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Special Safety Concerns of Transgenic Agriculture and Related Issues
Briefing Paper for Minister of State for the Environment, The Rt Hon Michael Meacher (April 1999)

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 [40].

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 [41]. When fed to pregnant mice, large fragments of the DNA are found in the nucleus of cells of the foetus and the newborn [42].

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 [43]. 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 [44].

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 [45]. 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 [46]. 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 [47]. 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 [48]. 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 [49]. 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 [50]. 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 [51].

Malaysia is in the grip of a national emergency due to a serious outbreak of viral diseases crossing from pigs to humans [52]. 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* [53].

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 [54].

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 [55].

1. Previous submissions to UKHSE and European Commission, available from M.W.Ho.
2. See Ho, M.W. (1998,1999). *Genetic Engineering Dream or Nightmare? The Brave New World of Bad Science and Big Business*, Gateway Books, Bath; also Ho, M.W. and Steinbrecher, R. (1998). Fatal Flaws in Food Safety Assessment: Critique of The Joint FAO/WHO Biotechnology and Food Safety Report, *Environmental and Nutritional Interactions* 2, 51-84; and references therein.
3. See Old,R.W. and Primrose, S.B. (1994). *Principles of Gene Manipulation* (5th ed.), Blackwell Science, Oxford, or similar texts.
4. See ISAAA Report, 1998.
5. See Ho and Steinbrecher, 1998 (note 2) and references therein.
6. Gould, F., Tabashnik, B., Hutchison, W., Ferro, D., Andow, D. and Whalon, M. (1998). Recommendations for developing and implementing resistance management plans for Bt-toxin-producing crops. In *Now or Never* (M. Mellon and J. Rissler, eds), pp. 13-8, Union of Concerned Scientists, Cambridge, Mass.
7. See Ho and Steinbrecher, 1998 (note 2).
8. Walden, R., Hayashi, H. and Schell, J. (1991). T. DNA as a gene tag. *Plant J.* 281-8.
9. Smith, V. (1998). More T-DNA than meets the eye. *Trends in Plant Science* 3, 85.
10. Conner, A.J. (1995). Case study: food safety evaluation of transgenic potato. In *Application of the Principles of Substantial Equivalence to the Safety Evaluation of Foods or Food Components from Plants Dervied by Modern Biotechnology*, pp. 23-35, WHO/FNU/FOS/95.1, World Health Organization, Geneva, Switzerland.
11. Reviewed by Doerfler, W., Schubbert, R., Heller, H., Kämmer, C., Hilger-Eversheim, D., Knoblauch, M. and Remus, R. (1997). Integration of foreign DNA and its consequences in mammalian systems. *Tibtech* 15, 297-301.
12. See Ho and Steinbrecher, 1998 (note 2).

13. See Ho, 1998, 1999 (note 2); Ho, M.W., Meyer, H. and Cummins, J. (1998a). The biotechnology bubble. *The Ecologist* 28(3), 146-153, and references therein; Ho, M.W., Traavik, T., Olsvik, R., Tappeser, B., Howard, V., von Weizsacker, C. and McGavin, G. (1998b). Gene Technology and Gene Ecology of Infectious Diseases. *Microbial Ecology in Health and Disease* 10, 33-59.
14. See Ho and Steinbrecher, 1998 (note 2), Ho *et al*, 1998a (note 13); also Traavik, T. (1999) *Too early may be too late, Ecological risks associated with the use of naked DNA as a biological tool for research, production and therapy*, Research report for Directorate for Nature Management, Norway.
15. Padgette, S.R., Taylor, N.B., Nida, D.L., Bailey, M.R., MacDonald, J., Holden, L.R., and Fuchs R.L. (1996). The composition of glyphosate-tolerant soybean seeds is equivalent to that of conventional soybeans. *Journal of Nutrition* 126, 702-16.
16. Hammond, B.G., Vicini, J.L. Hartnell, G.F., Naylor, M.W., Knight, C.D., Robinson, E.H., Fuchs, R.L. and Padgette, S.R. (1996). The feeding value of soybeans fed to rats, chickens, catfish and dairy cattle is not altered by genetic incorporation of glyphosate tolerance. *Journal of Nutrition* 1126(3) 717-26.
17. Are vegetables making you ill? Press Release, Food for Thought Food Allergy Testing, 19 March, 1999.
18. Pusztai, A. (1998). SOAEFD flexible Fund Project RO818 Report of Project Coordinator on data produced at the Rowett Research Institute (RRI); see also Goodwin, B.C. (1999). Report on SOAEFD Flexible Fund Project RO818, Jan. 23, 1999.
19. See Cann, A.J. (1997). Principles of Molecular Virology, 2nd ed., Academic Press, London; see also Cummins, J. (1998). A virus promoter used in the majority of genetically engineerd crops. Available from the author at <jcummins@julian.uwo.ca>.
20. Xiong, Y. and Eikbush, T. (1990). Origin and evolution of retroelements based upon the reverse transriptase sequences. The Embo Journal 9, 3363-72
21. Smerdon, G., Aves, S. and Walton, E. (1995). Production of human gastric lipase in the fission yeast. *Gene* 165, 313-8; Vlack, J., Scoulten, A., Usmany, M., Belsham, G., KlingeRoode, E., Maule, A., Vanlent, M. and Zuideman, D. (1990). Expression of Cauliflower Mosaic Virus Gene I. *Virology* 179, 312-20.
22. Assad, F.F. and Signer, E.R. (1990). Cauliflower mosaid virus P35S promoter activity in E. coli. *Mol. Gen. Genet*. 223, 517-20.
23. See Goodwin, 1999 (note 18).
24. See, for example, Mellon, M and Rissler, J. (1998).*Now or Never* , Union of Concerned Scientists, Cambridge, Mass.
25. Traavik, 1999 (note 14).
26. See Ho, 1998, 1999 (note 2); Ho *et al*, 1998b(note 13); Traavik, 1999 (note 14).
27. Asante-Appiah E. and Skalka, A.M. (1997). Molecular mechanisms in retrovirus DNA integration. *Antiviral Researh* 36, 139-56.
28. See Ho *et al*, 1998b (note 13) and references therein.
29. See Traavik, 1999 (note 14).
30. Bergelson, J., Purrington, C.B. and Wichmann, G. (1998). Promiscuity in transgenic plants. *Nature* 395, 25.
31. Hoffman, T., Golz, C. & Schieder, O. (1994). Foreign DNA sequences are received by a wild-type strain of *Aspergillus niger* after co-culture with transgenic higher plants. *Current Genetics* 27: 70-76; Schluter, K., Futterer, J. & Potrykus, I. (1995). Horizontal gene-transfer from a transgenic potato line to a bacterial pathogen (*Erwinia-chrysanthem*) occurs, if at all, at an extremely low-frequency. *Bio/Techology* 13: 1094-1098; Gebhard, F. and Smalla, K. (1998). Transformation of *Acinetobacter* sp. strain BD413 by transgenic sugar beet DNA. *Appl. Environ. Microbiol*. 64, 1550-4.
32. De Vries, J. and Wackernagel, W. (1998). Detection of nptII (kanamycin resistance) genes in genomes of transgenic plants by marker-rescue transformation. *Mol. Gen. Genet*. 257, 606-13; see also Gebhard. and Smalla, 1998 (note 31).
33. Schlutter *et al*, 1995 (note 31).
34. Reviewed by Lorenz, M.G. and Wackernagel, W. (1994). Bacterial gene transfer by natural genetic transformation in the environment. *Microbiol. Rev*. 58, 563-602.
35. See Ho *et al*, 1998a (note 13) and references therein.
36. See Ho *et al*, 1998a (note 13) and references therein.
37. See MacKenzie, D. (1999). Gut reation. *New Scientist*, 30 Jan., p.4.
38. See Ho *et al*, 1998b (note 13).

39. See Ho *et al*, 1998a (note 3).
40. See Ho and Steinbrecher, 1998 (note 2).
41. Schubbert, R., Lettmann, C. & Doerfler, W. (1994). Ingested foreign (phage M13) DNA survives transiently in the gastrointestinal tract and enters the bloodstream of mice. *Mol. Gen. Genet.* 242: 495-504; Schubbert, R., Renz, D., Schmitz, B. and Doerfler, W. (1997). Foreign (M13) DNA ingested by mice reaches peripheral leukocytes, spleen and liver via the intestinal wall mucosa and can be covalently linked to mouse DNA. *Proc. Natl. Acad. Sci. USA* 94, 961-6.
42. Doerfler, W., Schubbert, R., Heller, H., Hertz, J., Remus, R., Schrier. J., Kämmer, C., Hilger-Eversheim, K., Gerhardt, U., Schmitz, B., Renz, D., Schell, G. (1998)*APMIS* Suppl. 84, 62-8.
43. See Traavik,, 1999 (note 14).
44. See Ho *et al*, 1998b (note 13) and references therein.
45. Vaden V.S. and Melcher, U. (1990). Recombination sites in cauliflower mosaic virus DNAs: implications for mechanisms of recombination. *Virology* 177, 717-26; Lommel, S.A. and Xiong, Z. (1991). Recombination of a functional red clover necrotic mosaic virus by recombination rescue of the cell-to-cell movement gene expressed in a transgenic plant. *J. Cell Biochem*. 15A, 151; Greene, A.E. and Allison, R.F. (1994). Recombination between viral RNA and transgenic plant transcripts. *Science* 263, 1423-5; Wintermantel, W.M. and Schoelz, J.E. (1996). Isolation of recombinant viruses between cauliflower mosaic virus and a viral gene in transgenic plants under conditions of moderate selection pressure. *Virology* 223, 156-64.
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.
53. See Ho et al, 1998b (note 13) and references therein.
54. See Traavik, 1999 (note 14).
55. See World Scientists' Statement signed by a substantial number of scientists all over the world, calling for a global 5 year moratorium on environmental releases of transgenic crops and products, ban on patents on living organisms, cell lines and genes, and an independent public enquiry on the future of agriculture and food security for all, Institute of Science in Society.

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ISIS News 1, July 1999, ISSN: 1474-1547 (print), ISSN: 1474-1814 (online)

Glyphosate linked to cancer

A recent population-based study conducted in Sweden between 1987-1990 and including follow-up interviews clearly links exposure to Roundup Ready herbicide (glyphosate) to non-Hodgkin's lymphoma and strongly suggests glyphosate deserves further epidemiological studies.

Reference: Hardell, H. & Eriksson, M. (1999). A Case-Control Study of Non-Hodgkin Lymphoma and Exposure to Pesticides. *Cancer* 85, No 6.

AR

GM pollen harms monarch butterflies

Researchers at Cornell University published a study in *Nature* which found that pollen from GM Bt corn could have lethal effects on the larvae of monarch butterflies if it lands on milkweed, the plant upon which they feed.

Forty-four percent of the larvae were killed after 4 days, whereas no mortality occurred in larvae fed nontransgenic pollen. The Cornell University researchers say their results “have potentially profound implications for the conservation of monarch butterflies” and believe more research on the environmental risks of biotechnology in agriculture is essential.

Reference: Losey, J.E. et al (1999). Transgenic pollen harms monarch larvae. *Nature* 399, 214.

AR

GM soya fails to perform

A review of 8,200 university based trials of transgenic soya varieties. It reveals that Roundup Ready Soybeans produce lower yields compared to their non GM counterparts. The average yield drag in RR soybeans was 6.7% and in some areas of the midwest the yield average was 10% higher in conventional varieties compared to Roundup Ready varieties.

