechanisms silencing integrated GM genes, but loss of part or all of the GM construct has also been observed, even during later generations of propagation (see for example, Register *et al*, *Plant Molecular Biology* 25, 951, 1994).

Finally, a GM gene in *Arabidopsis* was found to be up to 30 times more likely to spread than the same gene created by conventional induction of mutation (Bergelson *et al*, *Nature* 395, 25, 1998). But no investigations were done to determine if this was associated with instability of the GM construct.

The instability and invasiveness of GM constructs are supported by direct and indirect evidence, while no evidence exists for the long term stability of the GM inserts with regard to structure and location in the plant genome. On grounds of safety and efficacy, such evidence

should have been provided before approvals for releases were granted.

Can Viruses Cross from Plants to Animals?

The CaMV debate continues…

The question of whether viruses can cross from plants to animals was raised in the course of the debate on the hazards of the cauliflower mosaic virus (CaMV) promoter used in practically all GM crops.

There is indeed evidence that viruses may have crossed from plants to animals. Also, similar viral sequences have integrated into the genomes of both plants and animals, which suggests that sequences may have moved from animals to plants. Transposons (jumping genes related to viruses) and endogenous viruses are now found in the genomes of all higher organisms, plants and animals included. All these recent findings have important implications for the ecological and health impacts of GM crops, which have not been adequately addressed by our regulatory authorities.

The *circoviruses* are small single stranded DNA viruses that cause serious infections of the digestive systems of humans and other animals, particularly pigs and chickens. They also cause infections in clover, banana and coconut. There is evidence that vertebrates may have acquired circovirus from exposure to plant sap (1). The similarity between plant and animal circoviruses was noted earlier (2,3). As the virus group is quite recently discovered, it is not known when the shift from plants to animals may have occurred, nor whether the virus may still migrate between plants and animals at present. Plant and animal circoviruses are related to the plant geminiviruses (4) which have been used in genetic engineering but not commercially.

The soya genome contains several hundred copies of a large retroviral sequence called SIRE (Short Interspersed Repetitive Element), which is related to retrotransposons called *copia* in the fruitfly and *Ty1* in yeast (5, 6). Retroviruses are RNA viruses that replicate via reverse transcription, ie, making a complementary copy of DNA from the RNA. A retrotransposon is a jumping gene that uses reverse transcription to spread itself around the genome. In other words, SIRE is a plant retrovirus related to the retrotransposons of fruit flies and their food, yeast.

Plant pararetroviruses, which include the cauliflower mosaic virus, have also been found to be integrated into plant chromosomes at high copy numbers (7), and virus infection may result from endogenous pararetrovirus in plants (8,9). Pararetroviruses are DNA viruses which use reverse transcription to multiply itself.

Foamy viruses are found in animals including human beings, which are integrated into the chromosomes during each cycle of replication. These resemble plant pararetroviruses in that they infect as double stranded DNA (10, 11). Foamy virus is associated with human thyroid infection leading to Graves disease (12). The foamy virus and plant endogenous pararetrovirus are very similar, and possibly related. Switching of such viruses between plants and mammals took place some time in our evolutionary past, but whether or not it can still take place is not known, and should be investigated.

The use of pararetrovirus promoter sequences from cauliflower mosaic virus (CaMV) in essentially all commercial GM crops and those undergoing field trials has not been subject to risk assessment in the light of all these and other recent findings suggesting it may be unsafe (13, 14, 15). Government agencies such as USDA, and scientists advising the UK government argue there is no risk from the CaMV promoter because the virus has been eaten with infected cabbages (16). Furthermore, they also imply that as so many copies of retrotransposons and pararetroviral sequences are already in the plant genomes, each of which has a promoter, then adding a few copies of CaMV promoter will not make any difference (17). These arguments have been answered in full (see ref. 15 in particular). Some key points are summarized here.

The CaMV promoter in the intact virus and the intact viral genome is a stable integral part of the virus and is very different from the isolated CaMV promoter in GM constructs, which are notoriously unstable and prone to break and join with other genetic material. This increases their propensity for horizontal gene transfer. A *prima facie* case that the CaMV promoter in the intact virus is not the same as the one in GM construct is that while the intact virus is specific for plants in the cabbage family, the latter is promiscuous, and works in many, if not all species (look out for the next episode soon!).

The integrated viral and retrotransposon sequences may have intact promoters, but again the promoter is a stable integral part of the element; furthermore, most of the elements are inactive, which means that their promoters are chemically modified to be non-functional.

The CaMV promoter has a recombination hotspot (18) a site at which it is likely to break and join with other genetic material. Consequently, GM constructs with CaMV promoter(s) will be more prone to horizontal gene transfer and recombination

The CaMV promoter is promiscuous in function, and is active in all plants, algae, yeast and *E. coli.* Thus, any gene linked to it will be expressed continuously at high levels in all these species to which it is transferred.

The CaMV promoter has a modular structure, and is interchangeable in part or in whole with the promoter of other viruses to give infectious viruses.

Adding a CaMV promoter, prone to recombination, to genomes laden with sleeping pararetroviruses and retrotransposons can only increase the chances of re-activating infectious viruses and creating new viruses that may cross from plants to animals.

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JC &MWH

Defence Against Genome Invaders

Plants have a wide-range of defences against genome invaders – foreign genetic material. That's why GM plants are often unstable. A review of the processes is presented by researchers.

"The widespread occurrence of transgene inactivation in plants and classical cases of

silencing of duplicated sequences in fungi suggest that all genomes contain defense systems that are capable of monitoring and manipulating intrusive DNA. Such DNA might be recognized by its structure, its sequence composition relative to that of its genomic environment and possibly by its disruption of normal biochemical functions."

Although methylation, especially of repeated sequences, is widely associated with gene inactivation, other mechanisms may be involved, including modification of chromatin structure. Elimination of inactivated intrusive DNA (presently best documented for filamentous fungi) may also contribute to genomic defense mechanisms in plants. " It is likely that, like viral and other infectious RNAs, alien RNA is also recognized by cellular defense systems."

