

## About the Author

My name is Roger Morton. I am a former molecular biologist of 10 years experience. I have worked in Australia, Europe and the USA. I retired from my position at an Australian academic institution 2 years ago and now work for a Commonwealth government department as an information technologist. I hold no pecuniary interests in any biotechnology companies nor am I employed by the biotechnology industry.

I am writing this response to a submission made by Dr Mae-Wan Ho of ISIS (Institute for Science In Society) to the GM Science Debate in a document titled "ISIS' Reply to ACRE's Response on Chardon LL (Revised & Updated)" (1) I make this submission as a private commonwealth citizen and receive no remuneration from this work.

## Horizontal Gene transfer?

Dr Ho seizes on any research that shows that horizontal gene transfer happens and then asserts that the dangers of this occurring are greater with GM plants than with conventionally bred plants. She bases this assertion on her belief that transgenic DNA exhibits behaviors inherently different from non-transgenic DNA. As I will show - at no time does she ever present any credible evidence to support this belief.

## DNA transfer to the Foetus?

In her GM Science Review submission (1) Ho cites a paper by Hohlweb and Doerfler (2) with the comment "*ACRE has also omitted to mention that transgenic DNA can pass through the gut and placenta into the blood stream ending up in some blood cells, liver and spleen cells, and some cells of the foetus and newborn. This work goes back to the early 1990s and is still continuing.*" [emphasis added]

The paper that Ho cites does not show that transgenic DNA can enter the blood stream and pass to the foetus. On the contrary this paper finds that:

*"mice have been continuously fed daily with Green Florescent Protein DNA for 8 generations and have been examined for the transgenic state by assaying DNA isolated from tail tips, occasionally from internal organs of the animals, by PCR. The results have been uniformly negative and argue against the germline transfer of orally administered DNA."*

This study also examined the fate of the naturally occurring Rubisco gene in the intestines of mice fed soybean leaves. I quote from the abstract to this paper *"plant-associated, naturally fed DNA is more stable in the intestinal tract than naked DNA"*.

Hohlweb and Doerfler also state that *"Rubisco gene-specific PCR products have also been amplified from spleen and liver DNA"*. Why isn't Dr Ho concerned about the consumption of Rubisco genes?

I am not sure why Dr Ho has quoted the 2001 Hohlweb and Doerfler paper. Perhaps it is because she has assumed that it is like these authors previous papers – the results of which she has previously quoted in other ISIS documents [for example (3)]. In fact the 2001 Hohlweb and Doerfler paper cites their previous research and then makes the comment "We have now chosen a natural scenario". So it is plain to see that the work of these authors now confirm what risk assessors have said in the past – data from artificial diets of large amounts of naked DNA is not relevant to the situation where DNA is consumed in foods.

### **ISIS redefine "Transgenic DNA"**

I would like to ask anyone reviewing any ISIS submissions to the GM science review to be very careful about how ISIS uses the term "transgenic DNA". When they use the term "transgenic DNA" to describe the type of DNA that was used in an experiment quite often the research article will in fact be about naked DNA. ISIS will justify this by redefining what is meant by "naked DNA".

At the ISIS web site (4) is the following definition:

'Naked' nucleic acids are DNA/RNA produced in the laboratory and intended for use in, or as the result of genetic engineering (Traavik, T. 1999).'

So Ho and her ISIS colleagues define Naked DNA to mean any DNA made in the laboratory for the purposes of genetic engineering. In other words according to ISIS scientists transgenic DNA = Naked DNA.

By this definition a transgene incorporated into a plant chromosome and covered with histones and other chromatin proteins is "naked DNA".

ISIS' definition of 'naked DNA' differs from what the general scientific community understand to be meant by the term 'naked DNA'. To the general scientific community 'naked DNA' is DNA from any source

(whether created by recombinant DNA techniques or not) that has been purified away from the large amounts of nuclear proteins that normally surround the DNA in the living organism. ISIS scientists do not appear to understand that transgenic DNA – DNA made by joining the DNA of two different organisms together in the laboratory – can be naked – when it is in a pure form in the laboratory or it can be non-naked – when incorporated into an organism. Similarly non-transgenic DNA can be naked – when someone purifies a sample of it in the laboratory – or it can be non-naked – when it is present inside an organism.