Furthermore, the analysis shows that farmers use 2 to 5 times more herbicide measured in pounds applied per acre on RR soybeans compared with other weed management systems. RR herbicide use exceeds the levels on many farms using multi-tactic weed management systems by a factor of 10 or more.
Reference: Evidence of the Magnitude and Consequences of the Roundup Ready Soybean Yield Drag from University-Based Varietal Trials in 1998. by U.S. agronomist Dr. Charles Benbrook, author of Pest Management at the Crossroads and former Executive Director of the Board on Agriculture for the US National Academy of Sciences. Ag Biotech Infonet Technical Paper Number 1 July 13 1999. website http://www.biotech-info.net/RR_yield_drag_98.pdf

AR

ISIS News 2, September 1999, ISSN: 1474-1547 (print), ISSN: 1474-1814 (online)

New genetic engineering technique claims to overcome current hit or miss transgenic technology

One of the major problems with current transgenic technology is that it results in random gene insertions and rearrangements. The new technique, 'chimeroplasty' claims to change a single base at a predetermined position in a specific gene in the plant cell.

However, it may not be as precise as claimed and introduces new dangers. (Thanks to Suzanne Wuerthiele for drawing our attention to this paper.)

The findings: A technique of directed gene conversion involves introducing an inverted repeat of a sequence of 25 bases composed of DNA and modified RNA residues, which forms a stable hairpin. The sequence is homologous to that of the target gene, except for the base to be substituted, which is in the middle of the sequence of 25 bases. When introduced into the cells by a gun that shoots tiny gold particles coated with the hairpins, the hairpins will basepair with both strands of the target sequence in the gene. DNA mismatch repair enzymes will then convert the sequence of the gene to that specified by the hairpin.

Using this technique, the researchers attempted to convert a gene in tobacco coding for the enzyme acetolactate synthase to a herbicide-resistance phenotype, by changing the codon (CCA) for proline at amino-acid position 196 to CAA for glutamine and CTA for leucine respectively, with two different hairpin constructs.

The results show that the target sequence was converted, but not at the base intended. Conversions were at neighbouring bases. For example, ACA for threonine was obtained instead of CAA intended, and TCA for serine resulted, as well as ACA and TCA instead of the intended CTA.

Another complication is that the gene is capable of undergoing spontaneous mutations to herbicide resistance. The directed mutation rates were up to 20-fold those in controls, but were variable from experiment to experiment.

Our comment: It is very unlikely that the technique is as precise as claimed. The mere act of introducing nucleic acid sequences into the cells by bombardment with a particle-gun will trigger injury responses that can cause nonspecific recombination. In addition, the technique depends on imprecise basepairing between the target sequence and the introduced
hairpin. Can one be sure that nontarget sequences are never affected? The hairpins themselves are a hazard to biodiversity and health if released into the environment. All kinds of unintended gene conversions could take place in species exposed to the constructs, including human beings.

Reference: Beetham, P.R., Kipp, P.B., Sawycky, X.L., Arntzen, C.J. and May, G.D. (1999). A tool for functional plant genomics: Chimeric RNA/DNA oligonucleotides cause in vivo gene-specific mutations. *PNAS* 96, 8774-8778.

"The Herbicide, Glufosinate, used with millions of acres of GM crops including corn, canola and soy, causes birth defects on exposure of father alone as well as mother!" Prof. Joe Cummins warns

Joe Cummins has written a number of previous notes on the danger of the herbicide ,glufosinate, used with GM and normal crops and on the false claims by officials of EU , US and Canada that the herbicide has no harmful side effects. Previous evidence showed that pregnant females who have eaten food containing the herbicide gave birth to children with birth defects, as well as defects in behavior and learning. Learning defects were also experienced by young children exposed to the herbicide.

Recent studies showed that fathers exposed to glufosinate gave birth to children with birth defects while most other pesticides did not produce the same effect.

Reference: Garcia,A.,Benavides,F.,Fletcher,T. and Orts,E. (1998). Paternal exposure to pesticides and congenital malformations. *Scand J Work Environ Health* 24, 473-80.
Joe Cummins comments: The glufosinate birth defects suggest that the large chemical companies have undue influence over government bureaucrats . Such bureaucrats turn their backs on clear evidence of danger from pesticides and promote dangerous genetic engineering.

New study on prevalence and distribution of viruses in natural populations and implications of widespread multiple viral infections with respect to the release of transgenic plants expressing virus-derived genes.

A quote from this paper: "The presence of transgenic virus resistant plants expressing viral proteins or virus-derived nucleic acids introduces a substantially new dimension into the dynamics of plant-virus co-evolution, even though virus-derived nucleic acids are normal constituents of plant populations. There is a possibility that the spread of virus-derived transgenes through seed and pollen will substantially alter the distribution of viral nucleic acids, for example, gene flow might reduce temporal and spatial variation in the incidence of virus-derived nucleic acids and so increase the potential for recombination."
Reference: Raybould et al (1999) The prevalence and spatial distribution of viruses in natural population of Brassica oleracea . N*ew Phytol* 141, 265-275.

AR

"Virus-Resistant Crops Could Help Weeds" Says Professor Alison Power

Genetic engineering cereals to resist the barley yellow dwarf virus (BYDV) might indirectly cause farmers difficulties in controlling related weeds. A report presented at the Ecological Society of America1s annual meeting indicates that the resistance engineered into oats could spread to wild oats, a weed. Transgenic barley and oats that can resist BYDV have been developed, but there is concern that because these crops can hybridize with wild relatives, that the introduced genes will escape into related weeds.

Alison Power, an ecologist at Cornell University says that if wild oats gain resistance to BYDV, they could become a much larger problem for farmers, and might also disrupt natural habitats, outcompeting other native species.
Power grew oats and wild oats in greenhouses and infected them with the BYDV. She found that infected wild oats did not perform well: they were much thinner and had shorter roots than uninfected controls and infected oats. Infected wild oats also produced fewer seeds than normal.
"A BYDV-resistant transgene transfer seems likely to help wild oat survivability," concludes Power.
Reference: Contact: Alison Power, Department of Ecology and Evolutionary Biology,E331A Corson Hall, Cornell University, Ithaca, NY 14853,USA. AR

How a genetically engineered microorganism was found to kill wheat seedlings

A microcosm study showed that a genetically engineered microorganism (GEM) killed wheat seedlings through impacts on the soil biota. This important study was first reported in 1997, but the full paper has only just been published. Read all about it.
Reference: Holmes M T et al (1999). Effects of Klebsiella planticola SDF20 on soil biota and wheat growth in sandy soil. Applied Soil Ecology 11 (1999) 67-78.

ISIS News 3, December 1999, ISSN: 1474-1547 (print), ISSN: 1474-1814 (online)

GM Soya and Increased Soya-associated Allergy

Scientists at the York Nutritional Laboratory have announced that soya food allergy among the British public have unexpectedly risen 50% between 1998 and 1999. Soya is now in 9th position on the list of top serum reactive (test for allergenicity) foods, up from 14th place in 1997. This finding coincides with the large increase in imported foods from the US containing GM soya.

Monsanto's GM soya, approved in 1996, was found to contain a 26.7% increase in a major allergen, trypsin-inhibitor, which is also a growth inhibitor. Consistent with this result, the growth rate of male rats was found to be inhibited by the GM soya. Monsanto has not tested all possible allergens. These results warrant a complete withdrawal of GM soya, at least until evidence that it is safe is obtained.
Reference : Personal Communication, Mark Varey, York Nutritional Laboratories.
Padgette S.R. et al (1996) The composition of glyphosate-tolerant soybean seeds is equivalent to that of conventional soybeans. *Journal of Nutrition* 126, 702-16 AR

Troubled GM Soya Not Substantially Equivalent

Once again, Monsanto's Roundup Ready GM soya is shown up to be not 'substantially equivalent' to non GM counterparts. Bill Vencill of the University of Georgia in Athens examined the effects of heat on GM soya in laboratory growth chambers. In soil temperatures of 25 C or less during the day, both GM and non GM varieties grew the same. But in warmer soils, the GM beans had stunted growth and in soils reaching 45 C the differences were marked - lower heights, yields and weights, and the stems cracked and split open in every GM soya bean plant. This phenomenon exposes the plant to secondary fungal infections and explains what may have happened to crops during the two hottest growing seasons in southern states, when there were substantial crop losses by farmers growing GM soya.

These results indicate changes in plant physiology caused by the insertion of transgenes, which make the plant resistant to glyphosate - Monsanto's Roundup. It has been shown that GM plants carrying these genetic alterations produce 20 per cent more lignin, which is the tough, woody form of cellulose. The bacterial enzyme that imparts resistance to glyphosate affects a major metabolic pathway in the plant, which sends lignin production 'into overdrive' says Vencill. This unexpected 'side effect' may have been what caused the GM plants to be more brittle. Resistance to gluphosinate, by contrast, uses a gene that enables plants to break down the herbicide, and such GM plants were not effected by heat in this way. Monsanto declined to comment but said that farmers could avoid the problem by choosing a variety of GM soya that is better suited to hot conditions.

Reference: NewScientist, News, November 20, 1999, "Splitting Headache" by Andy Coghlan.

Our comment: These physiological problems with Monsanto's Roundup Ready GM soya beans clearly demonstrate the inadequacy of the principle of substantial equivalence. In our 2nd update of concerns on the WSS, we report Marc Lappe's findings that Monsanto's GM soya is non substantially equivalent in having a reduced phytoestrogen content compared to its non GM counterparts. The insertion of transgenes into a plant cell causes major unpredictable, unintended, which cannot be detected by current tests purporting to establish 'substantial equivalence' and hence gain regulatory approval as being safe for human consumption. AR & MWH

Sleeping Viruses Lurk in Plant Genomes

A new study provides evidence of repeated integration of pararetroviral-like sequences into the genome of tobacco at a copy number of approx 10 000. Therefore, plant pararetroviruses may integrate much more commonly into host chromosomes than has been previously thought. Furthermore, the insertions are thought to have occurred by illegitimate recombination. Plant viral sequences were thought to integrate rarely, if at all, into host genomes and this new evidence calls for a reconsideration of this view. This has considerable implications for plant genome evolution as integrated pararetorviral DNA could act as an insertional mutagen or contribute strong constitutive promoters to neighboring plant genes or could accumulate to generate a new repetitive sequence family.

Reference; Jakowitsch J et al (1999) Integrated pararetroviral sequences define a unique class of dispersed repetitive DNA in plants. PNAS Vol 96 No23 pp 13241-13246

Our comment: This paper provides evidence that dormant viral DNA may be much more widespread in plant genomes than previously thought. It highlights the need for more extensive research in this area and it also has a bearing on the ecological impact of GM plants - although the authors of this paper fail to mention this. The CaMV 35S promoter is a pararetroviral-derived sequence used in most transgenic constructs, where it is integrated at random into the host genome. Furthermore, it contains an independent recombination hotspot and may therefore recombine with dormant viral sequences, which are in some instances integrated at extraordinarily high copy numbers and are also highly recombinogenic - as shown by the amount of illegitimate recombination events detected in this study. This may result in the reactivation of dormant viral sequences, novel epigenetic effects and other genetic disruptions, all of which are potentially hazardous and unpredictable (see Viral Gene Switch – A Recipe for Disaster? This issue).
(A.R.)

More on Bt-Toxin

A new study shows that Bt toxin is exudated from Bt-corn and remains active in the soil, where it binds rapidly and tightly to clays and humic acids. It also retains insecticidal properties and is protected against microbial degradation by being bound to soil particles. This research shows that the Bt toxin persists in various soils for at least 234 days (the longest time studied). Unlike the bacterium, which produces the toxin in a precursor form, Bt corn contains an inserted truncated

cry1Ab gene that encodes the active toxin. Larva of the tobacco hornworm were used to verify that the toxin was active and when placed on a medium containing exudates from Bt corn, stopped feeding and began to die after 2 to 3 days, and had a mortality of 90 to 95 percent after 5 days.