Most of the knowledge of defence mechanisms against foreign genetic material came originally from observations in bacteria. But over the years, similar mechanisms are uncovered in higher organisms. These include:

- Cytoplasmic nucleases (enzymes) which break down invading genetic material
- DNA methylation for inactivating the foreign genes
- Modification of histones (proteins) bound to foreign genes
- Genomic surveillance systems capable of searching and debilitating repeated sequences or foreign sequences which are out of place
- Gene-silencing mechanisms which pick out genes that have similar or homologous sequences
- Post-transcriptional gene silencing which breaks down the transcripts of foreign genes
- Selective elimination of duplicated sequences, including integrated viral sequences in mammalian transformed cells

The three major events postulated to occur in response to invading DNA or RNA are "detection, inactivation and elimination". These events work against the stability of transgenes.

This review covers interesting aspects of genome 'architecture' and the structure of 'chromatin' (the association of DNA with histones and other proteins involved in packaging the DNA into chromosome) which affect the fate of the integrated foreign genes. Most transposition and viral integration intermediates share certain structural features that may be prime targets for DNA methylation. Genomes appear to be made up of isochores – very long stretches of DNA with high compositional homogeneity, either GC rich or AT rich. This makes it possible to detect inserted genes that are compositionally different.

Stable integration and expression of introduced genes are essential for genetically engineered crops, and thus "transformation constructs must be designed to avoid host surveillance processes." The review outlines some design strategies for avoiding host surveillance suitable for *Agrobacterium*-mediated transformation methods as well as for biolistics and other direct DNA-mediated procedures, "provided that conditions for obtaining plants with few transgene copies can be established."

These design strategies include introducing gene sequences that are different from those in the plant to avoid gene-silencing which work on duplicated sequences, and adding either GC rich or AT rich flanking sequences to direct it to the appropriate isochore.

Reference: Kumpatla, S.P., Chandrasekharan, M.B., Iyer, L.M., Li, G. and Hall, T.C. (1998). Genome intruder scanning and modulation systems and transgene silencing. *Trends in Plant Sciences* 3, 96-104.

Our Comments: This review explicitly acknowledges the problem of transgene instability in plant genetic engineering and suggests design strategies to overcome different mechanisms that break down, search out, inactivate and eliminate invading genetic material. It deals realistically with the fact that transgenes are recognized to be invaders by crop plants (see also "Transgenes are Genome Invaders", this issue), which is denied by Conrad Lichtenstein, a pro-biotech molecular biologist (see "Corporate Science on the Offensive", this issue).

Transgene instability not only compromises the agronomic performance of GM lines, it has important ecological and health consequences. Structural instability of GM-inserts, due to excision mechanisms or the instability of GM constructs - not explicitly covered in this review - will also give recombinations and rearrangements within the host plant genome that may alter the plant's metabolism towards the production of harmful metabolites. It makes unintended, secondary horizontal spread of transgenes more likely.

Transgenes *are* Genome-invaders

Transgenes are recognized as genome-invaders by the host plant. The host plant mounts defence mechanisms against transgenes which are normally used against viruses.

'Post-transcriptional gene silencing' is a defense mechanism in plants similar to 'quelling' in

fungi and RNA interference in animals. It silences foreign genes (ie, inactivates it) after the gene is transcribed into RNA, by preventing the RNA being translated into protein. Four genes are found to be required for post-transcriptional gene silencing in *Arabidopsis*. One of these, *SDE1*, is a plant homolog of *QDE-1* in the fungi, *Neurospora crassa* that codes for an RNA-dependent RNA polymerase (an enzyme which makes a complementary copy of an existing RNA). The researchers propose that SDE1 polymerase synthesizes a double-stranded RNA which initiates post-transcriptional gene-silencing. According to this idea, when a virus induces post-transcriptional gene silencing, the virus-encoded RNA polymerase will produce the double-stranded RNA, and therefore has no need for plant *SDE1*.

Plants defend themselves from invading DNA or RNA primarily by inactivating the RNA messages of the invader. Virus RNA is recognized during replication because it is double stranded, and double-stranded RNA is otherwise unusual in plant cells. The double-stranded RNA is destroyed by another plant enzyme (RNAse). To be successful, a virus must have a gene that inactivates the plant gene coding for the enzyme that destroys the replicating virus. Transgenes (GM-constructs) are recognized by the plant cell as foreign invaders because the plant cell has an RNA dependent RNA polymerase (resembling the virus replication enzyme) that converts the messenger RNA of the transgene into double stranded RNA. The double-stranded RNA is then attacked and destroyed by the same cellular defence mechanisms that work against replicating viruses.

To be successful, the transgene must evade the plant's defences against invading foreign nucleic acid.

Reference: Dalmay, T., Hamilton, A., Rudd, S., Angell,S., and Baulcombe, D.C. (2000). An RNA-dependent RNA polymerase gene in *Arabidopsis* is required for posttranscriptional gene silencing mediated by a transgene but not by a virus. *Cell* 101, 543-553.

Our Comment: Transgene silencing and the defense against foreign nucleic acid illustrate a fundamental aspect of the organisms in that they must defend their genetic identity and integrity against a multitude of foreign nucleic acids. As transgene constructs become better designed to overcome host defence mechanisms, they will be more powerful genome invaders. Their potential for successful horizontal gene transfer and recombination will concomitantly increase, and so will their ecological and health hazards. It is time to pause and reflect. JC & MWH

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CaMV Promoter Active In Animal and Human Systems

Since the publication of our original paper on the CaMV promoter, we have been subjected to personal abuse and attack of the kind meted out to many other scientists who refuse to be intimidated into a 'scientific consensus' by the corporatized scientific establishment.

In a continuing campaign to mislead and obfuscate, the pro-biotech brigade have been re-cirulating again and again the same scientific critique of our paper which we have already rebutted in full in an article published in the same issue of the Journal.

But the worse is yet to come. Plant genetic engineers, including our critics, have been telling us that the CaMV promoter is safe because it is a plant promoter that only works in plants and plant-like species. We have now found in the scientific literature more than 10 years old that the CaMV 35S promoter is active in frog eggs as well as in extracts of a human cell line. It means that if the CaMV promoter ends up in our genome, it could well have unpredictable, untoward genetic effects.

