REPORT FOR THE CHARDON LL HEARING

HAZARDS ARISING FROM THE USE OF THE CaMV 35S PROMOTER IN GENETIC ENGINEERING

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INTRODUCTION

Most genetically modified crops now being grown contain a stretch of genetic material, the 35S promoter, from the cauliflower mosaic virus, hereafter referred to as the CaMV 35S promoter. Some scientists claim that this foreign introduction is harmless and cite the fact that human beings have been eating cauliflowers and other vegetables of this family, together with this virus, for many years without ill effects. Other scientists point out that the virus as used in genetic modification is no longer intact and, in this fragmented form, has the potential to cause harm by spreading to other plant species and even to animal species, including human beings. A grave consequence could, over time, be the creation of new, highly invasive and highly virulent diseases of mankind.

The debate between the scientists has come to a focus in a series of three papers published in the journal *Microbial Ecology in Health and Disease*. The principal arguments of these three papers, and also of a more recent paper, will be described.

The papers under discussion are (in the reference style of Chardon LL Hearing Documents (CHD)):

Paper 1: (CHD vol. 8, near end)
Mae-Wan Ho, Angela Ryan and Joe Cummins,
'Cauliflower Mosaic Viral Promoter --- A Recipe for Disaster?',
1999, *Microbial Ecology in Health and Disease*, **11**, 194-197)

Paper 2: (CHD vol. 16, about 2/3 way into the volume)
R. Hull, S.N. Covey and P. Dale,
'Genetically modified plants and the 35S promoter: assessing the risks and promoting the debate',
2000, *Microbial Ecology in Health and Disease*, **12**, 1-5)

Paper 3: (CHD vol. 8, near end)
Mae-Wan Ho, Angela Ryan and Joe Cummins,
'Hazards of transgenic plants containing the cauliflower mosaic viral promoter',
2000, *Microbial Ecology in Health and Disease*, **12**, 6-11)

In addition, reference will be made to a fourth paper:

Paper 4: Mae-Wan Ho, Angela Ryan and Joe Cummins,
'CaMV 35S promoter fragmentation hotspot confirmed, and it is active in animals'
2000, *Microbial Ecology in Health and Disease* (in press).

RELEVANT CONCEPTS FROM MOLECULAR BIOLOGY

This section is provided for reference.

A *base* (in the context of genetic structure) contains nitrogen in one or two ring-shaped structure(s). There are five such bases, denoted by the letters A, C, G, T and U. Chemical bonding produces *base-pairs*: A couples with T (in DNA) or with U (in RNA), and C couples with G.

A *nucleotide* consists of a base, a sugar containing 5 carbons, and one or more phosphate groups. The carbons are numbered, and these numbers with a 'prime' mark attached are used to identify the positions of the carbons and also to distinguish the two ends of a chain of nucleotides: 3' end and 5' end.

DNA (**d**eoxyribo**n**ucleic **a**cid) is a double-stranded, helical chain of nucleotides. It is usually extremely long. Each strand can be used as a template to form a complementary strand of RNA (*i.e.*, can be *transcribed*), with the aid of an enzyme called *RNA polymerase*. Each nucleotide is identified by the letter describing the base it contains: A, C, G, T or U. It is the sequential ordering of these four letters that encodes genetic information.

RNA (**r**ibo**n**ucleic **a**cid) is formed by transcription of DNA, each nucleotide of the DNA being transcribed into the complementary nucleotide: A into U, and C into G.
One type of RNA, messenger RNA or mRNA, is then *translated* to produce proteins.

A *gene,* in traditional genetics, is a region of DNA that functions to *express* a particular characteristic of the host organism. More than one gene, however, may be involved in the expression of a given characteristic; and, conversely, a combination of genes may function together to express a characteristic. If a gene or combination of genes is removed from the normal location and re-positioned within the DNA, it can function in a different manner and express different characteristics.

A *genome* is the totality of genetic information in a cell or organism; or, alternatively, it is the DNA carrying this information.

A *promoter* is a section of DNA containing the site at which RNA polymerase binds to the DNA to begin the process of transcription; it is also the site for the binding of regulatory proteins. The rate of transcription is controlled here.

A *plasmid* is a small, circular DNA chain that is capable of independent replication.