The authors point out that 15 million acres of Bt corn were planted in the US in 1998. Bt toxin that is released into soil from roots would add to the amount of toxin introduced into soil from pollen and as a result of incorporating plant residues into the soil after harvesting the crop. Bt toxin in the rhizosphere will kill target pest but may also promote the emergence of toxin-resistant target insects. Moreover, receptors for the toxin can be found on beneficial non-target insects which will also be killed, and this will have an impact on other organisms in higher trophic levels, in other words, a major impact on biodiversity.

Reference: Deepak Saxena, Saul Flores, G, Stotzky (1999) Nature 402, 480, p 480.

Our comment: The ecological impacts of GM plants producing bt toxins are now clearly predictable, based on existing scientific evidence. The immediate withdrawal of all bt-crops is the only responsibble course of action.

AR

ISIS News 5, July 2000, ISSN: 1474-1547 (print), ISSN: 1474-1814 (online)

Swallowing the Tale of the Swallowtail

No “absence of toxicity” of Bt pollen

The paper which claims “absence of toxicity” of Bt-pollen under field conditions is faulty in experimental design and actually demonstrates that Bt-pollen is toxic in the laboratory.

A study in Cornell University last year (1) prompted widespread concern that pollen from Bt-corn may be harmful to the Monarch butterfly. Researchers from the University of Illinois now claims that a field study on the black swallowtail, *Papilio polyxenes*, shows that Bt-pollen is not toxic to this species (2).

The black swallowtail feeds on host plants found in narrow strips between roads and crop fields in midwestern USA. A day after the start of Bt-pollen release, researchers set up five rows of five potted host-plant beside a field of Bt-corn (Pioneer variety 34R07 expressing the CrylAB gene in its pollen), at various distances from the edge of the field. Pollen traps consisting of a microscope slide coated with vaseline was placed with each plant to measure total pollen deposited. A second set of potted plants were placed behind the first set three days later. Ten first instar larvae were put on each plant, and the number of live larvae on each plant recorded daily for 7 days.

However, no control experiments were set up. A proper control experiment would have consisted of a replicate set of potted host plants and larvae placed next to a non-GM corn field.

It rained during the 5th and 7th day of the first experiment, and during the 2nd, 4th and 5th day of the second experiment. Would that not have washed away the pollen from the surface of the leaves? If so, what relevance would the pollen counts - on greasy pollen traps - have on actual pollen ingested by the larvae?

Pollen counts decreased sharply with distance from the field as expected; but there was no correlation between pollen counts and mortality. Even though the larvae were counted everyday for seven days, the detailed counts were not given. Instead, the aggregate percentage mortality was presented. Not only were the mortalities high, they were also highly variable. The means ranged from 45 to 82%, and in many cases, the standard deviation in each direction was almost as large as the mean. It was obviously impossible to draw any conclusion from such an experiment. But they stated, “No significant relationships between larval survivorship or mass were detected either as a function of distance from the edge of the field or as a function of pollen deposition.” That was true, but the main reason may be that it was a bad experiment. They suggested that the high mortalities might be due to predation. If so, would mortality not be correlated with “larval mass”? Yet no such correlation was reported.

Back in the laboratory, they deposited different amounts of Bt and non Bt pollen on leaf-discs and fed each in a single dose to a first instar larva which was observed over the next three days. They found no effect with the Bt-pollen collected from the field, even at the highest dosage. But exactly how much Bt toxin did each larva consume? From the figures presented, it can be calculated that at the highest dose used - 10 000 pollen grains – the larva would have consumed only 1 picogram of Bt protein, ie, 1/1 000 000 000 000 or one trillionth of a gram, over the three days.

With another Bt-corn pollen - Novartis Max 454 - which expresses 40 times as much Bt protein, ie, 40 picograms, a highly significant increase in mortality was found on the third day: 80% compared with about 10% for the rest.

As the laboratory experiments involved feeding a single dose over three days, it gave no information as to the effects on mortality of cumulative doses over the entire life-cycle of the butterfly, such as it may experience in the field.

The claim of "absence of toxicity" in the title of this paper is thus misleading to say the least. It will be an abuse of science if this report were to be accepted as evidence that Bt-pollen is safe for black swallowtails.

1. Losey, J.E., Rayor, L.S. and Carter, M.E. (1999). Transgenic pollen harms monarch larvae. *Nature* 399, 214.
2. Wraight, C.L., Zangeri, A.R., Carroll, M.H. and Berenbaum, M.R. (2000). Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions. PNAS early Ed. MWH

Postscript:

The myth that swallowtails were unharmed by bt toxin continued to be perpetrated, especially in mainstream scientific journals. For example, it was reported in *Nature Biotechnology* 2000, 18, 701, under the headline, "Swallowtails unaffected by Bt toxin", where it stated the half-truth, "Although corn pollen accumulated on the leaves eaten by the larvae, there was no difference in butterfly health or mortality between the experimental and control groups."

To Bt or Not to Bt

How sound science makes the case

Since the publication of Losey's study in the journal *Nature* showing that Bt-corn pollen harms monarch butterflies, things have gone into a downward spiral for Bt-crops. Bt-corn is now banned in Austria, France and Germany, and Monsanto's Bt-potato division has been closed down by its new parent company, Pharmacia.

'Bt' is short for *Bacillus thuringiensis*, the soil bacterium providing the genes for making toxins that kill insects; different forms of which are incorporated into GM crops. The adverse environmental impacts of Bt crops are now well documented in the scientific literature, ranging from harm to non target organisms to the evolution of resistance in insect pests, making it necessary to plant a high proportion of non-Bt crop for 'resistance management'. Aberrant gene expression in the field results in low-dose varieties which are ineffective in pest control and foster resistance. Cross pollination with non GM varieties creates Bt-weeds, and the Bt-plants themselves cause major problems as volunteers. Active Bt toxin leaks from plant roots into the soil where it is not biodegradable and accumulates over time. This will have major impacts on soil health, with knock-on effects on all other trophic levels of the ecosystem. The recent report that a GM gene has transferred from GM pollen to microbes in the gut of bee larvae underlines the fact that Bt toxin genes, like all other GM genes, will spread out of control. The case for withdrawing all Bt-crops is now compelling.

The way the case has been built is exemplary of the power of good independent science, which is indispensable for sound policy decisions.

No less than eighteen Bt crops were approved for field testing by the US Dept. of Agriculture between 1987 and 1997 (1). Bt cotton was the first to be approved for commercial use (USA 1995), followed by corn, potato and tomato.

The first specific concerns on the safety of Bt crops were raised from within the scientific community in 1997 when Angelicka Hilbeck and colleagues (2) showed that lacewings fed on pests that have eaten Bt-maize took longer to develop and were two to three times more likely to die.

Organic farmers also started to voice their fears - they have been using the spores of *Bacillus thuringiensis* as an occasional insecticide spray. Their fear was founded in the rapid development of resistance to Bt toxin in pest populations continuously exposed throughout the GM plant's growing season, with the potential loss of their only organic insecticide. They were also worried about GM contamination via cross-pollination - now admitted as unavoidable by our regulators.

Bt toxins are active against insects in the Order of Coleoptera (beetles, weevils and styloplids) which contains some 28,600 species

Then came Losey's famous Monarch butterfly study (3), which was confirmed by another from the University of Iowa (4), showing that milkweed in and at varying distances from Bt crops in the field does cause an increase in mortality to Monarch butterflies. Milkweed samples were taken from within and at the edge of the Bt corn field and were used to assess mortality of first instar monarch, *D. plexippus* exposed to Bt and non-Bt corn pollen. Within 48 hours, there was 19% mortality in the Bt corn pollen treatment, compared to 0% on non Bt-corn pollen exposed plants and 3% in the no pollen controls.

In a desperate recent attempt to counter this evidence, the pro-biotech lobby has just released a story claiming that pollen from Bt corn does not harm the black swallowtail. This story has been thoroughly deconstructed (see "Swallowing the Tale of the Swallowtail", this issue).

The biotech industry is fully prepared to misreport research results in order to confuse and mislead the public. On Nov 2nd 1999, a scientific meeting took place in Rosemount, Illinois, to discuss Bt corn and monarchs. That same morning, all the major news desks round the US received a fax carrying a News article about the meeting - which had only just begun at that point - headlining 'Researchers conclude Bt corn poses little risk to Monarchs'.

Luckily, Carol Yoon of the NY Times was at the meeting and received word from her editor in New York. She asked the participants if they agreed with what was obviously a press release from industry. The answer from the floor was a resounding "No" - her report was the only accurate account of the meeting, but unfortunately, the majority of US citizens got the industries' take on it (5).

After months of heated debate on the effects of Bt on non-target insects, the US Environmental Protection Agency (EPA) convened a Scientific Advisory Panel (SAP) meeting in Dec 1999 and asked the panel to review EPA's non-target organism testing requirement, applicable to Bt crops. The panel found EPA requirements inadequate and urged the agency to substantially expand the scope and quality of the studies that it relies upon (6).

Plans for managing the development of Bt-resistance in insect pests have been actively debated in the scientific literature, and earlier this year, the EPA revised their original mandate and ruled for larger refuges of non GM crop planted with the GM crop. This was hailed as a step in the right direction and now refuges have to be at least 20%. But major controversies remain as to whether or not the refuges should be sprayed by conventional insecticides (7). A study in the University of Arizona (8) showed that boll worm larva fed on GM and non GM develop at different rates and it is highly unlikely that they will interbreed, dashing any hopes of diluting out or slowing down the evolution of resistance. These moths mate within three days of hatching and the males only live for a week. Also, dilution only works if the Bt-resistance is recessive, ie, requiring two copies of the resistance gene, and the EPA's resistance management program relies on the trait being recessive. Unfortunately, studies on the inheritance of Bt resistance showed that it is a dominant trait (9) as insects with only one copy of the resistance gene survive exposure to Bt. Low levels of Bt expression in Bt crops has also been documented and also serves to foster resistance.

In June1999, Monsanto applied for the first Experimental Use Permit on CRY3Bb transgenic corn, another Bt corn line aimed at corn rootworm. The application has been thoroughly assessed by an alliance of four independent non profit organizations (10), who report the most astonishing findings. The technical study submitted by Monsanto in July 1999 contained no molecular data, nor data on the breeding regime, for three different Bt lines. Data on the levels of protein expression in different tissues was included. But 300 corn plants were produced for two of the transformation experiments, and some of the critical measurements of expression levels were done on only two plants. Despite this, the data clearly indicate that different transformations led to significantly different levels and patterns of protein expression. Such differences are of crucial important in assessing efficacy, resistance management and non-target impacts, as well as changes in the microflora of the digestive systems of livestock and humans using the crop for food.

Monsanto then submitted its application in full in August 1999, moving from greenhouse-scale research to unrestricted field use within one year. In the covering letter they wrote; "Please note that approval of this registration by May 2000 would reduce the need for additional submissions and reviews for year 2000 field trials". This statement makes it blatantly obvious that Monsanto has no intention of investigating their findings any further with respect to health and environmental impacts. To date, the full application is still pending in the US, but has been granted commercial approval in Puerto Rico and Hawaii for this growing season.

In Dec 1999, Gunther Stotsky and colleagues (11) reported that Bt toxin is released into the rhizosphere – the area around the plant roots in the soil - in exudates from the roots of Bt corn,

where the toxin is protected from biodegradation and accumulates. This raised, for the first time, the question of what is happening underground? A total of 15 million acres of Bt corn were planted in the US in 1998, 20% of the total acreage. The leaked toxin enters the soil in an activated form - Bt transgenes are truncated to produce active toxin, unlike the precursor-form produced in the bacterium, which has to be cleaved in the gut of susceptible insect pests. Moreover, the toxin is expressed continuously, and hence exuded for extended periods of time.