We submitted a short paper to *Nature Biotechnology* which has been publishing the most despicable attacks on us, but they rejected it after a two months delay. This paper is now in press in *Microbial Ecology in Health and Disease*, practically the only scientific journal that would allow a fair debate in their pages. The paper is posted on ISIS' website.

It is nothing short of a scandal that the plant genetic engineers have not bothered to check whether the CaMV promoter is active in animals before they started to use it so widely. Those who are still supporting the use of the CaMV 35S promoter should be held legally responsible for any harmful consequences arising from it.

More on Instability of Transgenic Lines

Somaclonal variation (SCV) in transgenic plants may slow incorporation of introduced genes into commercially competitive cultivars, researchers warn (1).

Somaclonal variation in transgenic barley (*Hordeum vulgare* L.) was assessed by comparing the agronomic characteristics of 44 transgenic lines in the T2 generation to their non-transformed parent (`Golden Promise'). A second experiment examined the agronomic

characteristics of seven transgenic-derived, 'null' lines – those that were not transformed - in the T2 and T4 generations.

Compared to their nontransgenic, noncultured parent, Golden Promise, most of the transgenic lines were shorter, lower yielding, and had smaller seed, and the variability among individual plants was higher. The frequency and severity of the observed SCV was unexpectedly high, and the transformation procedure appeared to induce greater SCV than tissue culture in the absence of transformation. Attempts to understand the sources of SCV, and to modify transformation procedures to reduce the generation of SCV, should be made, the authors stated.

The publication above deals with a fundamental problem with genetically modified (GM) crops. In order to make a GM crop for commercial use, the GM tissue cells are grown up in tissue culture, from which whole plants are regenerated. During the culture process and during later generations of selected plants, genetic variability is rife. Both gene mutation and chromosome alteration are rampant. There seems to be something about the laboratory technique and the introduction of transgenes that causes gene and chromosome instability. The best available evidence suggest that the technology activates retrotransposons (retrovirus like gene clusters) that replicate copies that jump into other chromosomes or regions of a chromosome.

Retrotransposons make up from a few percent to 85% of the genome of a higher plant. However, it only takes a few retrotransposons activated from their normally dormant state to cause gene mutation by insertion or chromosome rearrangement by recombination between retro-transposons.

Our comments: Somaclonal variation in transgenic lines is shown to be due to both tissue culture and the transformation process. It confirms the inherent instability and unpredictability of transgenic lines that we have drawn attention to (2), which has significant implications for the safety of GM crops. As they are grown in the field, a range of hidden defects may continue to be generated that lead to toxicities and other untoward, unexpected side effects. The phenomenon has largely been overlooked in regulation on the safety of GM crops (3).

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3. See Ho, M.W. (1999). Biosafety Alert: submission to TEP on the molecular genetic characterization required for commercial approval of transgenic lines www.i-sis.org

JC & MWH

More Trouble for Transgenic Lines

Transgenes are found to be poorly expressed due to premature poly-adenylation (adding a poly-A tail) to the messenger RNA.

The *cry* genes that code for the insecticidal crystal proteins of *Bacillus thuringiensis* (Bt) have been widely used to develop insect-resistant transgenic plants. The *cry3Ca1* gene has been reported to code for a crystal protein that is particularly potent against the Colorado potato beetle (CPB). To explore the biotechnological potential of cry3Ca1 protein, researchers introduced this gene into transgenic potato plants under the control of the CaMV 35S promoter (1).

In the resulting transformants, the *cry3Ca1* gene was very poorly expressed. In fact, no full-length transcript (2300 nt) could be detected. Instead, only short transcripts of approximately 1100 nt were observed. Analysis of these short transcripts by Northern hybridization (for RNA), RT-PCR (reverse transcription followed by polymerase chain reaction) as well as by cloning and sequencing showed that they resulted from premature polyadenylation.

These processing events occurred at four sites within the *cry3Ca1* coding region (at positions 652, 669, 914 and 981 relative to the translation start site). The sites at which premature polyadenylation took place were not those that showed the highest degree of identity to the canonical AAUAAA motif. Together with other recent data, these findings suggest that premature polyadenylation is an important mechanism which can contribute to the poor expression of transgenes in a foreign hosts.

Premature polyadenylation occurs when the RNA message is terminated short of the stop signal, and is then polyadenylated. A prematurely ended poly-A message without a stop signal might result in the polyA tail being translated as a string of the amino acid lysine added to the growing peptide. Such products have problems being released from ribosomes, and once released, tends to be destroyed by the final protein processing system. However, unnatural peptides may also be produced and cause untoward problems.

Our Comments: Many important findings seem to be ignored in regulation of GM crops and swept

under the carpet in the debate over GM foods. This paper documents yet another mechanism that makes transgenic crops unreliable and economically non-viable.

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Top Research Centre Admits GM Failure

Scientists in UK's top GM crop research institute, the John Innes Centre, are finally admitting to the public that GM crops are no good. It amounts to pronouncing the death sentence on GMOs.

The John Innes Centre (JIC) is UK's leading plant research institute, publicly funded by the Biotechnology and Biological Sciences Research Council (BBSRC) to the tune of more than £10m in grants every year. It also houses the Sainsbury Laboratory and has research alliances with Zeneca and Dupont.

Not surprisingly, JIC has some of the most pro-GM scientists staunchly defending GM crops from critics like ourselves, even as they are pointing to the same problems in scientific papers published in specialist journals. For years, we have been drawing attention to the instability of GM constructs and GM lines. This raises serious safety concerns over the possibility that the GM genes could spread horizontally to unrelated species, creating new bacteria and viruses by recombination. More recently, we have also argued that the promoter from cauliflower mosaic virus (CaMV 35S promoter), which is in practically all GM crops already commercially grown or undergoing field trials, will make GM constructs and GM lines extra unstable, and hence greatly exacerbating the problems of horizontal gene transfer and recombination.