A *vector* in genetic engineering is a plasmid, *phage* (*i.e.*, a *bacteriophage* --- a virus (see below) that infects a bacterium and destroys it) or other virus into which foreign DNA is inserted for introduction into other cells.

A *construct* is a 'package' of the engineered gene(s) by themselves, or spliced into a vector.

A *virus* is a minute, invasive, intracellular parasite consisting of a core of nucleic acid, which may be double-stranded or single-stranded DNA or RNA, surrounded by a protein coat and, in some viruses, a further envelope. Viruses are unable to multiply or express genes outside the host cell and require host cell enzymes to aid DNA replication, transcription and translation.

A *retrovirus* is a virus that contains RNA and, contrary to the 'central paradigm' of molecular biology, replicates itself by reverse transcription (hence the prefix of the name) of RNA into DNA; the latter is then integrated into the host genome.

A *pararetrovirus* contains DNA and does not integrate into the host genome.
Cauliflower mosaic virus (CaMV) is a double-stranded circular pararetrovirus. It contains more than one promoter; the one of concern in this series of publications is the 35S promoter.

A *transposon* is a unit of DNA capable of moving within the same chromosome or to another chromosome in the same cell. Such transposition can result in the inactivation of genes into which the transposable elements become inserted and can also result in changes in the expression of nearby genes. Permanent insertions in germline cells result in heritable mutations.

A *retrotransposon* is a transposable element that becomes replicated in another piece of DNA by being first transcribed into RNA, which is then reversely transcribed into DNA; and this DNA is then inserted into the target DNA.

PAPER 1: A RECIPE FOR DISASTER?

(CHD vol. 8, near end: Mae-Wan Ho, Angela Ryan and Joe Cummins, 'Cauliflower Mosaic Viral Promoter --- A Recipe for Disaster?', 1999, *Microbial Ecology in Health and Disease*, **11**, 194-197)

On the basis of work on the cauliflower mosaic virus promoter presented by various other authors, this first paper of the series was published by Mae-Wan Ho, Angela Ryan and Joe Cummins. The message of this paper is that the genetic fragment of the cauliflower mosaic virus (CaMV) as used in genetic engineering poses grave hazards.

It is known that the promoter has three domains, including a region that operates mainly on leaves. The special importance of this region in the present context is that it contains subdomains, which are able not only to operate independently of one another but, taken together, can produce effects that are more than the sum of the effects of the individual subdomains. The modularity of the promoter allows various pieces of the promoter to be manipulated individually in order to construct hybrid or combination promoters. This has already been done in the laboratory, and it raises the possibility that recombination of the promoter elements with dormant viruses in transgenic plants could create new, infectious viruses.

The hazard of new viruses created through the use of the CaMV 35 S promoter is further enhanced by the fact that their action would not be confined to the species that are infected by CaMV in its natural form. The viral coat is removed before the promoter is inserted into a transgenic plant; and it is the coat that makes the virus specific to a small range of hosts when it is intact. The promoter shorn of the viral coat is capable of invading a range of other plant species. In new, alien genetic environments, the effects of the promoter cannot be foreseen. Whatever the effects may be, the subsequent impact on other plants and, indeed, on animals (including human beings) is even more unpredictable; the same authors report in Paper 4 that the CaMV promoter is active in animal and human cells.

The CaMV 35S promoter has been demonstrated to possess a 'recombination hotspot', a position at which other DNA can readily interact with and become joined to the promoter. This results in a high frequency of recombinations. It is not even necessary, as in normal recombination, that the genetic elements of the foreign DNA should be lined up in a sequence similar to that of the promoter: illegitimate recombinations between dissimilar sequences of DNA readily occur. Not only are previously unknown recombinations possible, but the fact that the promoter is so strong may cause cells continuously to overexpress the functioning of their genes, causing continual stress to the host. Overexpression of certain genes results in cancer.

A further danger arises from the fact that transgenic lines are known to be unstable. The high activity of the CaMV promoter, which is due to its hotspot, is likely to make its recipients even more unstable than those containing milder promoters.

The very properties that make the promoter so useful for getting foreign genes into plants and over-expressed also make it highly likely that secondary recombination events will be facilitated and that strange genes will be mobilised out of those plants and more widely into the environment. The CaMV promoter could interact with dormant viruses already in the plant to create new viruses that could result in new diseases. Particular concern centres on the similarity between the close genetic relationship of CaMV to viruses like hepatitis B, which affect human beings.

