Special Safety Concerns of Transgenic Agriculture and Related Issues

Briefing Paper for Minister of State for the Environment, The Rt Hon Michael Meacher

Contained use *versus* release to the environment

It is important to distinguish between *contained* use of transgenic organisms and their *release* to the environment. Contained use occurs inside a physical facility designed to prevent escape into the open environment. It can be controlled, in principle, and made as safe as possible (though the current regulation of contained use is far from adequate[1]). Release of transgenic organisms to the environment, by contrast, cannot be controlled nor recalled, which is why great care must be taken in advance of release.

Transgenic agriculture is new and raises special safety concerns[2]

The production of transgenic varieties - which features most prominently in genetic engineering agriculture - is a new departure from conventional techniques including selective breeding, mutagenesis (induction of gene mutations by chemical or physical means such as X-rays), cell fusion and tissue culture. It raises safety concerns different in kind from those of conventional techniques, and which are inherent to the processes used in creating transgenic organisms.

Typically, genes of one or more donor-species are isolated, and spliced into artificially constructed infectious agents, which act as *vectors* to carry the genes into the cells of recipient species.[3] Once inside a cell, the vector carrying the genes will insert into the cell's genome. A transgenic organism is regenerated from each *transformed* cell (or egg, in the case of animals) which has taken up the foreign genes. And from that organism, a transgenic variety can be bred. In this way, genes can be transferred between distant species which would never interbreed in nature.

The artificial vectors are typically made by joining together parts of the genomes of natural viruses that cause diseases and other genetic parasites, *plasmids* (pieces of usually circular DNA found in bacteria and yeasts, replicating independently of the chromosome(s)) and *transposons* (mobile genetic elements, or 'jumping genes' found in all species), which carry and spread genes for antibiotic and drug resistances, as well as genes associated with diseases. Most, if not all of the disease-causing genes will have been removed from the artificial vectors, but antibiotic resistance genes are often left in as 'selectable markers', so those cells which have taken up the foreign genes can be selected with antibiotics. While natural viruses and other genetic parasites are limited by species barriers to varying degrees, the artificial vectors made by genetic engineers are especially designed to cross species barriers and to overcome mechanisms in the cell that destroy or inactivate foreign DNA.

The foreign genes are typically introduced with strong genetic signals, *promoters* and/or *enhancers*, which enable the foreign genes to be expressed at very high levels continuously (or constitutively), effectively placing those genes outside the normal metabolic regulation of the cell, and of the transgenic organism resulting from the transformed cell. The most common promoter used in plants is from the cauliflower

mosaic virus (CaMV).

There are four special safety concerns arising from current transgenic technologies:
1. Effects due to the exotic genes and gene products introduced into the transgenic organisms.
2. Unintended, unexpected effects of random gene insertion and interaction between foreign genes and host genes in the transgenic organisms.
3. Effects associated with the nature of the gene-constructs inserted into the transgenic organisms.
4. Effects of gene flow, especially secondary, horizontal spread of genes and gene-constructs from the transgenic organisms to unrelated species.

Safety concerns of exotic genes

The exotic genes introduced into transgenic crops are often from bacteria and non-food species, and their expression is greatly amplified by strong viral promoters/enhancers. In practice, that means *all species interacting with the crop-plants* - from decomposers and earthworms in the soil to insects, small mammals, birds and human beings - *will be exposed to large quantities of proteins new to their physiology*. Adverse reactions may occur in all species, including immunological or allergic responses.

Herbicide-tolerance and insecticidal transgenic plants now account for 71% and 28% respectively of all transgenic crops in the world, with the remaining 1% carrying both traits.[4] These traits are associated with genes isolated from soil bacteria. The insecticidal bt-toxins, isolated from *Bacillus thuringiensis*, are often engineered into plants in a pre-activated form, and are already known to be harmful to bees directly, and to lacewings further up the food-chain. Another insecticide, the snowdrop lectin, engineered into potato, was found to be toxic to ladybirds fed on aphids that have eaten the transgenic potato.[5]

Because the bt-toxin genes are expressed continuously at high levels throughout the growing season, insect pests have already become resistant barely a few years after the transgenic crops were first released, so other pesticides have to be used.[6] This also deprived organic farmers of a biological pest control in the form of occasional sprays with suspensions of the soil bacteria producing the bt-toxins.