In organic farming the toxin is sprayed sporadically in an inactive precursor form, only becoming active in the gut of the target insects once ingested. Furthermore, it is sprayed onto the surface of plants where it is readily biodegraded. Stotsky suggests that the widespread planting of Bt crops is equivalent to adding large doses of active toxin to the soil, not only from the plant root but also from the plant residues ploughed in, as well as from pollen. There is at present no clear indication as to how soil communities might be affected by Bt toxin from root exudates. It may promote evolution of toxin resistant target insects. But receptors for Bt toxins are present in both target and non-target insects, therefore both
will be affected. Bt toxins are active against insects in the Order of Coleoptera (beetles, weevils and styloplids) which contains some 28,600 species, far more than any other Order (12). The widespread use of Bt genes in crops and the build up of active toxin in the soil will have long term ecologically risks to non-target species and organisms in higher trophic levels, such as birds.

Simultaneously, it was reported that Novartis had filed a patent for another insecticide to be used in conjunction with Bt crops (13). It turns out that the pest-control spectrum of Bt toxins is limited, and other pesticides have to be used, that have been shown to be very damaging to health. This discredits industry's claim that Bt is essential for reducing harmful pesticide use.

This April brought further reports on pockets of Bt-resistance among pests in GM fields, and of GM cotton plants turning up as weeds in other crops (14). The cotton boll weevil may make a come back if such volunteers are ignored. An entomologist at Clemson Univ. said, "I could look across soybean fields and see hundreds of these Bt cotton plants". A return of the cotton boll weevil to parts of the American Cotton Belt would be a disaster, considering it
cost $1.3 million to eradicate them by 1995.

The ecological interaction between organisms is complex and scientifically challenging. The behaviour of insects with regard to choice, of food can have important impacts. This aspect has been overlooked completely in environmental risk assessments of GM crops. Researchers at Rothamstead in the UK (15) have pointed out that killing non-target species is a risk not unique to GM technology, as conventional regimes actually kill insects in an indiscriminate manner that is equally unsustainable. They highlight the need to find alternatives to conventional practices and suggest that management and good husbandry of bio-control agents should act in an integrated manner to eliminate caterpillars.

The health assessment of Bt crops relies totally on past experiences with Bt sprays in organic farming. It is wrong to assume that Bt toxin in GM crops is the equivalent to what has been used for over thirty years on organic produce with no deleterious effects. As with all GM crops, comprehensive feeding trials have yet to be conducted and therefore there are no data supporting the safety of eating Bt crops. Furthermore, there is a general lack of scientific transparency with all GMOs and Bt-crops are no exception. Crucial data are withheld from the public domain under various confidentiality statements made by the biotech companies in their applications for license.

Leading US agronomist Charles Benbrook has just completed a comprehensive review on EPA's management of Bt-corn (16). It provides important insights into the structural and legal shortcomings in the approval process, the major among which was the failure to adhere to the precautionary principle.

The summary of findings reported by independent scientists investigating or evaluating environmental risks are sufficiently compelling to warrant the immediate withdrawal of all Bt crops from use.

1. ISB Environmental Releases Database for USDA APHIS website :
www.aphis.usda.gov/bbep/bp/index.html
2. Hilbeck, A., Baumgartner, M., Fried, P.M. abd Bigler, F. (1997). Effects of transgenic Bacillus thuringiensis-corn-fed prey on mortality and development time of immature Chrysoperla carnew (Neuroptera: Chrysopidae) Enivronmental Entomology 27, 480-487
3. Losey. J., Raynor. L., & Carter. M. E., (1999) *Nature* 399,214
4.See:http://www.ent.iastate.edu/entsoc/ncb99/prog/abs/D81.html
[Non-target effects of Bt corn pollen on the Monarch butterfly
(Lepidoptera:Danaidae) *L. Hansen, Iowa State University, Ames , IA 50011
and J. Obrycki, Iowa State University, Ames, IA 50011. Contact e-mail:

lrahnsen@iastate.edu]
5. First Hand Account ˆ Industry manipulation of Bt research, by Beck Goldburg, Environmental Defense Fund. Forwarded to Biotech Activists 11/05/99
6. The final report of the SAP panel is accessible at <http://www.epa.gov/scipoly/sap/1999/december/report.pdf>
7. Shelton, A.M., Tang, J., Roush, R.T., and E. Earle. (2000) "Field tests on managing resistance to Bt- engineered plants, *Nature Biotechnology*, Vol 18;399-342
8. Liu, Y-B., Tabashnik, B.E., Dennehy, T.J.,Patin, A.J., & Bartlett, A.C.
(1999) *Nature* 400:519.
9. Huang, F., et al. (1999) *Science* 284, 965-967
10. Comments Submitted to Docket No OPP-30487a: Registration application for CRY3BB transgenic corn modified to control the corn rootworm March 20 2000. On behalf of Environmental Defense, the Institute of Agriculture and Trade Policy, the Science and Environmental Helath Network, the Center for Food Safety, and the Consumer Policy Institute/Consumer Union.
11. Deepak Saxena, Saul Flores, G. Stotzky (1999) Insecticidal toxin in root exudates from Bt corn. *Nature* Vol 402 pp 480
12. Arnett, R.H., and R.L. Jacques. Guide to Insects, Simon and Schuster. 1981.
13. Genetically modified plants may still need pesticides, By Andy Coghlan and Barry Fox, *New Scientist*, 18.12.99
14. Pockets of resistance : A pest might make a comeback thanks to engineered weeds. *New Scientist*, By Andy Coghlan April 15 2000.
15. Poppy, G. (2000) GM crops:environmental risks and non-target effects, *Trends in Plant Scienc* 5 , 4-6.
16. Benbrook, C. and Suppen, S. (2000) Applying the Precautionary Principle in Assessing Transgenic Corn Technologies in the US <www.biotech-info.net/case_studies>

AR

No Vaccines in Food Plants!

A recent review considers the development of plants expressing antigens of bacteria and viruses a particularly promising approach to vaccine development. The first human clinical trial for an edible plant vaccine was approved (by the US Food and Drug Administration) and carried out in 1997. GM potatoes expressing an *E. coli* diarrhea toxin gene (the B subunit of *E.coli* heat-labile toxin LT-B) constitutively (ie, continuously and in all parts of the plant) were taken orally by human volunteers in Phase I/II clinical trials.

Each received raw potato cubes from a random sample of non-GM control tubers or GM tubers. Eleven received 50 -100 g of GM potato while three received 50 g of non-GM potato. Ten of the 11 who ate the GM potatoes showed a significant rise in LT-B antibodies, whereas no LT-B specific antibodies were detected in the controls. The serum antibody levels induced by ingestion of the GM potatoes were comparable to those measured when volunteers were challenged with 10^6 virulent enterotoxigenic *E.coli* (ETEC) bacteria.

Thus, GM potatoes expressing the recombinant LT-B protein proved capable of inducing an immune response in humans when taken orally. Phase I and II trials are currently in progress with GM potatoes expressing hepatitis B surface antigen (HBsAg) as a booster for the commercial hepatits B vaccine, and GM potatoes with Norwalk virus virus-like particles (VLPs) as a vaccine against viral diarrhea. All three trials successfully induced systemic and mucosal immune responses without the aid of adjuvants (additional agents that stimulate immune response), and there were no adverse effects observed.

Reference: Amanda M Walmsley, A.M. and Arntzen, C.J. (2000). Plants for delivery of edible vaccines. *Current Opinion in Biotechnology* 11:126-129.

Our Comments: Food crops should not be used for vaccine production. First of all, they will readily contaminate crops that are used as food. This point has been made previously (Ho, M.W. and Steinbrecher, R. (1998). Fatal Flaws in Food Safety Assessment, *Environmental & Nutritional Interactions* 2, 51-84). For example, it is assumed potatoes do not spread by pollination or by over-wintering tubers. Actually, both modes of transfer are known. Genes for the vaccines may also spread horizontally by sucking insects and by transfer to soil microbes. The genes and proteins may be released during plant wounding or breakdown of roots and rootlets and pollute surface and ground water. The vaccines may provoke allergic responses if humans or other mammals or birds are repeatedly exposed to the allergen.

In addition, many instances of recombination between viral transgenes and viruses have already been reported (reviewed in Ho *et al* (2000). *Microbial Ecology in Health and Disease* (in

press)). Have these plants been assessed for their ability to generate recombinant viruses? When genes of viruses infecting human beings are incorporated into plants, are we not increasing the potential for generating new recombinant viruses that may cross from plants to human beings? (see "Can viruses cross from plants to animals?" This issue).

Vaccine production in plants may be a good idea. *But it should be done in plant tissue culture under strictly contained conditions and not in crops grown in the open field*. JC & MWH

Bacterial Genes and Autoimmune Responses

Bacterial DNA can trigger autoimmune responses, and so can synthetic oligonucleotides.

Over the past few years, it has become recognized that along with structural components and products of bacteria, bacterial DNA is also capable of signaling danger of infection to cells of the immune system. Particular DNA sequences (CpG motifs), which are abundant in prokaryotic (bacterial) but not in mammalian DNA, cause the activation and stimulation of immune cells. Research has been catalyzed by the finding that certain synthetic oligodeoxynucleotides mimic the action of bacterial DNA (see ISIS News#2, "Gene therapy and naked DNA vaccines can trigger autoimmune reactions"). Immuno-stimulation induced by bacterial DNA or synthetic oligonucleotides is being used therapeutically to condition or modify ongoing immune responses.

For example, CpG motifs have been used as vaccine adjuvants as well as instructing agents to selectively induce primary (Th1) immune responses involving T- helper cells, inflammation and cellular immunity. Hence, CpG motifs might be used in future as adjuvants and/or immunomodulatory agents in an attempt to treat or prevent undesired humoural cell response including allergy (associated with IgE antibodies).*

Reference: Heeg K and Zimmermann S (2000). CpG DNA as a Th1 Trigger. *Int Arch Allergy Immunol*. 121, 87-97.

Our Comments: Practically all transgenic crops have bacterial genes. Not much thought has been given to the potential impact of these bacterial DNA sequences in crop plants as they are digested by mammals or taken up in through wounds as plant juices or breathed in as pollen or crop dust. Recent research in gene therapy and DNA vaccines show that DNA can indeed be delivered into cells by oral ingestion, skin application or nasal inhalation (Reviewed in Unregulated Hazards: 'Naked' and 'Free' Nucleic Acids, ISIS and TWN Report, Jan. 2000 www.i-sis.org). Bacterial DNA not only produces immunity but stimulates inflammation and autoimmune responses. Autoimmune diseases include diabetes, Lupus , arthritis and multiple sclerosis.

It may be supposed that people and animals have bacteria in their guts but these bacteria normally pass through the digestive system protected by their thick cell walls. Bacterial DNA incorporated into the cells of the food crops is not so protected, and will be subject to digestive breakdown to generate fragments that may trigger autoimmune reactions. Whether these bacterial DNA danger signals are good or bad for mammals is something that should be known before we expose the world a to an avalanche of transgenic crops.

JC

Note added by Editor

The following was posted to ISIS by Bili Goldberg (BiGoldberg@aol.com)

Apparently, according to an abstract in a recent FASEB Journal, the use of synthetic CpG oligonucleotides (ODNs) as DNA vaccine adjuvants and in plasmid vectors (including HIV vaccines) may be fraught with problems with the finding of inhibitory CpG ODNs.

Ashman *et al*. *FASEB Journal* 2000, 14:A963 (Abstract 46.6) state:

"These results imply that the design of CpG-based vaccine adjuvants and plasmid vectors for DNA immunization must not only include stimulatory ODN sequences but avoid inhibitory ones."

GM Crops and the Ecology of Microbes

The widespread horizontal gene trafficking among bacteria makes it highly likely that GM constructs in GM crops will spread to microbial populations in all environments.