Two items in the latest annual report from JIC are note worthy. The first [1] reveals that GM barley lines became unstable and variable in later generations of field trials. The researchers conclude, "The results show that transgenic lines need to be examined over a number of generations under field conditions to obtain the necessary data on transgenic stability and agronomic performance". They also call for "detailed molecular and genetic analysis". ISIS has been demanding the same for years, along with other scientists.

The second item [2] concerns the CaMV 35S promoter. When ISIS pointed out the dangers of this promoter in the scientific journals, we were reviled and attacked. Our fiercest critic was leader of a research group in the JIC that had discovered the promoter has a 'recombination hotspot', a breaking point that makes it much more likely to recombine. Now, two years later, the same group admits the need to avoid recombination hotspots such as that in the CaMV 35S promoter as well as the 'origin of replication' in the plasmid serving as vector for the GM construct, which is also often integrated 'accidentally' into GM crops.

The authors of the second report also suggest the development of 'clean DNA' technology as a possible solution to the problem. But that amounts to pronouncing the death sentence for all GMOs. All GM crops currently on the market or under review contain the CaMV 35S promoter and many, also the plasmid backbone, including the origin of replication.

The Director of JIC and the scientists concerned have issued a strongly worded denial since our press release. (Readers can judge if our reading was justified from the full texts reproduced in ISIS' press release.) Apart from attacking ISIS, and repeating all the objections that we have already replied to in detail in published papers, they said essentially that the issue was one of agronomic performance and not safety. Furthermore, they claim that their transgenic rice lines are stable. We beg to differ, and do watch out for our reply.

Joe Cummins has replied personally to the tired old point they raise and again, that people have been eating lots of CaMV in infected cabbages for thousands of years without ill-effects. He writes, "It is worth pointing out that virus infected crucifers are not tasty items and they are avoided by most animal predators including humans. JIC may be exceptional in consuming large quantities of virus infected crops and certainly those laboring at the institute are worthy of fuller study"!

(We thank Mark Griffiths for drawing our attention to the items in the JIC Report. For the detailed quotes from the JIC report see ISIS Press Release www.i-sis.org.)

1. Harwood, WA, Hardon J, Ross SM, Fish L, Smith J and Snape JW. Analysis of transgenic barley in a small scale field trial. *John Innes Centre & Sainsbury Laboratory Annual Report* 1999/2000, p.28.

2. Christrou, P, Kohli A, Stoger E, Twyman R, Agrawal P, Gu X, Xiong J, Wegel E, Keen D, Tuck H, Wright M, Abranches R and Shaw P. Transgenic plants: a tool for fundamental genomics research. *John Innes Centre & Sainsbury Laboratory Annual Report* 1999/2000, p.29.

MWH, AR & JC

ISIS News 9/10, July 2001, ISSN: 1474-1547 (print), ISSN: 1474-1814 (online)

Scrambled Genome of Roundup Ready Soya

New 'event-specific' molecular characterization devised by Belgian government scientists "highly important in the context food safety control". Monsanto's Roundup Ready soya was found to have scrambled and unknown genes. Is it legal? Is it safe? ***Dr. Mae-Wan Ho*** *and* ***Prof. Joe Cummins*** *report.*

Belgian government scientists point out that as labeling of foods containing GM ingredients is mandatory in all European member states, there is urgent demand for reliable and easy identification methods. Furthermore, in both Europe and the United States, companies seeking to market a GMO must submit a technical dossier, and any new and relevant information has to be brought to the attention of the competent authorities.

Up to now, however, most GMO identification methods depend on detecting gene fragments and sequences that are *within* the construct, but does not allow anyone to "discriminate between different GMOs containing the same insert and to identify different GMOs containing different copy numbers of the same insert." The scientists stress the need for "*event specific*" identification and analysis, in other words, *a method that gives unequivocal identification of the specific transgenic line that has resulted from a single transformation event. It also requires that the transgenic line must be genetically stable in the first place.*

With that in mind, they have devised a method for characterising the junctions between plant and insert DNA. We welcome the idea that such a detailed characterisation should be included in the technical dossier, which can be used to check the accuracy of the technical dossier submitted by the company. Monsanto's Roundup Ready soya was analysed as a test case.

Monsanto's technical dossier submitted for commercial approval claimed that RR soya had a single insert with the intended order of genes. It turns out not to be the case. Not only is the gene order of the insert itself scrambled, the plant DNA at the site of insertion is also scrambled, and there is a large 534 bp fragment of unknown origin in there as well.

The scientists, however, let Monsanto off the hook by suggesting that the genome scrambling took place *at* insertion. But there is not a shred of evidence for that. Either Monsanto was wrong or presented false data; or there must have been secondary scrambling. Either way, both Monsanto and our regulators were culpable in the approval process.

They state, "In the future, problems concerning the inaccurate description of transgene events can be avoided through a detailed characterisation of the transgene plant DNA junctions submitted to the competent authorities. These characterisation of the junction regions will also give the best information for the development of line specific identification methods, which are at least from a European standpoint of high importance in the context of food safety control." That is precisely what ISIS has been pushing for since 1999 and before [2]. It also follows that RR soya must be withdrawn immediately for safety reasons.

1. Windels P, Taverniers I, Depicker A, Van Bockstaele E and De Loose M (2001). Characterisation of the Roundup Ready soybean insert. *Eur Food Res Technol* DOI 10.1007/s002170100336, © Springer-Verlag.
2. Ho, M.W. (1999). Biosafety Alert. Submission to Biotechnology Group fo the Trans-Atlantic Economic Partnership on the Molecular Characterisation Required for GMOs. www.i-sis.org

Questionable 'Stability' at JIC

Scientists at the UK John Innes Centre attacked ISIS for quoting their annual report indicating that transgenic plants are unstable and that the CaMV 35S promoter should be phased out [1]. They claim they have evidence that transgenic rice lines are stable. ***Mae-Wan Ho*** *shows how their claim is not borne out by their own evidence.*

Scientists at JIC assure us that the CaMV 35S promoter is safe on grounds that people have been eating cabbages infected with the virus all the time. We have shown in detail in at least two further publications [2,3] why the CaMV 35S promoter in the viral genome and the intact virus is not the same as the cut-out promoter in GM constructs integrated into transgenic plants. But the JIC scientists have persistently failed to cite our papers or to reply to them. It would be tedious to repeat our arguments here. So, let's look at the new point raised by one of them, Paul Christou, who claims their recent publication [4] shows transgenic rice lines are stable. Their claim to stability is not at all supported by the data presented.