The safety of genes and gene products introduced into transgenic agriculture must be thoroughly assessed in advance. In particular, the introduction of vaccines and industrial chemicals into agricultural crops, including food crops should be banned, as it will have devastating effects on wild life and human beings.[7] An acceptable and feasible alternative is to engineer *cultured plant cells* for those purposes *under contained use conditions*.

Safety concerns of random unpredictability

The special safety concerns of unpredictability come both from the random, uncontrollable insertion of foreign genes into the host genome[8] and from the unpredictable interaction of exotic genes with host genes. Tranformations with the T-DNA from the Ti-plasmid of *Agrobacterium* have been the most widely used vector system for plants. The assumption is that only the T-DNA - located between left and

right borders in the Ti-plasmid - is inserted into the plant genome. However that has proven not to be the case; unintended transfer of parts outside the borders occur frequently.[9] Furthermore, T-DNA can be inserted in a truncated or rearranged form, in single copies or tandem repeats at one or more sites, perhaps reflecting the instability of the gene constructs (see below); and insertion mutagenesis (mutations of host genes due to insertion *within* the genes) is relatively common.[10] The inserted DNA may also influence other genes downstream or up-stream of it. For example, its strong promoter(s)/enhancer(s) may activate or inactivate host genes. Such influences are known to spread very far into the host genome from the site(s) of insertion.[11]

Interactions between introduced genes and host genes are bound to occur, as no gene functions in isolation, and in particular because the foreign genes are being continuously over-expressed. The transgenic organism is, in effect, under constant metabolic stress, which may have many unintended effects on its physiology and biochemistry, including increase in concentrations of toxins and allergens. Another frequent unintended effect is transgenic instability due to gene silencing, or secondary mobility of the introduced genes.[12]

On account of the unpredictabilities and randomness inherent to the technology, every time the same vector system is used to introduce the same genes into the same plant variety, a different transgenic line results. Furthermore, there is no guarantee that the transgenic line retains its identity in subsequent generations, as transgenic organisms typically do not breed true, possibly due to the instability of the unnatural gene constructs in the insert (see below).[13]

It has been argued that unpredictability and randomness are not unique to transgenesis, but also result from conventional mutagenesis. However, the unpredictability and randomness differ in kind for the two cases. No novel genes will result from mutagenesis, only alleles (different forms) of the same genes. Mutagenesis does not introduce novel gene constructs containing gene-expression cassettes with strong viral promoters/enhancers or antibiotic resistance marker genes. Mutagenesis also does not give *position* effects, due to random gene insertion by the vector carrying the foreign genes; nor unpredictable *pleiotropic* effects, due to functional interactions of over-expressed foreign genes with host genes.

Examples of unexpected, unintended toxicities and allergenicities are already known, even for cases where the organism's own genes are being increased in copy number, details of which can be found in earlier publications.[14] I draw your attention to Monsanto's transgenic soya, which was approved by the UK Novel Foods Committee for our market since 1996 as 'substantially equivalent' and therefore safe. It was found, nevertheless, to have a 26.7% increase in a major allergen, trypsin-inhibitor, which is also a growth inhibitor.[15] Consistent with this result, the growth rate of male rats was found to be inhibited by the transgenic soya.[16] This raises the question as to whether the transgenic soya is responsible for the reported recent increase in soya allergy.[17]

The findings of Dr. Arpad Pusztai suggest that the major toxicities of two transgenic potatoes lines engineered with snowdrop lectin are due to the transgenic process, and not the lectin.[18] The two transgenic lines are different from each other, and from subsequent generations of each line, underscoring the unpredictable, unstable nature of transgenic varieties. Pusztai's experiments are the first comprehensive safety-testing of

any transgenic food/feed ever undertaken. They cannot, and should not, be lightly dismissed.