Eukaryotes ('higher' organisms which sequester their genomes in a nucleus) evolve principally through the modification of existing genetic information passed on in normal reproduction. Bacteria, however, have obtained a significant proportion of their genetic diversity through the acquisition of genetic material from distantly related organisms. Such horizontal gene transfer produces extremely dynamic genomes in which substantial amounts of DNA are introduced into and deleted from the chromosome. These lateral transfers have effectively changed the ecological and pathogenic character of bacterial species.

Large blocs of DNA are acquired by bacteria by taking up naked DNA molecules, by mating, by plasmid exchange and by virus (the transduction process). For example the disease causing

genes for both cholera and anthrax are located on transferable plasmids and can also be spread by bacterial viruses in nature. The evolutionary biology of bacteria is dominated by horizontal gene transfer.

Reference: Ochman, H., Lawrence, J.G. and Groisman, E.A. (2000). Lateral gene transfer and the nature of bacterial innovation. *Nature* 405, 299 - 304

Our Comments: Large blocs of bacterial/viral DNA are introduced into all transgenic crops now released to the environment in field tests or commercial production. The bacterial genes include antibiotic resistance markers, replication origins (of plasmids), expressed genes for herbicide or insect resistance and other bacterial plasmid genes. Such genes are released back into the bacterial *milieu* during digestion in the gut of predators from human to insect, and as crop residues in the soil. The ecological and health impacts of such gene releases from the millions of acres of GM crops have largely been ignored by those charged with protecting health and the environment.

JC

ISIS News 6, September 2000, ISSN: 1474-1547 (print), ISSN: 1474-1814 (online)

CaMV Promoter Active In Animal and Human Systems

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

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

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

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

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

Ban Biological Weapons and Agent Green!

Clinton admits that US' plan to use Fusarium to eradicate drug crops in Colombia may have an impact on biological weapons proliferation. ***Joe Cummins*** *reviews the scientific literature showing why that is the case. Please write to your Government to give them this information, and demand a total ban on this and other similar biological weapons.*

The United States government is considering using biological control agents to eradicate coca plants in Columbia. Because of its illicit coca crop, Colombia is on the front line of US biological warfare plans. Other projects to develop biological agents to kill opium poppy and marijuana are also funded by the US and the British Governments.

Clinton overruled the US Congress to decouple the link between Colombian acceptance of Agent Green and the overall implementation of the US 1.3 billion dollar bilateral assistance package for Plan Colombia.

Clinton states that the US will not use Agent Green until "a broader national security assessment, including consideration of the potential impact on biological weapons proliferation and terrorism, provides a solid foundation for concluding that the use of this particular drug control tool is in our national interest." That implies it is still on the cards.

The preferred biological control agent is the fungus *Fusarum oxysporum* a common plant pathogen. To be effective and safe for application, strains of the common pathogen would have to be selected and those strains would have to be supremely resistant to mutation and sexual gene exchange because small changes in a few genes can alter host range and the range of side effects on animals. The best available scientific evidence suggests that those goals of genetic conservatism and stability are unattainable, and that widespread saturation of a geographical area with this plant pathogen may not only impact on food crops, but on human health and a wide range

of mammals and birds.

Fusarium oxysporium is a fungus without a reproductive cell (without sexual spores) but one well known to have very active genetic recombination following fusion of mycelia (the fungal mat). Mitotic recombination (recombination during ordinary cell division) is common in asexual fungi (1).

The presence of several families of transposable elements (jumping genes) also contribute to mutation and chromosome rearrangement (2). Among the transposons is the *impala* element, a member of the *mariner* transposon family that is known to spread horizontally across fungi, plant and animals (3). Horizontal gene flow contributes to the variability in F*usarium*. There is no known way to control gene flow in *Fusarium* and such gene flow is the key to the success of the pathogen.

It is certainly ill-advised to drench a geographical area with a fungus known to infect humans or animals. In humans with normal immune systems, *Fusarium oxysporum* was associated with infection of skin and nails (4). A respiratory disease along with fungal infection of the liver was observed in a patient (5). People with undeveloped, aging or compromised immune system are highly sensitive to fungus infection. *Fusarium oxysporum* is associated with Kaschin-Beck (KB) disease, an early aging disease affecting numerous people in China and Russia, and the disease also strikes mammals and birds. For example, *Fusarium oxysporum* infected corn caused KB disease symptoms in chickens (6). *Fusarium oxysporium* infected grain caused KB symptoms in rats (7). and monkeys (8). The main onset of KB disease in humans is between the ages of 4 and 13 and the disease was twice as prevalent in boys than in girls (9).

We cannot allow the US Government to spray a fungus associated with such a serious disease. It is tentamount to waging biological warfare on the people of Columbia and their neighbours.

In conclusion, *Fusarium oxysporium* is unlikely to eradicate coca in Columbia but there is a reasonable chance that it will spread a horrific disease among young humans and animals.

1. Taylor,J, Jacobson,D, Fisher,M. (1999). The evolution of asexual fungi. *Ann Rev. Phytopath* 37,197-246.
2. Hua-Van,A, Daviere,J, Kaper,F, Langin,T and Daboussi,M (2000).Genome organization in *Fusarium oxysporum*: clusters of class ii transposons. *Curr Genet* 37,339-47.
3. Hua-Van,A, Hericourt,F, Capy,P, Daboussi,M and Langin,T (1998). Three highly divergent subfamilies of the impala transposable element coexist in the genome of the fungus *Fusarium oxysporum*. *Mol. Gen Genet* 259,354-62.
4. Romano,C, Miracco,C and Difonzo,E. (1998). Skin and nail infections due to *Fusarium oxysporum* in Tuscany, Italy. *Mycoses* 41,433-7.
5. Sander,A, Beyer,U and Amberg,R. (1998). Systemic *Fusarium oxysporum* infection in an immunocompetent patient with an adult respiratory disease syndrome (ARDS) and extracorporal membrane oxygenation (ECMO). *Mycoses* 41, 109-11
6. Chu,Q, Wu,W. Cook,M and Smalley,E. (1996). Elevated plasma glycosaminoglycans in chickes with tibial dyschondriaplasia induced by a *Fusarium oxsyporum* isolate. *Avain Dis* 40, 715-9.
7. Fu,Z and Zhang,S (1993). Effects of cereals from Kaschin-Beck's disease endemic area on fibrillogenesis in vitro of cartilage type II collagen in rats. *Chung Hua Yu Fang I Hsueh Tsa Chih* 2,77-80.
8. Zhang,G and Lui,J. (1989). An experimental animal model of Kashin-Beck disease. *Ann Rheum Dis* 48,149-52.
9. Zhai,S, Kimbrough,R, Meng,B, Han,J, LeVois,M, Hou,X and Yin,X. (1990). Kashin-Beck disease: a cross sectional study in seven villages in the People's Republic of China. *J Toxicol Ennviron Health* 30,239-59.

For more information, please see The Sunshine Project www.sunshine-project.org

Bt Pollen Lethal to Monarch Butterflies - Confirmed

A new study from Iowa State University shows Bt corn pollen naturally deposited on common milkweed in a corn field causes significant mortality to monarch butterfly larvae (1).

Larvae fed on milkweed plants naturally dusted with Bt pollen suffered significant higher rates of mortality after 48 hours exposure compared to larvae fed on leaves with no pollen, or on leaves with non-Bt pollen.

The highest mortality rates occur on milkweed plants in corn-fields or within 3 meters of the edge. But quantification of wind dispersal beyond the edge of fields predicts mortality may be observed at least 10 meters from Bt corn field borders.

The study also investigated sub-lethal effects and found continuous exposure to Bt toxin influences developmental time and adult characteristics to various degrees. The study found sub-lethal ingestion of Bt toxins caused reduction in adult lipid levels and may indicate that larva fed less, or did not digest nutrients efficiently. Migratory adult monarchs rely on lipids for energy and a lower level of lipids, carried over from the larval stage, could reduce their ability to reach Mexico.

Reduced adult weight and smaller wing lengths was also observed and similarly could decrease the ability of adults to complete migration.

Fifty percent of over-wintering adults in Mexico originate from central US, an area of concentrated corn production. In 1998, approximately 3.6 million hectares of Bt corn were planted and predictions are that by 2003, this area will have extended to 12 million hectares (1/3 of total US corn acreage).

The study concludes that because monarch larvae, milkweeds and transgenic pollen overlap spatially and temporally in the central US, Bt corn pollen will have a negative effect on monarch larvae. Larvae developing in late summer will be exposed to Bt pollen for most of their development and cumulative exposure to Bt toxin could raise mortality rates even further by preventing successful migration.

These findings indicate that Bt crops can have adverse effects on food webs that are not corn and the widespread planting of transgenic corn represents a significant mortality factor for non-target species. Ecological impact assessments must be evaluated more fully before Bt crops are planted over extensive areas.

The US EPA has convened an independent review board to assess the ecological impact of Bt crops. But they have also extended licence for plantings until 2002, despite calls for a ban.

1. Hansen L. C., Obrycki J.J. (2000) Field deposition of Bt transgenic corn pollen: lethal effects on the monarch butterfly, *Oecologia*, published online. AR

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GM Cotton Fails in Indonesia

Contrary to Monsanto's claim, its GM cotton succumbed to drought and insect attack while indigenous variety thrived, ***Mae-Wan Ho*** *discovered while on visit to Jakarta.*

Monsanto planted 500 hectares of GM cotton within 9 districts of Sulawesi, Indonesia in open 'field trials'. This came to light when the company invited journalists to one of the sites where it claimed the GM cotton out-performed the indigenous variety planted side-by-side. Konphalindo, a public interest organ-isation dedicated to environmental protection, demanded information from the Department of Agriculture, especially the risk assessment required for approval of the field trials. That was six months ago. The Department of Agriculture provided no information on risk assessment, despite repeated requests. Konphalindo wrote a letter to the top national newspaper *Kompas*, which triggered investigations by its journalist.

It transpires that the GM cotton failed to out-perform the indigenous variety in *all* but one of the 9 districts. Worse yet, the GM cotton succumbed to drought and the brown hopper. Vivid photographs showed the browned-out GM cotton field next to the lush green field of indigenous cotton, which is resistant to both drought and the brown hopper. One of the photos appeared in *Kompas* (8 Feb.) under the headline, "GM cotton in Sulawesi Suspected Illegal". Hira Jhamtani, founder of Konphalindo, said, "If Monsanto hadn't boasted of their 'success', we would never have found this out. We suspect that no safety assessment had been carried out at all."

Konphalindo had halted the commercialisation of Monsanto's GM cotton last October with the help of information provided by ISIS, which drew attention to strongly worded advice against the approval of Monsanto's GM cotton given by UK Government scientists. They warned of antibiotic resistance genes that would make gonorrhoea untreatable (see "Monsanto's GM Cottons & Gonorrhoea", this issue).

News has come that the Indonesian Government Department of Agri-culture has just granted commercial approval for the GM cotton, but the Department of Environment is opposed. So a fierce fight is expected in the Indonesian Cabinet, and a lot will now depend on civil protest action.

Monsanto's GM Cottons & Gonorrhea

Strongly worded advice against the approval of Monsanto's GM cotton was given by UK Government scientists warning of antibiotic resistance genes that would make gonorrhea untreatable.

The information is in the archives of the UK Advisory Committee on Novel Foods and Processes (ACNFP) which vats applications for commercial approval of novel foods and animal feed. The advice was given in February 1999 (but was only published last year by the UK Ministry of

Agriculture, Fisheries and Food). At around the same time, the European Union rejected Monsanto's application for the sale of the GM cottons in Europe. The reason? The gene *aad*, which confers resistance to the antibiotics streptomycin and spectinomycin, is present in both Bollgard (insect-protected) and Roundup Ready (herbicide tolerant) GM cottons.

The bacterium responsible for gonorrhoea, *Neisseria gonorrhoeae*, could acquire the *aad* gene from GM plant materials during infection of the mouth and small and large intestine as well as the respiratory tract. *N. gonorrhoeae* could also get the gene indirectly from other bacteria in the internal and external environments of animals and human beings, which have taken up the gene from GM plant materials. Those other bacteria can serve as a reservoir for antibiotic resistance genes.