When transgenic potatoes were fed to rats in a laboratory by Dr Arpad Pusztai and his collaborators to determine whether the genetic modification would have any effect on the rats, the internal organs (examined after the rats were killed at the end of the experiment), were found to have been adversely affected. The potatoes contained T-DNA from *Agrobacterium* (the most commonly used vector for plant transformations) and also the CaMV 35S promoter. The conclusion was that 'a significant part of the toxic effects of transgenic potatoes with snowdrop lectin was due to the "construct or the genetic transformation (or both)"'.

In summary, the highly invasive nature of the CaMV 35S promoter and its possession of a 'hotspot' for recombination, even with normally unsuitable DNA, make it very probable that it will be transferred horizontally, *i. e.*, by direct contact with other organisms, and that it will cause unpredictable, large-scale rearrangements of genes. The authors urge that 'all transgenic crops containing CaMV 35S or similar promoters which are recombinogenic should be immediately withdrawn from commercial production or open field trials. All products derived from such crops containing transgenic DNA should also

be immediately withdrawn from sale and from use for human consumption or animal feed.'

PAPERS 2 AND 3

PAPER 2: THE CRITICS RESPOND
(CHD vol. 16, about 2/3 way into the volume): R. Hull, S.N. Covey and P. Dale, 'Genetically modified plants and the 35S promoter: assessing the risks and promoting the debate', 2000, *Microbial Ecology in Health and Disease*, **12**, 1-5)

PAPER 3: REPLY TO THE CRITICS
(CHD vol. 8, near end): Mae-Wan Ho, Angela Ryan and Joe Cummins, 'Hazards of transgenic plants containing the cauliflower mosaic viral promoter', 2000, *Microbial Ecology in Health and Disease*, **12**, 6-11)

These two papers will be discussed simultaneously, each objection raised by the critics being immediately followed by the rebuttal. For convenience, the critics' arguments are numbered here, although no such numbering was applied in the paper itself. The ordering is that in which the objections appear in Paper 2. Objections that are essentially repetitions of previously stated objections in Paper 2 have not been repeated.

1a) Paper 2, page 1, column 2, paragraph 2, line 4
page 4, column 2, paragraph 2, line 1
'A survey of a local market ... showed that about 10% of the cauliflowers and cabbages were infected with CaMV. ... The virus infects most cells of the plant and produces about 105 particles per cell.'
The concluding paragraph of the paper begins:
'From the arguments above, there is no evidence that the CaMV 35S promoter will increase the risk over those already existing from the breeding and cultivation of conventional crops.'

1b) Reply from Paper 3, page 7, column 1, paragraph at bottom, line 1
'The intact, encapsidated [coated] 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 in the first instance. So eating the intact virus (objection 1 above) is of little consequence. However, the naked or free viral genomes may be more infectious and have a wider host-range than the intact virus. Human T-cell leukemia viral genomes formed complete viruses when injected into the bloodstream of rabbits... .Similarly, the genomes of the human polyomavirus BK (BKV) gave a full-blown infection when injected into rabbits, despite the fact that the intact BKV is not infectious ... . [I]t is recently found that integrated viral sequences in genomes of dead cells are much more readily transferred horizontally to the genomes of live cells that have taken up the fragmented DNA ...

'There is a world of difference between viral genomes containing the CaMV 35S promoter as an integral part of the virus --- adapted to the virus and to the host over millions of years of evolution --- and the CaMV 35S promoter taken out of context, joined up with a strange gene and inserted into a strange genome.'

2a) Paper 2, page 2, column 2, paragraph 2, line 1

'The one group of animal pararetroviruses, the hepadnaviruses, contains the human-infecting hepatitis B virus... . These viruses have a very different genome organisation to plant pararetroviruses, and ... there are major differences ... in the details of the replication mechanism ... .'

2b) Reply from Paper 3, page 10, column 2, paragraph 1, line 1

'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 [a section abounding in TA base-pairs, where transcription to RNA begins]) in common, if not more. It is therefore possible ... for host protooncogenes and proviral sequences to become activated and reactivated ... . Also, completely new cross-species viruses may arise from recombination between elements and motifs of the CaMV 35S promoter and those of animal viruses, dormant or otherwise ... .'