Why do ISIS affiliated scientists define naked DNA in this way? I have long wondered this myself. But now it is obvious to me. Now that Naked DNA and transgenic DNA are synonyms Dr Ho can make statements like this:

“scientific reports (5) dating back to the early 1990s had already indicated **transgenic DNA** could pass through the intestine and the placenta, and become incorporated into the blood cells, liver and spleen cells and cells of the foetus and newborn “ [Quoting ISIS from (6) Emphasis added]

In (6) ISIS references Doerfler and Schubert 1998 (5) – which is a study where large amounts of purified M13 bacteriophage were fed to mice. Is this a study which uses naked DNA. But because of ISIS redefinition of naked DNA to mean transgenic DNA Ho justifies her statement.

### **No regulations governing the release of Naked nucleic acids into the environment?**

ISIS claims on their web site that: “*There is no regulation governing the release of naked nucleic acids into the environment.*” (4) This is totally false. If naked nucleic acids are as ISIS defines – “*nucleic acids produced in the laboratory and intended for use ... in genetic engineering*” then they are totally regulated – that is the point of the UK's current GM science review is it not? – to see if the regulations are to be changed.

### **Putative DNA transfer to gut bacteria**

Dr Ho says “*Among the many ignored research reports on horizontal gene transfer submitted to ACRE [8] are some commissioned by the UK government showing that transgenic DNA in food has transferred to gut bacteria after only a single meal*”

This is the following unpublished reference: Netherwood et al (7)

In this paper the authors detect the DNA from GM plants in colostomy bags of people who have eaten GM soy. However, *“despite exhaustive attempts, [the authors] were unable to isolate the bacteria harboring the transgene by colony blot hybridisation or a PCR pooling strategy”*.

It is doubtful that this paper provides any evidence for transgenic DNA transfer to gut bacteria. In this paper the authors are suggesting that the DNA in the bacteria survives 6 passages through culturing but they could not isolate the individuals with the genes. For the DNA to survive 6 passages in the bacteria and still be detectable by PCR it would need to be incorporated into the bacteria and provide some selective advantage. Otherwise it would be diluted to nothing (0.05ml was diluted into 10mls at each subculturing – thus the original stock is diluted  $10^{13}$  fold - ie ten thousand billion fold). If the DNA was incorporated into the bacteria and provided a selective advantage then PCR pooling should have allowed the bacteria containing the gene to be isolated. Thus, I conclude that the PCR results were faulty. I note that the authors do not show any of the PCR results suggesting that they might be not very convincing. I also note that this paper has not been published suggesting it has been rejected at the peer review stage.

### **Agrobacterium and human cells**

The ISIS submission continues *“Agrobacterium was found to transfer genes into human cells in much the same way that it transfers genes into plant cells. This work has not been followed up, nor has ACRE called for further research.”*

I note that there is no reference for this work but it probably refers to Kunik et al 2001 (8)

This study shows that a naturally occurring bacterial species can insert DNA into human cells under laboratory conditions. Since *Agrobacterium* is a common soil microbe and since the *Agrobacterium* used in plant genetic engineering is not released into the environment how does the discovery of this fact alter the risks associated with GM crops? We have a situation where the environment is already full of potentially human-cell-transforming *Agrobacterium* and GM crops do not add to this population.