Streptomycin is mainly used as a second-line drug for tuberculosis. But it is in the treatment of gonorrhea that spectinomycin is most important. It is the drug of choice for treating strains of *N. gonorrhea* already resistant to penicillin and third generation cephalosporins, especially during pregnancy.

About 60% of the cotton harvest consist of cotton seed. Cotton seed oil is extracted for human consumption, while the residue, cotton seed cake is fed to animals. Although the Government advice was aimed at cotton seed, there are other hazards arising from the use of the GM cotton itself, which may be why it was rejected by the EU.

"Cotton is used in women's sanitary napkins and tampons, in babies' nappies, in bandages and other wound dressings." Dr. Elizabeth Bravo, a biologist from Accion Ecologica, Ecuador, reminds us. No one has checked if such cotton contains DNA.

Both GM cottons are being grown in millions of hectares in the United States and China, and exported to other countries. They are also planted to a smaller extent in Argentina. And Monsanto is trying to introduce them into Bolivia and other Latin American countries as well as India and Thailand. Illegal plantings of at least 500 hectares have already been discovered in Indonesia.

Why is this important scientific advice from UK Government scientists kept in the archives for more than a year before it was published? It could have, and should have, prevented millions of hectares of transgenic cottons from being planted.

All cotton crops should be destroyed, and no more should be planted. Meanwhile, people should avoid using GM cotton products, especially in tampons, babies' nappies and wound dressings. GM cotton seeds certainly should not be used in food or feed.

To see the MAFF document, go to http://www.foodstandards.gov.uk/maff/archive/food/novel/cotton.htm

Bt is Toxic

Recently there has been considerable international concern about the contamination of the human food chain with StarLink corn containing *Baciullus thuriniensis* (Bt) toxin Cry 9. Bt Cry 9 toxin had evident allergenicity in test animals, and had been approved for use in animal feed alone, but was found to have contaminated corn and corn products destined for human consumption.

Bt toxins are the products of a number of genes and genes that differ between Bt varieties. The United States and Canada judge that each toxin gene product must be considered safe for human consumption until it is proven otherwise. A study that recently came to light [1] shows that a widely used Bt toxin actually damages the mammalian ileum (the final part of the small intestine, where food stays the longest). Damages to the ileum can produce chronic illness such as fecal incontinence and/or flu like upsets of the digestive system.

The researchers studied the effects of both GM potatoes carrying the CryI gene of *Bacillus thuringiensis* var. kurstaki strain HD1, as well as non-GM potatoes spiked with the toxin from the same strain of bacterium. Groups of five one-month old male mice were fed daily for 2 weeks on a diet of either the GM potatoes or the non-GM spiked by soaking the diced potatoes for 30 minutes in a suspension of the toxin (1 g per litre). The control group was fed non-GM potatoes for the same duration. Light and electron microscopic structures of the ileum in the three groups were compared.

Both the groups of mice fed GM potatoes or spiked potatoes revealed certain common features such as the abnormal appearance of mitochondria, with signs of degeneration and disrupted short microvilli (microscopic projections on the cell surface) at the surface lining the gut. However, in the group of mice fed on the spiked potatoes, several villi (projections of the intestinal lining, each made up of many cells, not to be confused with microvilli above) appeared with an abnormally large number of cells (151.8 in control group compared to197 and 155.8 in the spiked and GM-fed groups, respectively). Fifty percent of these cells were overgrown with multiple nuclei. The mean

area of the cells was significantly increased (105.3 □m2 in control group compared to 165.4 and 116.5 in the spiked and GM-fed groups, respectively).

Several forms of secondary digestive vacuoles were recognised in these cells. These changes were confirmed with the scanning electron microscope which revealed a remarkable increase in the perimeter of the cells (23 □m in control group compared to 44 and 28 in spiked and GM-fed groups, respectively). The basal lamina along the base of the cells was damaged at several foci. Several disrupted microvilli appeared in association with variable-shaped fragments of broken cells. In addition, the Paneth cells (secretory cells) in the spiked-fed group were highly activated and contained a large number of secretory granules. These changes may suggest that spiked potatoes resulted in the development of hyperplastic (overgrown) cells in the mice ileum.

Although milder changes are reported in the structural configuration of the ileum of mice fed on GM potatoes, the authors recommend that "thorough tests of these new types of genetically engineered crops must be made to avoid the risks before marketing".

It seems clear that the damages to the mice ileum are due to the bt toxin. What is not clear from this paper is the amount of toxin expressed in the GM potatoes compared to the amount in the spiked potatoes.

It is also of interest that the results here are similar to those obtained by Ewen and Pusztai in experiments with GM potatoes expressing the snowdrop lectin. As Pusztai points out, bt is also a lectin. It suggests that all lectins may have detrimental effects on the gut and should never have been used in GM crops.

1. Fares NH and El-Sayed AK. Fine structural changes in the ileum of mice fed on □endotoxin-treated potatotes and transgenic potatoes. *Natural Toxins*:1998: 6: 219-33.

JC & MWH

Kanamycin Still Used and Cross-Reacts with New Antibiotics

Kanamycin resistance genes are approved as selectable markers on grounds that the antibiotic is no longer in use. ***Prof. Joe Cummins*** *tells us that on the contrary, kanamycin is still in use and that cross-reaction between kanamycin and other related antibiotics is commonplace.*

The antibiotic kanamycin is used extensively in crop genetic engineering as a selectable marker. A selectable marker is a gene inserted into a cell or organism to allow the modified form to be selectively amplified while unmodified organisms are eliminated. In crop genetic engineering the selectable marker is used in the laboratory to identify cells or embryos that bear the genetic modifications that the engineer wishes to commercialize. The selection gene is used once briefly in the laboratory but thereafter the genetically modified (GM) crop has the unused marker gene in each and every one of its cells.

There is a well-known cross-resistance between antibiotics of a particular type. A mutation to resistance to an antibiotic may cause resistance to some or all of the members of the antibiotic family [1]. Kanamycin is a member of the aminoglycoside family of antibiotics. Cross-resistance between kanamycin and other aminoglycosides including streptomycin, gentamycin and tobramycin was found to vary markedly between bacterial isolates. All of the antibiotics mentioned are used to treat human diseases. The aminoglycoside antibiotic neomycin was found to cross react with kanamycin B in inhibiting Rnase, P ribozyme, 16s ribosomal RNA and tRNA maturation [2].

Along with cross resistance to aminoglycoside antibiotics, pathogenic bacteria frequently develop multiple drug resistance transmitted on a single plasmid. For example, the cholera pathogen *Vibrio cholerae*, first isolated from India, Bangladesh and Thailand [3], was found to have a plasmid resistant to tetracycline, ampicillin, chloramphenicol, kanamycin, gent-amycin, sulphaethoazole and trimethoprim. Pathogenic bacteria do aquire plasmids with multiple antibiotic resistance genes in areas where the antibiotics are used extensively.

In spite of the belief of many genetic engineers that kanamycin is no longer employed in medical applications, there is evidence that the antibiotic is used extensively in North America where crops bearing kanamycin resistance are marketed without warning labels. Kanamycin is used prior to endoscopy of colon and rectum [4] and to treat ocular infections [5]. It is used in blunt trauma emergency treatment [6] and has been found to be effective against *E coli 0157* without causing release of verotoxin [7].

In conclusion, extensive use of kanamycin resistance marker genes in genetically modified crops is unjustifiable in the face of current medical applications.

1. Onaolapo J. Cross-resistance between some aminoglycoside antibiotics. *Afr J Med Sci* . 1994, 23,215-9.

2. Mikkelsen N, Brannvali M, Virtanen A and Kirsebom L. Inhibition of P RNA cleavage by aminoglycosides. *Proc. Natnl Acad Sci USA*, 1999, 96,6155-60.
3. Yamamoto T, Nair G and Takeda Y. Emergence of tetra-cycline resistance due to a multiple drug resistance plasmid in *Vibrio cholerae* 0139. *FEMS Immunol Med Microbiol* 1995, 11,131-6
4. Ishikawa H, Ikuko A, Minami T, Shinmoura Y, Ojo H and Otani T. Prevention of infectious complications subsequent to endoscopic treatment of the colon and rectum. *J Infect Chemother*, 1999, 5, 86-90
5. Hehl E, Beck R, Luthard K, Guthoff R and Drewelow B. Improved penetration of amono-glycosides and fluoroquininolones into the aqueous humour of patients by means of acuvue contact lenses. *Eur J Pharmacol*, 1999, 55,317-323.
6. Yelon J, Green J and Evans J. Efficacy of an interperitoneal antibiotic to reduce incidence of infection in the trauma patient a prospective randomized study. *J AmCollSurg,* 1996, 182,509-14.
7. Ito T, Akino E and Hiramatsu K. Evaluation of antibiotics for enterohemorrhagic Escherichia coli 0157 enteritis effect of various antibiotics on extracellular release of verotoxin. *Kansenshogaku Zasshi*, 1997, 71,130-5.

Gene Therapy with Your Salads, Anyone?

A virus is simultaneously being genetically modified to kill insect pests and to transfer genes into human cells in gene therapy. ***Prof. Joe Cummins*** *points to a major gap in biosafety regulation.*

Do our biosafety regulators know that a certain virus is being genetically modified to control plant disease and to serve as a gene carrier or vector for human gene therapy? This is the baculovirus, a virus previously thought to infect only insects, but has since found to get into all kinds of mammalian cells including those of human beings. Farm workers spraying the crop with anti-insect baculovirus and the public eating the crop not properly washed may both become genetically modified as a result.

Pests that infect and cause disease symptoms in both crops and human cells have never been described. Yet natural viruses that infects and slowly kills insects are also known to infect humans, but the infected humans do not seem to have symptoms. However, when the virus is genetically modified to eradicate insect pests, it may cause disease symptoms in those spraying crops or eating the sprayed crop. The baculovirus manipulated for insect control and for human gene therapy have both proven genetically unstable, and are prone to recombination and deletion at high frequency [1]. Such genetic instability has been noted repeatedly by those studying the virus, the genetic instability of the virus makes toying with it like playing with explosives.

Natural baculovirus, in contrast, is very stable and may remain dormant in the environment for years before infecting insects. The virus alone has a relatively low killing power and slow action. When a gene for a potent toxin such as scorpion toxin or a gene affecting a juvenile hormone is added to the virus, it kills faster and fewer insects survive infection. Numerous field tests of modified virus sprayed on crops have been done, despite protests from the public. Soon after GM baculovirus was developed for insect-control, the virus was discovered to be capable of infecting human liver cells and produced relatively little toxicity to the infected cells. For that reason, baculovirus vectors were developed to treat liver disease, and even to transfer genes to the human brain [2]. The fact that baculovirus can infect human liver or brain cells seems to have been ignored by those developing the virus for commercial pest control. There has been a great deal of pressure to hasten approval of the GM baculovirus for pest control especially in the United States and Canada, where human populations have already been used as guinea pigs for GM crops.

Ecological impacts of recombinant baculovirus insecticides have focussed on baculovirus containing scorpion toxin because that construction has been most widely used [3]. Impacts on non-target insects are simply extrapolated from findings on insects of related phylogeny, a practice that is full of pitfalls, for simply adding and deleting genes can change the host range of the resultant baculovirus in unpredictable ways [4]. Furthermore, the recombinant baculoviruses were very persistent, and capable of reshaping an ecosystem.

The scorpion toxins used with recombinant baculovirus have been selected to avoid toxicity to humans, and as much as possible, to non-target animals. However, the allergenicity other harmful effects in human liver infection has not yet been investigated.