3a) Paper 2, page 2, column 2, paragraph 4, line 8

Retroviruses, pararetroviruses and retrotransposons are highly co-ordinated structures, and 'perturbation of the sequence leads to loss of infectivity or functionality. ... In most cases, it is only when closely related sequences are added to or exchanged within the genome that viability is retained.'

3b) Reply from Paper 3, page 6, column 1, paragraph 2, 4th line from bottom

'The suspected mechanism of recombination [of the CaMV promoter] --- double-stranded DNA break repair --- requires little or no DNA sequence homologies. Finally, recombination between viral transgenes and infecting viruses has been demonstrated in the laboratory'.

[Comment by EN: Note the words 'In most cases' in the objection, implying that sometimes the sequences need not be closely related.]

4a) Paper 2, page 2 , column 2 , paragraph 5, line 3

'[T]here are several different human retroviruses and no instance of recombination has been found between them. This indicates that there are many constraints on natural recombination.'

4b) Reply from Paper 3, page 7, column 2, paragraph 3, line 1

'As Hull et al. ... emphasize, there are many constraints on natural recombination, and natural recombination between viruses is very rare ..., possibly because each viral genome has a natural integrity and stability resulting from millions of years of evolution. The 35S promoter in the virus does not transfer into genomes because pararetroviruses like CaMV, do not need to integrate into host genomes to complete their lifecycle;... . Nevertheless, some pararetroviral sequences have been found integrated into plant genomes ... .'

5a) Paper 2, page 3, column 1, paragraph labelled 'a', line 1

'[T]here are more than 105 copies of the 35S promoter in each cell of a plant naturally infected by CaMV, in contrast to the one to a few copies of the 35S promoter in each cell of transformed plants. ... In spite of these high numbers of both 35S promoter and retrotransposons no cases of natural recombination leading to new viruses have been found in spite of intensive research on these virus groups.'

5b) Reply from Paper 3, page 8, column 1, paragraph 1, 1ine 11

'A ... report stated that recombination between transgenes and infecting virus in CCMV [cowpea chlorotic mottle virus] was much more frequent than recombination between co-infecting viruses ..., despite the fact that transgenic sequences occur at very low concentrations with co-infecting viruses ... .'

'As all the experiments involved recombination between defective virus and transgene, it was thought that under natural conditions, when viruses are not defective, no recombinant viruses would be generated ... .' However, such recombination was demonstrated and at least one such recombinant virus 'was more virulent than the wild type'.

'Recent findings ... indicate that the viral RNA-dependent RNA polymerase' of several types of virus can recognise a certain region of the transgene mRNA and 'use them as transcription promoters ... . These findings have important implications for the safety of viral resistant transgenic plants in general.'

'It has been noted in experiments involving CaMV ..., that the frequency of recombination is much higher than for other viruses.'

page 7, column 2, paragraph 4, line 1

'It is ...clear that recombination between viral transgenes and infecting viruses can occur. A number of studies have demonstrated that plant viruses can acquire a variety of viral genes from transgenic plants. It indicates that the viral transgene, isolated from the virus and integrated into the host genome, cannot be equated with the same gene in the virus itself. ...

page 7, column 2, paragraph 5 (last), line 1

'Defective red clover necrotic mosaic virus lacking the cell-to-cell movement gene, and hence not infectious, recombined with a copy of that gene in transgenic Nicotiana benthamiana *plants to regenerate infectious viruses. ... Transgenic* Brassica napus *[oilseed rape] containing CaMV gene VI ...recombined with a complementary part of the virus missing that gene ..., and gave infectious recombinants in 100% of the transgeneic plants.'*

Two other experiments are mentioned: one produced 'infectious recombinants that expanded the host range of the virus' and in the other a segment of a gene 'recombined with defective virus missing that gene.'

7a) Paper 2, page 3 , column 1 , paragraph labelled 'b', halfway down

'There is uncertainty concerning the stage of transformation at which the recombination described by Kohli *et al*. ... occurred. [The experiments of Kohli *et al*. on transgenic rice identified the CaMV 35S promoter as the 'hotspot' at which many illegitimate recombinations took place.] They did not distinguish between recombination taking place during the process of transformation and recombination taking place after the sequences had been integrated. There is accumulating evidence of rearrangements of DNA during transformation ... . In most cases, these rearrangements result in the non-functioning of the transgene and are selected out in the early stages of analysis of the properties of the transformed lines. Furthermore, the construct used for transformation by Kohli *et al*. had

three copies of the 35S promoter, one in inverse orientation in relation to the other two. The presence of repeated sequences in transformed integrants, and especially inverse repeats, also tends to lead to gene silencing ..., a condition which would be selected against in developing the transgenic line.'