## The hazards of CaMV 35S promoter

Here Dr Ho refers to an earlier ISIS publication (9) which states:

*Kumpatla and Hall (10). analyzed a transgenic rice locus and confirmed that fragmentation and recombination occur frequently within the CaMV 35S promoter, but not in the wheat plant ubiquitin promoter used in another transgenic cassette. This indicates that the CaMV promoter is not like any other promoter. Six out of seven recombination junctions in the CaMV promoter map near the 19 basepair palindrome identified as a recombination hotspot by Kohli et al .*

To suggest that a recombination hotspot being found in the CaMV 35S promoter makes this promoter “not like any other” means the authors are ignorant of the fact that it is well known that recombination rates are higher in transcriptionally active regions – ie many promoters display the same properties – see Thomas and Rothstein 1989 (11) for an example.

It is also well known that transposable elements – natural and widespread genetic elements – cause recombination hot spots – for example Askew et al 1993 (12).

The ISIS submission refers to some published data (13) which suggested that “the transgenic DNA containing the CaMV 35S promoter may be fragmenting and promiscuously scattering throughout the genome”. ISIS do not point out that this data was later retracted by the journal (14).

ISIS then take this non-evidence further and suggest that “*promoters like CaMV, which shows a propensity to fragment, and could therefore jump around the genome, has the potential to cause cancer*”.

Does ISIS need reminding that the CaMV promoter we are talking about is inserted into PLANTS? What evidence can ISIS present that plants even contract cancer? Plants do not contract cancer. Even if they could – who cares if some crop plants have some sort of “plant cancer”? Cancer is not contagious. This is a blatant exercise in fear mongering.

Dr Ho attempts at blatant scare-mongering continue with the following comments – not backed up with any scientific argument at all:

*The two recent cancer victims of gene therapy uncovered within months of each other [22, 23, 24] should make us wary about eating anything that has transgenic DNA. ... GM constructs are similar, whether used for genetic modification of human cells in gene therapy or for genetic modification of plants and animals.*

Note that the bare statement at the end of this passage is not backed up by anything at all. Gene therapy vectors are nothing like genetically modified plants. People are eating genetically modified plants. They are not eating the vectors used to transform plants.

What relevance does gene therapy have? Let us compare gene therapy to eating GM crops.

1. Gene therapy. Infect someone with a virus designed to insert DNA into their cells in the hope that this might cure them from a deadly disease. This is risky because the DNA might insert in an anti-oncogene and cause cancer. This is why patients for gene therapy must have no other treatment options available before being considered.
2. Eat a GM crop. DNA enters stomach and small intestine. DNA digested into nucleotides. Nucleotides absorbed.

The process of making a GM crop is risky *for the individual plant cell* you are transforming because the transgene might go into a vital gene. However, it is not risky for some one eating the GM crop because the transgene behaves like all the other genes in the plant once it is inserted. Dr Ho is being very disingenuous here in trying to suggest that all "*GM constructs are similar*". She is trying to say that a virus engineered to infect DNA into a patient is the same as a soybean plant with a Bt gene growing in the field.

### **Transgenic DNA and transgenic instability**

Under the heading "Transgenic DNA and transgenic instability" Ho calls into question ACRES statement that "There is also no evidence in the literature to support the idea that transgenic DNA is inherently less stable than native DNA." and says:

*"Instability of transgenic DNA is so well known that it is a textbook topic, as I have pointed out to ACRE and other government science advisors time and again."*

Dr Ho has indeed pointed this out time and time again because I have read her doing so. However, pointing it out time and time again does not make it so. In her submission here Dr Ho fails to reference the text-book she is referring to. However Dr Ho has referenced Old and Primrose "Principles of Genetic Manipulation" in other ISIS publications in the context of "instability" (15).

An examination of the data documented in the text book chapter ISIS cites reveals that the data applies equally well to "natural" DNA as it does to transgenic DNA.

For example Old and Primrose refer to data about DNA molecules being unstable because of repetitive sequences. Old and Primrose says: "*A common feature of these deletions is the involvement of homologous recombination between short direct repeats*". I.e DNA is unstable if it has repeat regions.