There is no case for regarding transgenic lines constructed with the same methods and involving the same gene constructs and plant varieties as a class, as far as safety assessment is concerned. Each resulting transgenic line is different, with different unexpected, unintended characteristics. Therefore, before each line is authorized for release into the environment, it must be thoroughly characterized with respect to the site(s) of foreign gene insertion. There must be evidence, supported with the appropriate molecular genetic and other scientific data, that the line is stable in gene expression and gene insert(s) under a reasonable range of conditions of growth for at least five generations. Appropriate toxicity/ allergenicity testing must be done on human volunteers. There is a very strong case that transgenic foods should be as stringently tested as new drugs.

Safety concerns of gene constructs

Foreign genes are typically introduced as 'gene expression cassettes' each with a strong viral promoter/enhancer accompanying a gene. Safety concerns have been raised not only over the high levels of constitutive foreign gene expression discussed above, but over the viral promoters themselves. One viral promoter used in practically all transgenic plants is from the cauliflower mosaic virus (CaMV), which is closely related to human hepatitis B virus, and less closely, to retroviruses such as the AIDS virus.[19] The CaMV promoter can drive the synthesis of related viruses.[20] It is functional in most plants, in yeast, insects[21] and *E. coli*.[22] Two kinds of potential hazards exist within the transgenic plant itself: the reactivation of dormant viruses, and recombination between the CaMV promoter and other viruses, dormant or otherwise, to generate new, super-infectious viruses or viruses with broadened host-range.

The safety of CaMV promoter has never been assessed before it was widely used. As it is active in practically all species, and as horizontal gene transfer from the transgenic plant to unrelated species is now known to happen (see below), all the genes linked to this promoter will be actively over-expressed in any species to which the gene expression cassettes happen to be transferred. In addition, the reactivation of dormant viruses which are in all genomes, and the generation of new, super-infectious viruses may also occur in those species. Signs suggestive of viral infection in the tissue of rats fed transgenic potatoes have been reported to be among the findings of Pusztai's group.[23] The potential ecological damages due to the spread of the cauliflower mosaic viral promoter alone warrants an immediate moratorium on further environmental releases of transgenic crops and products that might contain transgenic DNA. There is urgent need for an independent enquiry and targetted research on the hazards of CaMV and other similar promoters.

Safety concerns from the uncontrollable spread of transgenes and marker genes

Genes can spread from transgenic plants by ordinary cross-pollination to nontransgenic plants of the same species or related species, and also by secondary horizontal gene transfer to unrelated species.

The most obvious effects of cross-pollination already identified are in creating herbicide-tolerant, or insecticidal weeds and superweeds.[24] Another special hazard is the spread of the novel genes and gene-constructs for over-expression, as well as the antibiotic resistance marker genes which are in a high proportion of transgenic plants. This will multiply the unpredictable physiological impacts on the organisms to which the genes and gene-constructs are spread, and hence on the ecological environment.

Horizontal gene transfer is the very process that is exploited for creating the transgenic plants themselves. Secondary horizontal transfer from the transgenic plants may spread the novel genes and gene-constructs to unrelated species. This can, in principle, occur to all species that interact with the transgenic plants, either directly or indirectly: microbes in the soil and in other parts of the plants, worms, insects, arthropods, birds, small mammals and human beings. Horizontal gene transfer is the subject of a major report commissioned by the Norwegian Government's Directorate for Nature Management in 1995, which has now been up-dated and translated into English.[25]