The publication in question is a report of studies on 40 independent rice lines representing 11 cultivars, each containing 2 or 3 different transgenes (not 3 or 4 as it says in the text) delivered by particle bombardment. The transgenes were delivered either by cotransformation, ie, the genes

in two or more different vectors, or in the form of cointegrate vector, ie, all three genes linked together in one vector. The authors made the following claims.

1. "Approx. 75% of the lines (29/40) demonstrated Mendelian inheritance of all trangenes, suggesting integration at a single locus".
2. Levels of expression varied, but primary transformants showing high level expression of four of the transgenes, *gna* (snowdrop lectin), *gusA* (beta-glucuronidase), *hpt* (hygromycin resistance) and *bar* (tolerance to herbicide phosphinothricin) "faithfully transmitted these traits to progeny".
3. In the case of transgenes *cry1Ac* and *cry2A* (insecticidal endotoxins from *Bacillus thuringiensis* or bt), "transgene expression was stably inherited when primary transformants showed moderate or low-level expression".
4. The results show that "six transgenes (three markers and three insect-resistance genes) were stably expressed over four generations of transgenic rice plants".
5. The results showed that "transgene expression was stable in lines of all the rice genotypes" analysed.

Now, claims 4 and 5 are contradicted by claims 1 to 3 which indicates that at least 25% of the lines did not show Mendelian inheritance, a sign of instability, and that only some of the transformants gave stable expression in later generations. ***So we can dismiss claims 4 and 5 immediately, and concentrate on the first three. But even those are not borne out by the data presented. This seems to be a general problem with this publication. The text states what the data do not support, and I shall highlight some of the inconsistencies here.***

The experimental details are very sketchy. No genetic maps were given on the constructs or the plasmids used, nor whether any plasmid sequences are integrated into the transgenic plants. No genetic map of any of the inserts in the plant genome is given. Going by the information in Table 1 (p.391) and "Transforming plasmids" (p.389), I believe the 40 transgenic lines consisted of the following categories.

(a) Twelve lines from 5 cultivars transformed with the co-integrate vector containing three marker genes, all driven with the CaMV 35S promoter, but in which orientation, the paper does not say.
(b) Eight lines from 4 further cultivars co-transformed with *gna* driven by the maize ubiquitin promoter in one plasmid and *gusA* and *hpt* both driven by the CaMV 35S promoter in a second plasmid.
(c) Six lines from 2 of the 4 cultivars in (b) co-transformed with *gna* driven with the rice sucrose-synthase-1 promoter in the first plasmid and the same *gusA* and *hpt* second plasmid as in (b).
(d) Eight lines from two cultivars different from all the above, cotransformed with a plasmid carrying a bt toxin gene, *cry2A*, driven by the CaMV 35S promoter, and a second plasmid as in (b).
(e) Six lines from the same cultivars as (d), cotransformed with a plasmid carrying the bt toxin gene, *cry1Ac*, driven by the maize ubiquitin promoter and a second plasmid with *hpt*, driven by the CaMV 35S promoter.

There is little or no overlap between the transforming genes/plasmids and cultivars, so it is impossible to tell if all the transgenes behave the same in all cultivars. ***The claim in the title "multiple heterologous transgenes show similar behaviour in diverse genetic backgrounds" is unjustified, to say the least.***

The transgenic lines were characterised as follow.

- enzyme assays to detect *bar* and *gusA* expression
- western blots to detect the protein products of *crylAc*, *cry2A* and *gna*
- polymerase chain reaction (PCR) to detect the DNA of all six transgenes
- Southern blots to detect the plant genomic fragments that have integrated the transgenes after the genomic DNA is cut with restriction enzymes with *unique* sites in each of the transforming plasmids used. This means that for single copy of the non-rearranged insert at a single site, only one, or at most two bands should be present. The presence of more than two bands is an indication of repeats and rearrangments.

There are two kinds of transgenic instability, functional instability as in gene silencing, and structural instability as in rearrangement and loss of transgenes. Of the analytical methods used, only Southern blot is really informative on the structural instability of the transgenic lines. Southern blot data were presented on only four R3 lines from category (a) involving the cointegrate vector carrying three marker genes. Instability is also indicated whenever the progeny of transgenic lines deviate significantly from Mendelian ratios. ***However, and this is important, failure to significantly depart from the Mendelian ratio is not a sign of stability, unless corroborated by quantitative molecular data.***

On p. 390, it states that,

"Genetic analysis confirmed that all lines carried a single transgenic locus with one to seven copies of the three-gene construct." And

"R2 and R3 plants of lines K496-4 and K496-1 consistently lacked detectable GUS activity although the primary transformants showed moderate GUS activity, indicating that the *gusA* transgene had undergone silencing. Plants from C549-1 consistently lacked detectable PAT activity; however in this case the primary transformants also lacked PAT activity. Southern blot hybridisation of K496-4, K496-1 and C549-1 genomic DNA revealed banding patterns identical to those of the primary transformants showing that there had been no loss or rearrangement of the genes during transmission from Ro to R1. Stable inheritance of the *gusA*, *hpt* and *bar* transgenes was also found for the remaining (non-silenced) lines, from Ro through to R3 (Fig. 3)."