Naturally occurring DNA molecules with repeat regions are also subject to recombination. A prime example being the so-called "junk DNA" of eukaryotes. This fact is revealed by the variation in the length of tandem repeats of repetitive DNA used as markers for gene mapping in eukaryotes. This fact provides the basis for the DNA fingerprinting technique known as VNTR. This technique can be used for DNA fingerprinting in plants (16) and human beings (17). This shows that that repeat regions in natural DNA are also subject to recombination. The fact some DNA is subject to recombination has nothing to do with whether the DNA is transgenic. It is dependant on the repetition of DNA sequences not on the transgenic nature of the molecule.

So even if some transgenes were structurally unstable this would not be any added risk over the risk already entailed in eating foods since structurally unstable sequences are wide spread in all organisms.

Old and Primrose also talk about other situations where plasmid molecules are subject to deletions. It references data reporting deletions in NATURAL *E.coli* chromosomes. It also refers to instability due to the NATURALLY occurring transposable elements - nothing to do with transgenic molecules.

Another situation where deletions form mentioned by Old and Primrose is when attempts are made to express proteins at high levels in *E.coli* from chimeric plasmids. If the protein is toxic to the cell then there is high selection pressure to form mutants which have deletions of the chimeric plasmid. This is merely a process of natural selection - it is

due to the toxic nature of the gene product not to the "transgenic nature" of the DNA molecule. This has zero relevance to the situation in transgenic plants. Transgenic molecules producing non-toxic proteins are completely stable. (Witness the ability of scientists to exchange plasmids with each other and get the same results with them. Witness the ability of different sequencing labs to sequence shared clones and get 100% matches – a regular part of genome sequencing quality control.)

The fact is that transgenic DNA is stable except under exceptional circumstances. This fact would be understood by anyone who has worked with transgenic DNA in the laboratory because it would be based on everyday first-hand experience with plasmids.

Ho continues *"Furthermore, there is such a vast literature on transgenic instability [27] that ACRE's denial here is embarrassing. I will only cite the report by another group in JIC [28] documenting instability arising in later generations of transgenic barley lines:"*

Here Dr Ho quotes the "members only" section of the ISIS web site [her reference 27] so I have no way of following up on her further "data".

However, Dr Ho does quote one example (her reference 28) where transgenic lines of plants show unstable gene expression. However, Dr Ho confuses instability in gene expression in transgenic lines with instability of the transgenic DNA. So what if some transgenic lines show unstable expression? Only stably expressing lines are released – due both to regulations and commercial good sense.

Dr Ho then states *"Despite that, the only criterion on which ACRE asserts transgenic lines are stable is transgene expression."*

The vast literature on transgene instability Dr Ho mentions also refers to instability of transgene expression. So it would seem to be quite correct for ACRE to assess lines on the basis of stability of transgene expression. If what Dr Ho is worried about is the structural instability of the gene sequence itself then surely this is going to be reflected in instability of expression of the transgene – a re-arranged transgene does not express the gene product. So monitoring stability of transgene expression covers both situations.

*There is still no event-specific characterisation in successive generations to document true genetic stability. If the company*

*had submitted event-specific characterisation in its original application for commercial approval, it would be an easy test to carry out on Chardon LL maize today, to see if the insert has remained unchanged in structure and location in the plant genome.*

*Such a test was applied to Roundup Ready soya in 2001. Roundup Ready soya failed the test, the foreign insert was considerably scrambled compared to Monsanto's original submission (18).*

This is false. The paper by Windels et al (18) simply produces more sequence information on the transgene locus than Monsanto did in their original submission – Windels et al sequenced further out into the flanking DNA of the insertion site. The insertion site has always had “scrambled” DNA.

de Loose, one of the authors on this paper, has been quoted as saying “*the discrepancy was simply a case of technology now allowing scientists to examine DNA in more detail than previously. The product itself has not changed*” (19)

The Windels et al data is not evidence of instability of the transgene locus after the GM plant has been made and has no food safety implications. This gene rearrangement has always been in the transgenic line and has thus been subjected to numerous food safety tests.