Several factors make it more likely for the foreign genes that were introduced into the transgenic plants to take part in secondary horizontal gene transfer than the plant's own genes.[26] First, the mechanisms that enable foreign genes to insert into the genome may enable them to jump out again, to re-insert at another site, or to another genome. For example, the enzyme, *integrase*, which catalyzes the insertion of viral DNA into the host genome, also functions as a *dis*integrase catalyzing the reverse reaction. These integrases belong to a superfamily of similar enzymes present in all genomes from viruses and bacteria to higher plants and animals.[27] Second, the unnatural gene constructs tend to be unstable, and hence prone to recombine with other genes. Third, the metabolic stress on the host organism due to the continuous over-expression of the foreign genes may contribute to the instability of the insert, as it is well-known that transposons are mobilized to jump out of genomes during conditions of stress, to multiply and/or reinsert randomly at other sites resulting in many insertion-mutations. Fourth, the foreign gene-constructs and the vectors into which they are spliced, are typically mosaics of DNA sequences from many different species and their genetic parasites, and hence more prone to recombine with, and successfully transfer to, the genomes of many species.[28] (However, DNA sequence homology is not required for successful horizontal gene transfer,[29] otherwise it would have been impossible to create many transgenic organisms in the first place.)

The potential hazards from secondary horizontal gene transfer to unrelated species are as follows.
Generation of new viruses by recombination between the viral genes or promoters and viruses in recipient species and in the general environment
Generation of new bacterial pathogens by recombination between the bacterial genes introduced and bacteria in recipient species and in the general environment
Spread of drug and antibiotic resistance marker genes among pathogens in recipient species and in the general environment
Random, secondary insertion of genes into cells of recipient species, with harmful position and pleiotopic effects, including cancer
Reactivation of dormant viruses that cause diseases by the CaMV and other viral promoters in recipient species
Multiplication of ecological impacts due to all the above.

There is evidence that a herbicide-tolerance gene, introduced into *Arabidopsis* by means of a vector, may be up to 30 times more likely to escape and spread than the same gene obtained by mutagenesis.[30] One way this could happen is by secondary horizontal gene transfer via insects visiting the plants for pollen and nectar.

Secondary horizontal tranfer of transgenes and antibiotic resistant marker genes from genetically engineered crop-plants into soil bacteria and fungi have been documented in the laboratory.[31] Successful transfers of a kanamycin resistance marker gene to the soil bacterium *Acinetobacter* were obtained using DNA extracted from homogenized plant leaf from a range of transgenic plants: *Solanum tuberosum* (potato), *Nicotiana tabacum* (tobacco), *Beta vulgaris* (sugar beet), *Brassica napus* (oil-seed rape) and *Lycopersicon esculentum* (tomato).[32] It is estimated that about 2500 copies of the kanamycin resistance genes (from the same number of plant cells) is sufficient to successfully transform one bacterium, despite the fact that there is six million-fold excess of plant DNA present. A single plant with say, 2.5 trillion cells, would be sufficient to transform one billion bacteria. Despite the misleading title in one of the publications,[33] a high "optimal" gene transfer frequency of 6.2×10^{-2} was found in the laboratory from transgenic potato to *Erwinia chrysanthem*, a bacterial pathogen. The authors then proceeded to 'calculate' a frequency of 2.0×10^{-17} under extrapolated "natural conditions". The natural conditions, are of course, largely unknown. There is no ground for assuming that such horizontal gene transfer will not take place under natural conditions. On the contrary, there is now a large body of evidence to suggest it can occur.

The genetic material, DNA, released from dead and live cells, is not readily broken down as previously supposed, but rapidly sticks to clay, sand and humic acid particles where it retains the ability to infect (transform) a range of organisms in the soil.[34] That means transgene-constructs and marker genes will be able to spread to bacteria and viruses with the potential of creating new pathogens and spreading antibiotic resistance genes among the pathogens. The bacteria and viruses in all environments essentially act as a reservoir for the genes and gene-constructs, allowing them to multiply, recombine and further spread to all other species.

DNA is not broken down rapidly in the gut as previously supposed.[35]That means genes can spread from ingested transgenic plant material to bacteria in the gut and also to the cells of all organisms ingesting the material.