Recombinant baculoviruses have also been constructed containing other genes, such as those coding for *Bacillus thuringiensis* (bt) toxins [5], which are known to produce allergic reactions in human beings and also harmful to rats [6]. A recombinant baculovirus has even been constructed containing an antisense fragment to the c-myc oncogene [7]. The c-myc oncogene is a modified form of an essential cellular gene. Thus, the antisense gene, which contains a DNA sequence complementary to the gene itself, may end up inactivating an essential cellular function.

Baculovirus vectors efficiently transfer genes into human liver cells [8, 9]. Hybrid baculovirus-adenovirus vectors have also been used to deliver genes to human cells [10].

In conclusion baculovirus vectors are being used to control insect pests because they are effective and persist for a long time in the environment. Baculovirus vectors are also being used in gene therapy of human liver and brain. These areas of research seem to exist as two solitudes and the risks of one are not evaluated in the context of the other. We may be treated to liver and brain gene therapy with our salad whether we need it or not.

1. Wu Y, Lui G and Carstens E. Replication, integration, and packaging of plasmid DNA cotransformation with baculovirus viral DNA. *J Virol*, 1999, 73,5473-80.
2. Sarkis C, Serguera S, Petres D, Buchet J-L, Ridet L, Edelman J, and Mallet. Efficient transduction of neural cells in vitro and in vivo by a baculovirus-derived vector. *Proc Natl Acad Sci* 2000, 97,: 14638-43.
3. Richards A, Matthews M and Christain P. Ecological con-siderations for the environmental impact evaluation of recombinant baculovirus insecticides. *Ann Rev. Entomol*, 1998, 43,493-517.
4. Thiem S. Prospects for altering host range for baculovirus bioinsecticides. *Curr Opin Biotechnol* 1997, 8,317-22.
5. Martens J, Knoester M,Weijts F, Groffen S, Hu Z, Bosch D and Vlack J. Characterization of baculovirus insecticides expressing tailored Bacillus thuringiensis Cry1A9b) crystal proteins. *J Invertebr Pathol*, 1995, 66, 249-57.
6. Fares NH and El-Sayed AK. Fine structural changes in the ileum of mice fed on endotoxin-treated potatotes and transgenic potatoes. *Natural Toxins*,1998: 6: 219-33.
7. Lee S, Qu X, Chen W, Poloumieko A, MacAfee N, MorinB, Lucarotti C and Krause M. Insecticidal activity of a recom-binant baculovirus containing an antisense c-myc fragment. *J Gen Virol* 1997, 78,273-81
8. Hofmann C, Sandig V, Jennings G, Rudolph Schlag P and Strauss M. Efficient gene transfer into human hepatocytes by baculovirus vectors. *Proc. Nantl Acad Sci USA*, 1995, 92,10099-103.
9. Boyce F and Bucher N. Baculovirus-mediated gene trans-fer into mammalian cells *Proc. Natnl Acad Sci USA*, 1996, 93,2348-52.
10. Palombro F, Mociotti A, Recchia A, Cortese R, Ciliberto G and LaMonica N. Site specific integration in mammalian cells mediated by a new hybrid baculovirus-adeno-associated virus vector. *J Virol* 1998, 72, 5025-34.

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Common Plant Vector Injects Genes into Human Cells

The genetic engineering com-munity has assumed that Agrobacterium, a commonly used gene transfer vector for plants, does not infect animal cells, and certainly would not transfer genes into them. But this has been proved wrong. ***Prof. Joe Cummins*** *warns of hazards to laboratory and farm workers.*

Agrobacterium tumefaciens is a bacterium that causes tumours to appear on the stems of infected plants. The bacterium causes the tumours by transferring genes to the cells of the infected plant cells from a tumour inducing plasmid (Ti). The Ti plasmid has virulence genes that determine attachment to cells and transfer of a segment of the plasmid, T-DNA, to the plant cell. The transferred DNA is integrated essentially randomly (no apparent sequence bias at the site of insertion) into the plant chromosomes and normally add bacterial genes that stimulate plant tumour cell growth.

In crop genetic manipulation (GM), the growth-stimulating genes that give rise to tumours are replaced by GM constructs, which include genes for antibiotic resistance, plant viral promoters and genes for desired crop traits such as herbicide tolerance.

Until quite recently, the genetic engineering community has assumed that *Agrobacterium* does not infect animal cells, and certainly would not transfer genes into them. But this has been proved wrong.

A paper published earlier this year reports that T-DNA can be transferred to the chromosomes of human cancer cells [1]. In fact, *Agrobacterium* attaches to and genetically transforms several types of human cells. The researchers found that in stably transformed HeLa cells, the integration event occurred at the right border of the Ti plasmid's T-DNA, exactly as would happen when it is being transferred into a plant cell genome.

This suggests that *Agrobacterium* transforms human cells by a mechanism similar to that which it uses for transformation of plants cells.

The paper shows that human cancer cells along with neurons and kidney cells were transformed with the *Agrobacterium* T-DNA. Such observations should raise alarm for those who use *Agrobacterium* in the laboratory.

The integrated T-DNA will almost certainly act as a mutagen as it integrates into human chromosomes. Cancer can be triggered by activation of oncogenes (ie, cancer genes) or inactivation of cancer-suppressing genes. Furthermore, the sequences carried within the T-DNA in the transforming bacterium can be expressed in the transformed cells (the viral promoter CaMV has been found to be active in HeLa cells [2]) and constructions currently being tested include pharmaceutically active human genes such as the interleukins [3].

It is clear that little has been done to prevent environmental escape of the transforming bacteria or to quantify such releases. In conclusion, a study of cancer incidence among those exposed to *Agrobacterium tumefaciens* in the laboratory and in the field is needed. It would be worthwhile to screen workers for T-DNA sequences.

1. Kunik T, Tzfira T, Kapulnik Y, Gafni Y, Dingwall C, and Citovsky V. Genetic transformation of HeLa cells by *Agrobacterium*. *PNAS USA*, 2001, 98, 1871-87.
2. Ho MW, Ryan A and Cummins J. CaMV 35S promoter fragmentation hotspot confirmed and it is active in animals. *Microbial Ecology in Health and Disease*, 2000, 12, 189.

See "GM AIDS virus more lethal" by Joe Cummins & Mae-Wan Ho *ISIS Report*, July 19, 2001 www.i-sis.org, also this issue.

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Mice Prefer Non-GM

A Dutch farmer left two piles of maize in a barn infested with mice, one pile GM, the other non GM. The GM pile was untouched, while the non-GM pile was completely eaten up. Incredible! Young undergraduate Hinze Hogendoorn devised his own laboratory tests and confirmed the finding, and more. ***Dr. Mae-Wan Ho*** *reports from her visit to Hilversum near Amsterdam, where he lives with his mum.*

Hinze couldn't find a single scientific report on animals being tested for preference of GM versus non-GM food on the web when he began. On extending his search to effects of GM foods on animals, he came across reports from companies developing GM foods, all declaring there were no adverse impacts. But he also came across independent researchers who have reported harmful effects, including Dr. Arpad Pusztai, who found GM potatoes damaged the kidney, thymus, spleen and gut of young rats. Hinze was disturbed, not just by the scientific findings, but by the fact that scientists opposing the big companies are so easily discredited. "Personally, I'm afraid these companies have too much interest invested in their products for their research to be creditable." That was another motivation for him to do his own experiments.

The 17 year-old was stumped at first, because he would have needed to go through a lot of bureaucracy to experiment on animals. However, he managed to rescue 30 female six-week old mice bred to feed snakes from a herpetology centre. The next problem was to find the appropriate food. He went to a website on the care of mice. Mice eat about 15% of their body weight every day, and they need a diverse diet. So he decided to give them a staple food along with the two foods that were to be compared, so they could really show their preference without being starved. For the staple, he used Rodent mix from the pet store, as well as some oatmeal and cereals guaranteed by their producers (Kellogg's and Quaker's) to be 'GM-free' in the Netherlands. For GM foods, he used maize and soya, and the corresponding organically grown versions as non-GM.Water was supplied for the mice to drink as they pleased. And he kept track of all the food consumed each day.

Large cages were used so the mice had plenty of room to move around. At the beginning, all the mice were weighed before they were put into the cage with four bowls containing GM and non-GM maize meal, and GM and non-GM soya meal respectively. The mice had not eaten for some time, but amazingly, they already showed very definite food preferences. The didn't like soya meal at all, GM or non-GM, and only one mouse was found feeding on non GM soya meal for one minute in the 10 minutes they were observed. In the same period, 4 to 8 mice could be found in the bowl with non-GM maize, compared to 1 to 3 in the bowl with GM maize.

For the next week, Hinze continued to give the mice GM and non-GM maize or soya chunks (which they did eat) in addition to their staple food, and measured the amount of each consumed daily over the next week. In all nine successive observations, more non- GM was eaten than GM for maize or soya. In sum, the mice consumed 61% non-GM and 39% GM food when given free choice. The results were highly significant, even though Hinze did not perform the statistical test.

For the next experiment, Hinze tested for the effects of GM food. By this time, however, one mouse had died for unknown reasons. So he removed another mouse from the experiment, assigned 14 to the group fed GM food and 14 to the group fed non-GM food after weighing them. Over the next 10 days, he kept track of the amount of food that the two groups consumed daily, and weighed the mice, halfway through and at the end of the experiments.

The group fed GM ate more, probably because they were slightly heavier on average to begin with, but they gained less weight. By the end, they actually lost weight. In contrast, the group fed non-GM ate less and gained more weight, continuing to gain weight until the end of the experiment. The results were statistically significant.

That was not the only difference observed. There were marked behavioural differences, though Hinze admitted, these were "subjective" and not quantitative. The mice fed GM food "seemed less active while in their cages". The differences in activity between the two cages grew as the experiment progressed, the mice in the non-GM cage were in the exercise wheel more often than those in the GM cage. Hinze also noticed that each time he came into the room, there tended to be more mice in the non-GM cage walking or climbing around than in the GM cage.

The most striking difference was when the mice were weighed at the end of the experiment. The mice fed GM food were "more distressed" than the other mice. "Many were running round and round the basket, scrabbling desperately in the sawdust, and even frantically jumping up the sides, something I'd never seen before." They were clearly more nervous than the mice from the other cage. "For me this was the most disconcerting evidence that GM food is not quite normal."

Another "interesting result" is that one of the mice in the GM cage was found dead at the end of the experiment.

He concluded, "At the end of everything, I must admit that the experiment has done nothing to soothe my qualms concerning genetically enhanced food." His results "do seem to agree with Pusztai's".

Hinze is tall and athletic, and definitely doesn't like GM food. He is pleased to have found all that out for himself, and suggests everyone should do the same.

He has put the scientists to shame, especially those who have condemned Pusztai's work, but have done nothing since to add to our knowledge.

A young activists group (Jongeren Milieu Aktief) presented the report Hinze has written to the Dutch parliament on 11 December, and is featuring it on their new website www.talk2000.nl

US Foodborne Illnesses Up Two to Ten Fold

Genetic engineered food has increased enormously in the United States since 1994. Figures released at the end of 1999 showed a two to ten-fold rise in food-related illnesses compared with 1994. A Swedish study throws new light and raise important questions on the safety of genetic engineered food. ***Dr. Mae-Wan Ho*** *reports.*

Food related illnesses are on the increase. At the end of 2000, more than 250 foodborne diseases were described, but in the vast majority of cases, the causal agent is unknown. Diarrhoea and vomiting are the most common symptoms, with serious after-effects that include blood poisoning, abortion, infections, blood in the urine, and death. Chronic disorders of the heart and nervous system can also result, as well as arthritis, renal disease, and disease of the digestive system [1,2].