Figure 3 (p.392), the only Southern blot data presented, is on just four lines in the R3 generation. The first line, K496-4 represented by seven plants, indeed showed identical banding patterns in all lines probed with *hpt*, *gusA* and *bar*, but four or five bands are present and the banding pattern for each enzyme is different. These are signs of multiple repeats and rearrangements. More seriously, the intensity of the bands varied more than ten-fold, suggesting that some plants may have had deletions of multiple copies, or else other plants have had amplified copies. The second line, K496-3 represented also by 7 plants did not have any hybridisation signal for three of the plants, while one had at least 20 times the intensity of the banding pattern. Of the remaining three, one had twice the intensity of the other two. These plants cannot be said to follow Mendelian inheritance. The third line K496-2 is represented by 2 plants only, one with multiple banding pattern and the other without any bands, again, not quite 'Mendelian'. That leaves only the fourth line, K496-1, represented by two plants, which showed identical multiple banding patterns in both plants which are different for all the enzymes, again indicating repeats and rearrangments. ***On the most generous interpretation of the data, there is evidence that one out of the 12 transgenic lines in category (a) may be stable to the R3 generation.***

On p. 395, it claims that,

"With the exception of the line K495-1, PCR analysis confirmed a 3:1 Mendelian segregation in all lines at the DNA level. Many lines also showed Mendelian segregation for protein expression, but the ratio was distorted for *gusA* in lines K496-1 and K496-3 due to silencing… Line C549-1 carried a single integrated copy of the construct, but *bar* gene expression was undetectable even in the Ro plant. Further analysis showed that the CaMV 35S promoter driving *bar* in this line was rearranged.."

However, no data were presented to show Mendelian segregation for any of the lines.

With regard to the transgenic lines with *gna* driven by two different promoters in categories (b) and (c) , it states on p391,

"In most of the lines (11/14), the three transgenes segregated together as a single Mendelian trait (3:1 ratio), indicating cointegration of the two cotransforming plasmids at a single locus. Three lines showed an aberrant 1:1 ratio."

However, Table 2 (pp.394-5) contains data only on 6 lines in the R1 generation, of which one gave the aberrant 1:1 ratio. At R2 and R3, only 4 lines are left. ***So, at most, there is evidence to support the stability of 4 out of 14 lines. Even these are questionable, if the standards of Fig. 3 are to go by. Quantitative PCR or Southern blot should distinguish between homozygous and heterozygous positives, so the proper Mendelian ratio to use is 1:2:1, and not 3:1.***

The same criticisms apply to the data in Table 3 (p.396) on inheritance of the transgenes in categories (d) and (e) above. Of the 13 (not 14 as claimed in the text) lines analysed in R1, 5 failed to give 'Mendelian' 3:1 ratio. Four lines remained in R2 and by R3, only two lines were left. ***So again, there is evidence of stability for at most 2 out of 14 lines.***

And yet it states on p.391,

"We analysed 6 *cry1Ac*-transgenic lines. All lines showed 3:1 segregation for the transgenes in the R1 and R2 generations." ***According to Table 3, two of the lines did not give 3:1 ratio in R1 and none were analysed in R2.***

And on p. 392,

"Most seedlings from lines expressing Cry2a at high levels (M7-10, M7-12, M7-13 and M7-14) died within two weeks of emergence, probably due to the toxic effects of high-level endotoxin expression."

The western blots of Cry1Ac and Cry2A (Fig. 4 C-D, p.393) showed multiple extra bands, differing between plants in the same line, hardly an indication of stability.

Another factor that already biases the data towards apparent stability is that the seeds were germinated in the presence of hygromycin, so all plants that had lost the *hpt* gene or in which the *hpt* gene was silenced would have been eliminated.

A generous interpretation of the data presented would suggest that 7 out of 40 (18%) transgenic rice lines may be stable to the R3 generation.

1. "Top research centre admits GM failure" ISIS News7/8, Feb. 2001 www.i-sis.org
2. Ho, M.W., Ryan, A. and Cummins, J. (2000). Hazards of transgenic plants with the cauliflower mosaic viral promoter. *Microbial Ecology in Health and Disease* 12, 6-11.
3. Ho, M.W., Ryan, A. and Cummins, J. (2000) CaMV 35S promoter fragmentation hotspot confirmed, and it is active in animals. *Microbial Ecology in Health and Disease* (in press).
4. Gahakwa D, Maqbool SB, Fu X, Sudhakar D, Christou P and Kohli A. Transgenic rice as a system to study the stability of transgene expression: multiple heterologous transgenes show similar behaviour in diverse genetic backgrounds. *Theor Appl Genet* 2000: 101: 388-99.

Admission from John Innes Centre Annual Report 2000:
GM crops are unstable and prone to recombination

"Data from the 1998 trial showed that transgenic barley lines performed as well as non-transformed control plants and controls from tissue culture-derived parents for several agronomic traits, including yield. For other traits, a significant difference was seen between transgenic and control lines. The transgenic lines were significantly shorter and also slightly later flowering […]. ***When we examined the next generation of the same transgenic line in the field during 1999, there was evidence that the transgenic plants were more variable compared to the controls than those in the 1998 field trial. This could be because somaclonal variation, resulting from the tissue culture and transformation procedures, and was more obvious in later generations. These results show that transgenic lines need to be examined over a number of generations under field conditions to obtain the necessary data on transgene stability and agronomic performance. Further field trials […] combined with detailed molecular and genetic analysis will allow us to increase our understanding of the transformation process*** *so that we are better able to assess the long term effects of genetic modification."*

> Harwood, WA, Hardon J, Ross SM, Fish L, Smith J and Snape JW. Analysis of transgenic barley in a small scale field trial. *John Innes Centre & Sainsbury Laboratory Annual Report* 1999/2000, p.28.

"Analysis of junctions between genomic and transforming DNA, and between individual plasmid molecules at integration sites, demonstrates the ***predominance of microhomology-mediated illegitimate recombination events involving regions with secondary structure. One such region occurs in the CaMV 35S promoter, widely used to drive transgene expression in plants. The plasmid backbone provides other such regions, including the origin of replication [...]. The influence of transgene rearrangements on expression and silencing has been understated in the past,*** *but our research may allow improved construct design to discourage rearrangements and improve transgene-expression stability."*

> Christou P, Kohli A, Stofer E, *et al.* Transgenic plants: a tool for fundamental genomics research. *John Innes Centre & Sainsbury Laboratory Annual Report* 1999/2000, p.29.