7b) Reply from Paper 3, page 9, column 1, last paragraph

'It is quite likely that stacking CaMV promoter in three successive expression cassettes, as Kohli et al. … have done will increase structural instability …. The recombinations and rearrangements they have observed in the different transgenic lines, however, may have occurred both before and after the primary transformation events, during propagation and selection in cell and plant culture. This is something that must be addressed by empirical observations.

[On the subject of stability:] page 9, column 1, paragraph 3, line 1

'Structural instability of transgenes is generally underestimated, as cells which lose transgenes are automatically eliminated during the selection process necessary for producing transgenic lines. However, instability may arise even in later generations of plant propagation ... . We are aware of no published data documenting the structural stability of transgenic lines in successive generations, even though phenotypic instability has been documented, for example, in transgenic bt-cotton commercially grown in Southern United States in 1996 ..., and Roundup cotton in 1997 ... . Physiological stress due to extremes of temperature, or drought, which can mobilize transposons, may increase transgene instability. The constitutive overexpression of transgenes linked to the CaMV 35S promoter, similarly is a metabolic stress-factor that may increase transgene instability ... .

[Comment by EN: Again, note the words 'In most cases' in the objection: 'In most cases, these rearrangements result ...'. Also note that it is left to 'selecting out' any undesirable properties that happen to become apparent during the trials: it is not as if all the transformants were uniform and as if the geneticists were certain in advance what the properties of the transformants would be. Dr Arpad Pusztai grew GM potato plants from a common origin and under the same conditions, yet found that each pot of potatoes had unique properties.]

8a) Paper 2, page 3, column 1, paragraph labelled 'c', line 2

For the 35S promoter in a transgenic plant to 'effect the activation of a dormant virus or create a new virus, the whole promoter would have to be either excised and reinserted precisely at the new site or its 3' end linked precisely with another gene.' The former possibility would require the existence of another hotspot, apart from the 19S hotspot, for which there is no evidence.

8b) Reply from Paper 3, page 10, column 1, paragraph 2, line 1

'Hull et al. ... may ... be mistaken to think that the entire CaMV 35S promoter has to be transferred before it can either lead to over-expressing of host genes or to reactivating or generating new viruses ... . On account of the modular construction of all promoters, it is already clear that many elements are common to many promoters, so much so that gene therapists are now making synthetic super-promoters by random recombination of different elements ... .[T]he transfer of parts of the CaMV 35S containing enhancer

or other elements into genomes may be sufficient to cause over-expression of genes or to reactivate dormant viruses or generate new viruses.'

page 9, column 1, paragraph 4, line 7:

'... [T]he transgenic construct typically contains multiple recombination hotspots. For example, most transgenic plants created with the Agrobacterium T-DNA vector will have transgenic DNA flanked by the left and right borders, both recombination hotspots. In addition, gene expression cassettes include terminators that are also recombination hotspots ... . So all or part of the transgenic DNA may be prone to horizontal transfer.'

page 9, column 2, paragraph 2, line 5:

'The integration of transgenic DNA into genomes is known to have many unexpected effects, including mutations, cancers (in the case of mammalian cells) and changes in DNA methylation, a chemical modification of DNA which can affect activities of host genes. The effects are known to extend far away from the site of insertion ... . Hull et al. ... 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 ... .'

9a) Paper 2, page 3, column 2, paragraph 1, at beginning and at end

'It is important to note that genetic recombination is a normal feature of conventional plant breeding and of all natural populations. ... Thus, recombination and hotspots for recombination are not unique features of the CaMV 35S promoter.'