The fact that there are rearrangements of the genome when a transgene is inserted needs to be compared to what happens in the genome of plants bred by conventional means. During conventional breeding there are many hundreds of recombination junctions introduced into plant chromosomes. Here are some examples (20, 21, 22). Are all these recombination junctions characterized in crops bred by conventional means? – No they are not. However, they are in GM crops. Ergo – GM crops are better characterized and more likely to be safe for consumption than non-GM crops.

Where is the increased risk?

### **ISIS “demolishes” criticisms ?**

In the GM Science Debate submission (1) under the heading “The hazards of CaMV 35S promoter” Dr Ho refers to criticism (23) of one of

her papers (24) on the supposed dangers of the 35S promoter and states "We published at least two further papers demolishing the criticisms" (25, 9).

I think it is up to others and not Dr Ho to decide if Dr Ho has "demolished" the criticism of their paper. Let's examine some of the arguments put forward in (25) and ask if they are demolition quality.

*"The intact, encapsidated CaMV, consisting of the CaMV genome wrapped in its protein coat, is not infectious for human beings nor for other non-susceptible animals and plants, as is well-known; for it is the coat that determines host susceptibility. So eating the intact virus is of little consequence."* [no reference given Quoted from (25) ]

What basis does Dr Ho have for this claim about the reasons for CaMV host specificity? What about when the virus de-coats itself for replication? If the viral coat protein is all that is stopping us coming down with CaMV then we all should have it by now because the virus is "naked" during its life cycle. It is totally bogus to suggest that the coat protein is the reason why CaMV does not infect human beings. CaMV is a plant virus and is specialized to infect plants. It contains an "aphid transmission factor" (26) - a protein involved in allowing it to transmit between plants via the aphids that suck on it. It contains a "movement protein" required for movement between cells via the plant plasmodesmata (26). Humans don't get attacked by aphids and they don't have any plasmodesmata to allow the transmission of CaMV. There is no risk that CaMV 35S promoter is going to cause human being to become infected with CaMV.

*"However, the naked or free viral genomes may be more infectious and have a wider host-range than the intact virus."* [Quoted from (25)].

This fact is irrelevant because we are neither talking about naked DNA nor a viral genome. We are talking about a small fragment of a viral genome (that contains no coding sequences) and we are talking about this fragment being inserted into plant chromosomes (ie non-naked).

Ho then attempts to rebut the criticism that recombination hotspots are not unique to the CaMV 35S promoter and exist in all plants by again quoting a text book by Old and Primrose (15). As I discussed above, the data from this text book is not relevant to the discussion because the phenomenon discussed in this text apply equally well to "natural" DNA as they do to "transgenic DNA". Ho then goes on simply

to reassert that the 35S promoter has a recombination hotspot. No attempt is made to actually address the criticism and show why this hot-spot makes plants containing it anymore dangerous than any other plant with a recombination hotspot. The criticism is not "demolished" – the criticism is essentially accepted since no data is offered to refute it.

*"The CaMV promoter in transgenic DNA is also quite different from the promoters of the plant's own genes (objection 3). Structurally, the plant's own promoters **will be expected to be much more stable than the CaMV promoter in transgenic DNA for the following reasons. First, the plant's genes and their promoters exist in an organized genome, where recombination is predominantly between homologous alleles, so each promoter will remain associated with alleles of the same gene after recombination.**" [Quoted from (25)]*

The fact is that that the 35S promoter will also exist in an "organized genome". Transgenes are made homozygous for the transgene before being released. Thus, recombination will be between homologous alleles with respect to the transgene too.

Next comes this bald assertion:

*"Second, each host gene and its promoter have been adapted to each other, structurally and functionally, in the context of the whole genome, for hundreds of millions of years, and **therefore expected to be much more stable than the transgenic DNA containing CaMV promoter.**" [Quoted from (25) emphasis added]*

This might be expected by Dr Ho, but I think we should be afforded some data to back this assertion up. Plant genomes modified by conventional breeding also have their million year old genome "messed up" – so why is it "expected" that they be more stable? In fact the degree of disruption of the genome is demonstrably lower in GM plants since only tiny fragments of new DNA are transferred compared to the huge amounts of foreign chromatin introduced during conventional breeding.