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Citizens' Vigil Exposes Bad Science in GM Field Trial

Local inhabitants of the Scottish Highlands witnessed another GM crop fail in front of their very eyes. Scientists who have approved the UK farm-scale field trials should be held to account. ***Dr. Mae-Wan Ho*** *reports.*

The temperature has dipped below zero for short spells since the beginning of November when the first snow arrived. It snowed again days before Christmas and also turned very cold. The children were taking advantage of a sunny break to decorate a Christmas tree by the freezing pond, their laughter bright and sparkling as fresh ice.

The Munlochy GM Vigil (munlochyvigil@tiscali.co.uk) had started up spontaneously in August after a local march and rally in protest of the GM crop trials approved by the Scottish Executive. That was despite repeated veto by the local inhabitants and their elected representative,

the Highlands Council (see "Beware corporate takeover of organics" ISIS News 11/12 www.i-sis.org).

Some local people went to see Jamie Grant's Roskill Farm near Munlochy on Black Isle, where Aventis' GM oilseed rape was to be planted. While they were there, a tractor appeared without warning, and started sowing the seeds, at which point, a dozen of the locals walked on to the field and sat down in front of the tractor, putting a stop to the sowing. The next day, many more people gathered at the field, and again, some put themselves in the tractor's path. This time, eleven were arrested, ten of whom have been charged with aggravated trespass and are awaiting trial.

Since then, a constant vigil had been kept near the field. An encampment has grown up at the site. The decisive act must have been the "raising of the yurt" towards the end of September, which now forms the central feature of the camp. The original yurt, as explained to me, is a round Tibetan house with a wooden frame, covered with fur. The Munlochy version consists of a hemispheric plywood frame set on top of a cylindrical trellised wall, one and one-half metres tall, enclosing a space about four metres in diameter. The whole is draped with bright blue-green canvas. An opening at the top lets through the chimney connected below to a stove for cooking and to provide heating against the cold nights.

From the camp, a campaign has grown up to "stop the crop". More than 3000 signatures have been collected. Jamie Grant has tried to get them off the site, but the Highlands Council decided they had a right to be there in peaceful protest.

Chain-saw operator Anthony Jackson, thirty-ish with long-blond hair worn loose, and Nigel Mullan, 46, visual artist and sculptor, are two of the main 'vigilantes'.

"There's a minimum of two or three of us constantly at the vigil. Then there are 30 to 40 regulars, the same number of supporters, and hundreds of friends and donors." Anthony said.

Across the road from the camp is the closely watched GM oilseed rape field trial. And nothing has escaped notice.

At the beginning of October, the field was sprayed with the herbicide glufosinate ammonium, as the GM oilseed rape is engineered to be tolerant to the herbicide, and it has also been treated with a fungicide. "It has rained quite heavily since the sprays and the runoff is directly into the Munlochy Bay. But the scientists have avoided sampling the water in the Bay." Nigel said. There is plenty of evidence that glufosinate is poisonous to a wide range of wild-life, and causes birth defects, which is why the herbicide is not approved for commercial use.

But it soon became clear that all was not well with the GM crop either. It was severely stunted compared with the control and commercial crops planted side by side. The GM crop was a quarter to a fifth the height of the control and commercial crops, and was noticeably more sparse and had more weeds growing in it.

"The control crop has substantial leafage and a closed canopy, thus restricting the amount of light available for weeds to grow," explained Anthony and Nigel. There was much more variation among the plants in the GM crop. Many of the leaves had turned yellow or had yellow edges. And one of the plants in the GM field had started to flower, "probably four months early".

In other words, the crop was showing typical signs of the genetic instability that has plagued many other GM crops (see "Scrambled genome of RR soya" and other articles, *ISIS News* 9/10 www.i-sis.org). This alone would invalidate any findings from the field trials, making the entire exercise pointless, particularly in the light of the new European Directive governing deliberate release of GM crops (see below).

The GM oilseed rape fiasco was reported in the local Highland News at the beginning of December. Aventis' response was that although the varieties used are "very similar", the GM crop was of a "different" variety from the control, a fine example of Orwellian 'doublespeak'.

And no wonder, this particular GM oilseed rape was approved as "substantially equivalent" (to non-GM oilseed rape) by the Scientific Committee on Plants in Europe. But that was before the European Directive for deliberate release was substantially strengthened last year (see "Europe's new rules could sink all GMOs" *ISIS News* 11/12 www.i-sis.org). This change of reference makes the farm-scale field trials obsolete, because they are unlikely to pass muster for commercial approval at the end.

According to the report by the Agriculture and Environment Biotechnology Commission, the objective of the farm-scale field trials is not to find out if the GM crops are safe. Yield is also not a relevant measure, even though some farmer experiencing such a drastic crop failure might well commit suicide. Both those aspects have already been "approved by the regulatory authorities". The farm-scale field trials are not designed to answer all key questions about GM crops. Only

"some key indicators of biodiversity" will be monitored to see if there are differences between the two halves of each field.

"This obviously makes a complete mockery of the science involved." Anthony and Nigel rightly conclude. The scientists who have approved such crops should be held to proper account.
To see more of the excellent pictorial evidence provided by Munlochy GM Vigil, visit ISIS website www.i-sis.org

Transgenic Pollution by Horizontal Gene Transfer?
Landraces of indigenous maize growing in remote regions in Mexico have been found contaminated with transgenic DNA. Molecular analysis suggests horizontal gene transfer mediated by CaMV 35S promoter. ***Dr. Mae-Wan Ho*** *reports.*
Researchers in University of California Berkeley reported in the journal *Nature* [1] that indigenous landraces of corn, growing in remote regions of Mexico, have become contaminated by transgenic corn.

This raised general alarm for two reasons. Mexico is the centre of diversity for corn, and transgenic contamination could easily wipe out the landraces. The fact that landraces in remote regions are contaminated means that contamination of other crops could be far worse. It could destroy both organic and non-GM corn crops, which are much in demand across the world, as consumers are overwhelmingly rejecting GM products.

The mystery remains as to how the landraces could have become contaminated. There has been a moratorium on commercial planting of transgenic corn in Mexico since 1998, though transgenic corn has been shipped to Mexico and elsewhere in the developing world as 'food aid'. Could the contamination have arisen in the usual way by cross-pollination? Government-approved plantings of transgenic corn before the moratorium were at least 60 miles away.