9b) Reply from Paper 3, page 10, column 2, paragraph 2, line 1

'New research in our critics' own research institute ... [is] revealing how plants naturally resist viral infections by making small antisense RNA [RNA of a sequence complementary to a target gene, aimed at inactivating that gene] of 25 nucleotides against viral genes. Exactly the same mechanism is directed against transgenes to silence them ... . The authors remark that the gene-silencing "may represent a natural antiviral defence mechanism and transgenes might be targeted because they, or their RNA are perceived as viruses." So much for the claim that genetic engineering is just like conventional plant breeding.'

page 8, column 2, paragraph 2, line 1

'Our critics believe that the recombination hotspot of CaMV 35S promoter in transgenic DNA is not unique, as all promoter[s] contain recombination hotspots and recombination in genomes is a normal event ... .

'It is well-known, however, that pieces of DNA taken out of context and recombined in novel configurations are likely to be unstable, so much so that structural instability of artificial vectors for genetic engineering --- made by joining pieces of DNA from different viruses, plasmids, transposons and other sources --- is a topic discussed in a text-book on genetic engineering ... . This instability also renders the artificial vectors prone to recombination and rearrangement.'

'The CaMV promoter is joined to a gene it has never been linked to before, to form an 'expression-cassette'. Several expression-cassettes are often stacked in series, and spliced in turn to an artificial vector, most often T DNA , which is also known to be flanked by recombination hotspots ... . Such a structure typical of transgenic DNA is recognised to be unstable and to have a propensity for rearrangements and for horizontal gene transfer ... .

This is stated explicitly in a recent scientific report commissioned by the UK Health and Safety Executive.'

Paper 4, column 1, paragraph 2

'Kumpatla and Hall… 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.'

10a) Paper 2, page 3, column 2, paragraph labelled 'e', line 5

'If the transgenic food was cooked the DNA would be denatured and be very unlikely to renature in an operable form. The next major problem is that the DNA containing the promoter would be exposed to nucleases [enzymes that break down nucleic acids] both from the plant cells when they were disrupted and in the animal's gut. ... The DNA would then have to pass into the gut cells and integrate precisely to activate the animal sequence.'

10b) [This point was not addressed in Paper 3. EN supplies the following extracts from GM-Free*, vol. 1, no. 4, 1999.]* GM-Free *quotes the following source for the extract below: Forbes., J.M. et al., 1998, MAFF Report no. CS0116, p.5, "Effect of Feed Processing Conditions on DNA Fragmentation"*

GM-Free*, page 3, column 2, near bottom*

A Leeds University study was commissioned by MAFF to '"address the necessary treatments to GM material if risks are to be removed". The study tested DNA in animal feed crops after they were subjected in the laboratory to the types of processing used in commercial feed mills, to see whether the DNA survived in a viable form. The results showed that:

'DNA was not destroyed by silageing the crops. The authors comment that "if there is a significant risk of transmitting a transgene ... in the gut of farm animals, it would seem sensible not to use [sileaged] crops as animal feed".

'Dry heat and steam heat processing needs to be carried out at temperatures of 95 C for at least 5 minutes for the DNA to fragment. The authors comment that "It would not be usual for feed materials to be heated to more than about 85 C during normal processing and pelleting." In other words, normal feed processing by dry heat and steam would not *destroy GM DNA.'*

[The same article in GM-Free *continues, on page 4, column 1, paragraph 1, line 8]*

'Certainly, the human and animal food industries and their regulators have resisted labelling oils and other derivatives of GM crops on the grounds that there is no DNA present. They sometimes add that even if there is DNA present, it would be too fragmented to be "viable".

'However, an interview we conducted with Dr Gordon Wiseman of RHM Technology, of High Wycombe, Buckinghamshire, casts doubt on these assumptions. RHM has developed a test which can detect GM DNA even in highly processed foodstuffs such as oils and lecithin. Dr Wiseman says that all soy lecithin and about 50 percent of oil samples contain enough DNA for his company to test for GM material.

'We told Dr Wiseman that consumers had been led to believe that highly processed derivatives like oils and lecithin contained no "viable" DNA. He said, "I'm sure plenty of people would like to believe that. But it's not true. The fragmentation is not always complete."'