Horizontal gene transfer between bacteria in the human gut has been demonstrated since the 1970s and similar transfers in the gut of chicken and mice in the early 1990s.[36] This is confirmed in new research showing that antibiotic resistant marker genes from genetically engineered bacteria can be transferred to indigenous bacteria at a substantial frequency of 10^{-7} in an artificial gut.[37] The transformed bacteria will constitute a reservoir of antibiotic resistance genes that may be passed onto pathogenic bacteria.

Mammalian cells are known to take up foreign DNA by many mechanisms, including conjugation, a process previously thought to occur only between microorganisms.[38] Studies since the 1970s have documented the ability of bacterial plasmids carrying a mammalian SV40 viral genome to infect cultured cells which then proceeded to make the virus. Similarly, bacterial viruses and baculovirus (of insects) can also be taken up by mammalian cells. Baculovirus is so good at gaining access that it is being engineered

as a vector for human gene therapy, at the same time that it is being engineered to control insect pests in agriculture.[39] We have called on all projects engineering baculovirus for agricultural use to be banned immediately.*

Viral and plasmid DNA fed to mice have been found to resist digestion in the gut. Large fragments passed into the bloodstream and into white blood cells, spleen and liver cells. In some instances, the viral DNA was found attached to mouse DNA and *E. coli* DNA, suggesting that it has integrated into the mouse cell genome and the bacterial genome respectively.[40] When fed to pregnant mice, large fragments of the DNA are found in the nucleus of cells of the foetus and the newborn.[41]

Viral DNA is now known to be more infectious than the intact virus, which has a protein coat wrapped around the DNA. For example, intact human polyoma virus injected into rabbits had no effect, whereas, injection of the naked viral DNA gave a full-blown infection.[42] Viral DNA is in practically all transgenic plants especially in the form of CaMV and other similar viral promoters, which, if integrated into mammalian cells may reactivate dormant viruses, generate new viruses by recombination, and also cause cancer.[43]

There is as yet no direct evidence that latent viruses can be reactivated in transgenic plants by the CaMV promoter, if only because the possibility has not been investigated. However, plants engineered with coat-protein and other genes from viruses to resist virus attack actually show increased propensity to generate new, often super-infectious viruses by horizontal gene transfer and recombination with infecting viruses.[44] This suggests that the viral promoters engineered into practically all transgenic plants may also take part in horizontal gene transfer and recombination to generate new viruses.[45] Once formed, the new viruses will spread by insects to other plants, unleashing wide-spread disease epidemics.

It has been argued that 'fluid genome' processes, which include horizontal gene transfer, have always operated in nature, and therefore, transgenic organisms cannot be said to pose a new threat. However, horizontal gene transfer has been relatively rare in our evolutionary past, both because natural species barriers prevent gene exchange, especially between distant species, and because there are mechanisms which inactivate or break down foreign DNA.[46] Furthermore, genomic fluidity is increasingly recognized to be part and parcel of the regulatory repertoire that keeps genes and genomes stable under ecologically balanced conditions while allowing rapid changes to take place under stress.[47] Genetic engineering biotechnology greatly accelerates the rate of horizontal gene transfer as well as enlarging its scope. It creates large numbers of arbitrary combinations of genes from different species and their pathogens, and uses increasingly sophisticated means to overcome species barriers.[48] It is foolhardy to be complacent about releasing great quantitites of such arbitrary combinations of viral and bacterial genes into the environment.