Corn pollen is heavy, so it does not travel far by air, and is short-lived. The researchers suspect imported transgenic corn was handed out by a government agency as food, and may have been planted by the recipients near the traditional crops.

Dr. Ignacio Chapela, one of the co-authors of the *Nature* report tells me that a campaign to discredit their research has already begun.

The January issue of the journal *Nature biotechnology* [2] carries a report on what the critics are saying. Scientist Tim Reeves at the International Maize and Wheat Improvement Centre in El Batan, Mexico, suggests that the research finding was an artefact, and claimed to have found no contamination of indigenous landraces in their own study, yet to be published. On the other hand, scientists working for the biotech industry's public relations are saying the finding was not surprising at all. Vivian Moses of Cropgen, a UK group funded by industry, was reported to have said, "The paper shows, in essence, that genes move around in nature, and this is hardly news." And Val Giddings of the US industry group BIO: " Should we be shocked to discover gambling in a casino?" The critics are contradicting each other in their haste to discredit the research.

Another criticism from industry and proponents is that the molecular evidence failed to show that the complete transgenic insert was transferred, but only isolated fragments. So, cross-pollination could not have been involved.

The *Nature* report actually offers evidence of something much more serious and insidious than cross-pollination. The contamination could have been due to horizontal gene transfer, a process that cannot be prevented or controlled, once the transgenic plants are released into the environment (see Box).

What is horizontal gene transfer?
A cell can pick up pieces of genetic material directly from its environment, and instead of digesting it as food, ends up inserting the genetic material into its own genome. The genetic material picked up could belong to the same species or to unrelated species. This 'illicit' gene trafficking is called *horizontal* gene transfer, to distinguish it from the *vertical* transfer that takes place in reproduction when transfer is from parent to offspring.

Horizontal gene transfer across species barriers is a rare event in nature, especially in multi-cellular organisms. Foreign genetic material is largely broken down or otherwise put out of action. And even after it has become inserted into the genome, it can still be thrown out.

Genetic engineering consists to a large extent, of *artificial* horizontal gene transfer. New combinations of genetic material from different species are made (recombined) in the laboratory. The artificial constructs are designed to cross all species barriers and to jump into genomes. They are also structurally unstable, consisting of many weak links, and tend to break and rejoin incorrectly, or to join up with genetic material from other genomes. In other words, the process of genetic engineering has greatly enhanced the potential for uncontrolled horizontal gene transfer.

The researchers collected 3 corn-cobs of native, 'criollo' landraces from fields in each of two locations of Sierra Norte de Oaxaca in South Mexico, more than 20 kilometres from the main mountain crossing road. A cob contains 150 to 400 kernels, each kernel resulting from an individual pollination event. They also obtained a bulk grain sample, Diconsa, from local stores of the Mexican government agency that distributes subsidised food throughout the country. These seven samples were analysed for transgenic DNA using probes for a piece of genetic material, the cauliflower mosaic virus (CaMV) 35S promoter, which is in all transgenic crops planted or sold commercially.

Four of the six samples of criollo landraces tested positive for the CaMV 35S promoter, whereas cob samples from blue maize of Cuzco Valley in Peru and seed samples from historic collection in Sierra Norte de Oaxaca both tested negative. The bulk grain sample Diconsa tested strongly positive, as strongly positive as the Roundup Ready maize and Bt-maize from Monsanto, confirming that unwanted transgenic food is being dumped as 'food aid' in many countries.

The Mexican government independently found transgenic contamination of land races in Oaxaca as well as in another state. Analysis of individual kernels on a single cob found 3-10% had transgenes, similar to the level found by the Berkeley scientists.

Two of the four criollo samples that tested positive for CaMV 35S promoter also tested positive for another piece of genetic material, the terminator (T-nos) *from Agrobacterium tumefaciens*, as did the Diconsa sample. In a third that tested positive for CaMV 35S promoter, *Bt* gene sequence was present.

The researchers then analysed the sequences at the site of insertion of the transgenic DNA, next to the CaMV 35S promoter. Each sample yielded 1 to 4 DNA fragments differing in size. The sequences found next to the CaMV 35S promoter were diverse. Two sequences were similar to synthetic constructs containing regions of the *adh*1 gene found in transgenic maize currently on the market, such as Novartis Bt11. Other sequences represented the criollo maize genome, including retrotransposon regions, whereas others showed no similarity to any GenBank sequence. (GenBank is a public database of all the genes that have been sequenced in some 50 000 genomes.)

It is true that simple cross-pollination cannot explain the fragmentary, diverse nature of the transgene contamination, as the critics have pointed out. Instead, that is a sign of horizontal gene transfer and recombination. Of the transgenic construct breaking and joining up again inappropriately, with genetic material from the same species or other species. It is significant that all the contaminated samples had acquired the CaMV 35S promoter, with the rest of the transgenic insert either missing or recombined.

This finding is consistent with our warning in 1999 that CaMV 35S promoter has a recombination hotspot, where it tends to fragment and join up with other DNA, and is hence expected to enhance horizontal gene transfer and recombination [3-5]. One possible scenario for horizontal gene transfer is if insects were to visit transgenic corn and native corn in succession. They could feed on the transgenic corn, take up and carry fragmented transgenic material to the native landraces. The transgenic material then becomes incorporated randomly into the plant cells, some of which subsequently develop into corn kernels.

We have demanded all transgenic crops with CaMV 35S promoter to be immediately withdrawn in 1999. Since then, the researchers who have discovered the CaMV 35S recombination hotspot have recommended that the promoter should no longer be used [6], but fell short of calling for existing crops containing it to be withdrawn.

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3. Ho MW, Ryan A and Cummins J. Cauliflower mosaic viral promoter – a recipe for Disaster? *Microbial Ecology in Health and Disease* 1999: 11: 194-197.
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5. Ho MW, Ryan A and Cummins J. CaMV35S promoter fragmentation hotspot confirmed and it is active in animals. *Microbial Ecology in Health and Disease* 2000: 12: 189.
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