Ho then quotes Hull et al (27): *"most, if not all of the retrotransposons are no longer mobile."* What relevance does the non-mobility of most retrotransposons have as to whether or not transgenes are unstable. Transgenes are not mobile genetic elements. They have no ability to act like transposons. On the other hand some retrotransposons are

definitely active in plants – this makes the genomes of all crop plants “unstable”. But we have not suffered a nightmare disaster.

Ho then quotes Smith (28) as evidence that "*transgenic DNA is recognized to be unstable and to have a propensity for rearrangements and for horizontal gene transfer*". Smith's one page paper reports the fact that sometimes the whole binary plasmid and not just the sequence between the borders of the T-DNA will be inserted into transgenic plants. This speaks nothing of DNA rearrangements and nothing of horizontal gene transfer.

Next Dr Ho states "*Structural instability involving secondary mobility or rearrangement of integrated transgenes is also a common cause of breakdown of transgenic lines*" and quotes (29). Structural instability might be a reason why a transgenic line might be rejected in the early stages of development. However, Dr Ho can not present any evidence that this is a reason for “breakdown” of commercial transgenic lines. By the time a line is released it is structurally stable. The paper Ho references here points out "*complex integration sites undergo either structural instability such as intrachromosomal recombination between multiple copies, sometimes resulting in loss of the transgene*" (29). Thus, we are again talking about recombination with repeat sequences. This is exactly what happens with naturally occurring repeat sequences and contributes no added risk.

Dr Ho states "*In addition, gene expression cassettes include terminators that are also recombination hotspots (as discussed in ref (30).*" [quoted from (25)]. I am glad Dr Ho is making this point because it makes my point for me. Gene expression cassettes do indeed contain transcription termination sequences – as do every single gene in every organism on the planet. So according to Dr Ho we should be frightened of gene expression cassettes because they are like every other gene on the planet.

Ho continues:

*"Hull et al (27) are mistaken to suppose that the CaMV promoter has to be placed exactly next to a gene in order to make it over-express. In a recent experiment in insertion mutagenesis using a synthetic mini-transposon, researchers discovered an event resulting in the over-expression of a host gene which is 164 basepairs away from the site of insertion"* (31).

It surprises me that Dr Ho does not understand that in the scale of genomes (Arabidopsis is 125 million basepairs) 164 basepairs IS EXACTLY NEXT to a gene.

*“Although CaMV 35S promoter and promoters of animal viruses do not have the same base sequence, they have at least one element (the TATA-box) in common. It is therefore possible therefore, for host protooncogenes and proviral sequences to become activated and reactivated.” [quoted from (25)].*

Here we are asked to believe that, because the 35S promoter has a TATA box, that makes it dangerous – it might activate protooncogenes. Is Dr Ho aware that nearly every gene in every plant and animal that humans consume contains a TATA box? By this reasoning every food we eat is dangerous.

The last part of the article (25) continues with more speculation along these lines. For example the 35S promoter is modular and therefore supposedly dangerous. All promoters are modular Dr Ho. Are all promoters dangerous?

This speculation about how the presence of the 35S promoter in plants increases the chances of the activation of oncogenes is meaningless when you consider that plants are already full of promoters and sequences that could do exactly the same thing. The addition of the 35S promoter does not add to this risk anymore than does introducing a new promoter by crossing a plant with another plant.

## **Conclusion**

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14 *Nature* 2002 416(6881) page 1 “Nature has concluded that the evidence available is not sufficient to justify the publication of the original paper.”

15 See <http://www.i-sis.org.uk/chardonLLtranscript.php> where ISIS references Principles of Genetic Manipulation: R.W. Old and S.B. Primrose 5th edition (1994), chapter 8, page 164 - Structural Instability. This text is also referenced in (25)

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