Already, the world is experiencing a public health crisis from the accelerated resurgence of drug and antibiotic resistance diseases over the past 20 years. Many factors are thought to be responsible, among them, environmental destruction, urbanization, the abuse and overuse of antibiotics in medicine and intensive agriculture. One factor which has not been considered is the development of genetic engineering biotechnology on commercial scales over the same period.[49] There is overwhelming evidence that the new

viral and bacterial pathogens have been created by horizontal gene transfer and subsequent recombination, which also spread drug and antibiotic resistance genes among the pathogens. Many of the horizontal gene transfer events have occurred very recently, as evidenced by the identity or near-identity of the same genes in unrelated species. New, cross-species viral agents, in particular, have been emerging in great numbers in recent years, with a trend towards increasing virulence and infectivity that has not been seen previously.[50]

Malaysia is in the grip of a national emergency due to a serious outbreak of viral diseases crossing from pigs to humans.[51] One virus associated with Japanese encephalitis, a member of the Flavivirus family, is spread by several species of the *Culex* mosquitoes. It was endemic to Malaysia, and sporadic outbreaks in the rural population have occurred between 1974 to 1992, with a few deaths. The recent outbreak since October 1998 involves a dramatic shift from endemic to epidemic form, resulting in the highest fatality rates recorded. Sixty-nine people have died, and close to 200 cases identified. Less than one-third of the cases is accounted for by the Japanese encephalitis virus. An additional virus identified is reminiscent of the Hendra virus belonging to the Paramyxovirus family, first isolated in Hendra, a suburb of Brisbane in Queensland, Australia, in 1994. It originated from race horses and is believed to spread by urine and other body fluids. Many questions are raised by the epidemic, including the possibility that it may be due to new recombinant virus(es) arising from horizontal gene transfer.

Many scientists have already called for phasing out antibiotic resistance genes in transgenic plants on grounds that they may spread horizontally and compromise treatments for infectious diseases. However, that does not address the emergence of the bacterial pathogens themselves, nor the plagues of new viruses and viral strains. Recent findings also reveal that *while disease-causing functions in bacteria are due to many genes, these genes are often clustered together in mobile units - pathogenicity islands - that transfer horizontally as a unit. Thus, non-pathogens can be converted into pathogens in a single step*.[52]

When is scientific evidence 'sufficient'?

When is scientific evidence considered sufficient to indicate that the risk is unacceptable? Risk is technically the extent of damage multiplied by the probability that the damage will occur. People take risk for a number of reasons: because they have to, or because there is overwhelming moral imperative for doing so, or because the likely benefits are compelling despite the potential damage. Not one of these reasons applies in the case of transgenic agriculture. On the contrary, existing scientific evidence pointing to the serious damages to health and the ecological environment that are likely to be incurred should compell us to call an immediate halt to the enterprise. That is in accordance with the generally accepted precautionary principle.[53]

Instead, scientists on the relevant advisory committees appear to have been operating on the *inverse* precautionary principle, according to which all processes and products must be approved unless proven absolutely unsafe. Arguments such as "no-one has been shown to have died from eating genetically engineered food yet" or "just because horizontal gene transfer happens in the laboratory does not mean it will happen in nature" go against the practice of good, sound science and are frankly irresponsible. It is like saying we have to wait for 8000 babies to be born with truncated limbs before

admitting there is sufficient evidence that thalidomide is harmful.

The most rational, responsible course of action is to impose a five year moratorium at the very least, in order to create space for desperately needed research, and more importantly, for an open wide-ranging debate on the future of agriculture and food security for all.[54]

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1footnote *Previous submissions to UKHSE and European Commission, available from M.W.Ho.

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[46]This possibility has been suggested by Cummins since 1994 (see Cummins, 1998, note19).

[47]See Ho *et al*, 1998b (note 13) and references therein.

[48]See Shapiro, J. (1997). Genome organization, natural genetic engineering and adaptive mutation. *TIG* 13, 98-104; also, Ho, 1998, 1999 (note 2).

[49]See Ho *et al*, 1998b (note 13) and references therein.

[50]Ho *et al*, 1998a (see note 13).

[51]Mahy, B.W.J. (1997). Emerging virus infection. *Viral Immunol*. 48, 1-2.

[52]Briefing from Third World Network, Penang, Malaysia, March, 1999.

53footnote [*]See Ho et al, 1998b (note 13) and references therein.

[54]See Traavik, 1999 (note 14).