GM-Free *quotes the following source for the extract below:*
*Mae-Wan Ho***, Genetic Engineering: Dream or Nightmare**, *Bath, Gateway Books, 1998, p.15, who cites Schubbert, R. et al., 1994,* Molecular and General Genetics, ***242**, 495-504, "Ingested foreign (phage M13) DNA survives transiently in the gastrointestinal tract and enters the bloodstream of mice" and Wahl, G.M. et al., 1984, Nature, **307**, 516-520, "Effect of chromosomal position on amplification of transfected genes in animal cells".]*

GM-Free, *page 4, column 3, paragraph 3, line 1*

'... Dr Mae-Wan Ho of the Open University says the claim that DNA is easily digested by enzymes in our gut is "not true". She says, "The DNA of a virus has been found to survive passage through the gut of mice. Furthermore, the DNA readily finds its way into the bloodstream, and into all kinds of cells in the body. Once inside the cell, the DNA may insert itself into the cell's genome (genetic material) and create all manner of genetic disturbances, including cancer'"

11a) Paper 2, page 3, column 2, paragraph labelled 'e', 4th line from bottom

'Brassicas are not the only crops which contain pararetrovirus sequences. All banana varieties ...have multiple copies of the sequences of banana streak badnavirus integrated into their genomes ... . In spite of exposure of humans to these pararetroviruses there is no evidence of any ill effects from them even in countries such as Uganda where bananas are the staple diet and HIV is rife.'

11b) Reply from Paper 3, page 7, column 2, paragraph 3, line 1

'[T]here are many constraints on natural recombination, and natural recombination between viruses is very rare ..., possibly because each viral genome has a natural integrity and stability resulting from millions of years of evolution.

12a) Paper 2, page 4, column 1, paragraph labelled 'g', first and last lines

'Plants contain many secondary metabolites which have evolved to provide defense mechanisms against herbivores.' Some of these have been shown to be carcinogenic to rodents but apparently do not cause cancer in human beings, which seem to be more adapted to them. 'Thus the overexpression of normal genes is very unlikely to cause cancers.'

12b) Reply from Paper 3, page 9, column 2, paragraph 3 (last), line 1

'... [T]he possibility of new toxins and allergens arising cannot be easily dismissed[,] on account of both position effects due to random insertion of transgenic DNA and pleiotropic effects due to functional interactions with host genes. The suggestion that potentially carcinogenic compounds occur in abundance in natural plants ... is irrelevant. These foods have been eaten for tens of thousands of years, and compounds which are carcinogens in isolation may have very different effects when eaten in combination with all the other constituents of the food itself.'

13a) Paper 2, page 4, column 1, paragraph 5 (last), line 4

'It is well known that viral sequences recombine naturally and that the vast majority of these recombinants are unsuccessful. Very occasionally new viruses arise, this being one of the major ways by which viruses evolve. However, this recombination is between viruses which occur in plant cells at very much higher concentrations than those of transgenic sequences, whether they be promoters or the transgenes themselves.'

13b) [The reply has already been made in point 5b above.].

SUMMARY OF PAPERS 3 AND 4

page 1, paragraph 2, line 1

'To recapitulate, we pointed out that the CaMV 35S promoter is promiscuous in function, and works efficiently in all plants, as well as green algae, yeast and E coli' and animal cells, including human cells. 'It has a modular structure, with parts common to, and interchangeable with promoters of other plant and animal viruses. It also has a recombination hotspot, flanked by multiple motifs involved in recombination, and is similar to other recombination hotspots including the borders of the Agrobacterium T DNA vector most frequently used in making transgenic plants. The suspected mechanism of recombination --- double-stranded break repair --- requires little or no DNA sequence homologies. Finally, recombination between viral transgenes and infecting viruses has been demonstrated in the laboratory.

'Transgenic constructs are already well-known to be unstable, and the existence of a recombination hotspot will exacerbate the problem. Consequently, transgenic constructs containing the CaMV promoter may be more prone to horizontal gene transfer and recombination than non-transgenic DNA. The potential hazards include genome rearrangement, insertion mutagenesis, insertion carcinogenesis [causing of cancer by insertion of a transgene], the reactivation of dormant viruses and generation of new viruses ... .'

page 10, column 2, paragraph 3 (last), line 1

'In signing on to the International Biosafety Protocol in Montreal in January [2000], more than 130 governments agreed to implement the precautionary principle. The available evidence clearly indicates that there are serious potential hazards associated with the use of the CaMV 35S promoter. ... [We call for] the withdrawal of all GM crops and products containing the CaMV 35S promoter, both from commercial use and from field trials, unless and until they can be shown to be safe.'