According to a report published at the end of 1999 [3], foodborne diseases cause approximately 76 million illnesses, 325 000 hospitalisations and 5 000 deaths in the United States each year. Known foodborne pathogens account for 14 million of the illnesses, 60 000 hospitalisations and 1 800 deaths. In other words, unknown agents account for approximately 81% of food borne illnesses and hospitalisations and 64% of deaths. Three pathogens, *Salmonella*, *Listeria* and *Toxoplasma* kill 1500 each year, more than 75% of those killed by known pathogens, while *Campylobacter*, *Salmonella* and *Shigella* top the list in known causes of foodborne illnesses.

To see foodborne illnesses in perspective, total illnesses from known pathogens are estimated at 38.6 million, and that includes 5.2 million (13%) due to bacteria, 2.5 million (7%) due to parasites and 30.9 million (80%) due to viruses. The breakdown for foodborne illnesses - in percentages of known etiological agents - is similar, with the highest proportion due to viruses.

The figures on foodborne illnesses are more than double those produced in 1994 [4], which were between 6.5 to 33 million illnesses per year. In terms of incidence, the increase is from 25 to 130 cases per 1 000 inhabitants in 1994 to 278 per 1 000 in 1999. Is the huge increase over the past five years real? Or is it simply a case of improved surveillance and reporting ?

For comparison, a Swedish study was undertaken in the Municipality of Uppsala of 186 000 inhabitants, based on enhanced surveillance and retrospective interviews in 1998-1999 [5]. A

total of 268 incidents were recorded, and 515 cases documented. This gives an incidence of 28 illnesses per thousand, which falls within the low end of the US estimate in1994. But that means the incidence of foodborne diseases in the US in 1999 is nearly ten times that of Sweden, as well as up to ten times higher than in 1994.

There are other aspects in the Swedish study comparable to the US. Thus, in 79% of the cases, the etiological agent was unknown, a proportion similar to the 81% reported in the US.

The breakdown in terms of known etiological agents, however, appears quite different. In Sweden, bacteria were found to cause 10% of the incidents and 25% of the documented cases, compared with 13% of cases in the US. Viruses, on the other hand, caused only 9% of both the incidents and documented cases in Sweden compared with 80% of cases in the US. As there is no reason to suppose that the countries differ in their ability to detect viruses, this discrepancy may well be significant.

The Swedish study suggests that the incidence of foodborne diseases in Sweden is similar to that of the United States in 1994, which is not surprising as both countries are presumably comparable in their food hygiene. But since then, the incidence in the United States has undergone an increase of between two to ten-fold. Such a large increase surely deserves to be thoroughly investigated.

Notably, genetically engineered food has increased enormously in the US since 1994, with proponents insisting there is no evidence that it has caused any harm. Health authorities should be on the lookout for new viruses and bacteria that could evolve by the horizontal transfer and recombination of viral and bacterial genes in genetically engineered crops.

1. "Foodborne infections", CDC Press Release December 2000.
2. Lindsay JA. Chronic sequelae of foodborne diseases. *Emerging Infectious Diseases* 1998, 3 www.cdc.gov/ncidod/EID/vol3no4/lindsay.htm
3. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C. Griffin PM and Tauxe RV. Food-related illness and death in the United States. *Emerging Infectious Diseases* 1999, 5, 607-25.
4. Foodborne Pathogens: risk and consequences. Ames (IA): Council of Agricultural Science and Technology, 1994.
5. Lindqvist R, Andersson Y, Linkback J, Wegscheider M, Eriksson Y, Tidestrom L, Lagerqvist-Widh A, Hedlund K-O, Lofdahl S, Svensson L and Norinder A. A one-year study of foodborne illnesses in the municipality of Uppsala, Sweden. *Emerging Infectious Diseases* 2001, 7 www.cdc.gov/ncidod/eid/vol7no3_supp/lindqvist.htm

Citizens' Vigil Exposes Bad Science in GM Field Trial

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

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

The Munlochy GM Vigil (munlochyvigil@tiscali.co.uk) had started up spontaneously in August after a local march and rally in protest of the GM crop trials approved by the Scottish Executive. That was despite repeated veto by the local inhabitants and their elected representative, the Highlands Council (see "Beware corporate takeover of organics" ISIS News 11/12 www.i-sis.org).

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

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

canvas. An opening at the top lets through the chimney connected below to a stove for cooking and to provide heating against the cold nights.

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

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

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

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

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

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

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

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

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

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

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

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

GM Crops Failed on Every Count

"GM crops have higher yields, improved performances, and greatly reduce the use of agrochemicals. Farmers like them because they increase income." ***Lim Li Ching*** *and* ***Jonathan Matthews*** *debunk every one these myths, documenting failures of GM crops around the world.*

Lower yields

Thousands of controlled trials have shown significantly decreased yields with GM crops.
A study based on 8,200 trials of soya varieties in US universities in 1998 [1] reports yield drags between top Roundup Ready (RR) varieties and top conventional varieties averaging 6.7%. In

some areas, best conventional varieties produced yields on average 10% higher than RR varieties sold by the same seed companies.

In May 2000, results of a two-year study by Nebraska University's Institute of Agriculture and Natural Resources showed RR soya yielded 6% less than their closest non-GM relatives and 11% less than high-yielding non-GM varieties [2]. The yield penalty was attributed to the gene insertion process.

Similar yield drags have been reported since 1997, and involve other GM crops besides soya.

- In 1997, the University of Purdue found that transgenic soya varieties yielded on average 12-20% less than unmodified varieties grown at the same locations [3].
- Research published in 1998 by the University of Arkansas and Cyanamid revealed reduced profit levels and lower yields for GM soya and cotton compared with unmodified varieties [3].
- The University of Wisconsin found GM soya yields from the 1998 harvest lower than non-modified varieties in over 80% of cases in trials across nine US states [4].
- In Iowa, a 1999 survey reported an average RR-soybean yield reduction of 4% in over 365 fields [5].
- A review of 40 trials of soya varieties in the north central region of the US in 1999 found a mean 4% yield drag in RR soya [6].
- In the UK, reports of crop trials from the National Institute of Agricultural Botany show yields from GM winter oilseed rape and sugar beet 5-8% less than high-yielding conventional varieties [7].

In summary, yield losses, not yield gains, are more commonly associated with transgenic crops compared to best available conventionally-bred cultivars and hybrids [8].

Yield drag in soya is associated with problems in root development, nodulation and nitrogen fixation, particularly in drought or infertile conditions, as the bacterial symbiont responsible for nitrogen fixation is sensitive to both Roundup and drought [9]. Furthermore, there is a metabolic cost to expressing herbicide-resistance or the Bt-endotoxin. For example, levels of proteins responsible for plant defence responses are depressed following Roundup application. Although these are eventually restored to normal, pathogens quickly infect the plants in sub-optimal growing conditions. This forces a diversion of energy to repair damage, resulting in an essentially irreversible tax on yields.

University of Minnesota economist Vernon W. Ruttan sums up: "Thus far, biotechnology has not raised the yield potential of crops" [10].

Yet, the power of distorted perceptions is such that, in an opinion poll of 800 farmers, 53% chose RR varieties over non GM varieties because of perceived higher yields. When actual data from their farms were analysed, exactly the opposite was found [5]. "It is interesting to note... that increasing crop yields was cited by over half the farmers as the reason for planting GM soya, yet yields were actually lower".

Poorer performances

To add to the yield woes, research at the University of Georgia has demonstrated that glyphosate resistant beans (glyphosate is the active ingredient in Roundup and other herbicides) perform poorly in heat stress conditions, with up to 40% crop losses [11]. At higher temperatures (above 25^{o}C), RR soybeans were stunted. In soils reaching 45^{o}C, the beans had lower heights, yields and weights. Worse, stems split open as first leaves emerged, possibly caused by changes in plant physiology due to insertion of genes conferring glyphosate resistance. Plants carrying these genetic alterations produced up to 20% more lignin, making them more brittle and prone to splitting.

Further, some glyphosate resistant soy crops in the US are damaged by glyphosate applications, emerging soybean leaves became yellow or lemon-lime green in colour [12]. While the phenomenon does not always translate to lower yields, soybeans under stress from disease, drought, nematodes, insect feeding, excessive moisture or injury from other herbicides 'will not be able to metabolize glyphosate as quickly, and soybean will show crop injury'.

And while it is not certain what causes such tolerance failure, 'gene silencing', is not unknown. A plant may use it to inactivate deleterious foreign genes and for regulation of growth and development, but the phenomenon can interfere with the expression of newly inserted genes, in particular if triggered by environmental stresses [13].

Yellowing of soybean leaves might also be a symptom of something more serious, such as stem and root diseases. Scientists from the University of Missouri have recorded a significantly

higher incidence of *Fusarium* (a soil fungus) on the roots of RR soybeans receiving glyphosate at recommended rates, compared to soybeans that did not receive glyphosate [14]. This was also true of the *Fusarium* that causes SDS (sudden death syndrome). Monsanto has disclosed that the foundation genetics for RR soybeans came from a line that is SDS-susceptible. And there could be potential yield impacts in subsequent seasons due to high soil *Fusarium* populations, resulting from continued use of glyphosate.

Bt resistance and more pesticides

The other big claim for GM crops is reductions in pesticide use. In reality, herbicide tolerant and Bt-transgenic varieties of GM crops are trapping farmers into more reliance on pesticides.

Recently, hundreds of hectares of GM cotton fields in Bulukumba, South Sulawesi, were destroyed by pests [15]. Officials said that there was "nothing to worry about", and a spokesperson from Monsanto (the GM Bollgard cotton seed supplier) asserted that "they are just larva which eat the leaves, but will not disrupt cotton production". But local farmers complained, pointing out that the supplier had claimed the cotton variety was resistant to all kinds of pests.

What happens when GM crops fail to deliver on their promise of pest resistance? Farmers in Australia are now being advised to spray additional insecticide on Monsanto's GM Bt cotton, INGARD, "under conditions of reduced INGARD plant efficacy" [16]. The latest official guidance [17] makes it clear that Bt cotton is in some circumstances failing to control the principal target pest it was introduced for, *Helicoverpa armigera*.

Even when GM crops express pest resistance, there is little evidence of reduced pesticide use. This is borne out by data on transgenic cotton - although to date one fourth of American cotton is produced with genetically engineered Bt varieties, no significant reductions in the overall use of insecticides were achieved [18]. In fact, those insecticides that could be replaced by Bt cotton make up a minor proportion of the insecticides used.

Similarly, with Bt corn, there is no independent evidence of a reduction in overall pesticide applications despite industry claims. Nor is there economic advantage in using Bt corn except in areas with very high pest infestation. Insecticide use on US Bt corn has in fact slightly increased, with insecticide targeting European corn borer rising from about 4% of acres treated in 1995 to about 5% in 2000 [19].

Herbicide use shows a similar picture. Although the cultivation areas of herbicide-tolerant cotton in the US have doubled annually over the past few years, herbicide use has shown little reduction. More revealingly, the sales of total herbicides that can be used with GM cotton have risen drastically since the introduction of herbicide-tolerant cotton [18].

While the Roundup Ready soybean system simplifies weed management, it entails 2-5 times more herbicide use than other weed management systems [1]. Tolerance to Roundup is emerging in several key weed species, contributing to increased chemical use. Unbiased field-level comparisons, drawing on official USDA data, show that RR soybeans require more herbicides than conventional soybeans, despite claims to the contrary [9, 19]. In 1998, total herbicide use on RR soybeans was 30% greater on average than on conventional varieties in six US states [9].

Furthermore, Roundup is only effective in controlling weeds to a certain extent. For example, with waterhemp, a member of the pigweed family, Roundup applied over RR soybeans is only effective until the weeds are about 12 inches tall [20]. And the weeds can easily grow two inches a day, quickly overwhelming a soybean crop. Farmers who don't respond quickly will experience crop loss.

Analysis thus shows that RR soybean systems are '…not likely to reduce herbicide use or reliance. Claims otherwise are based on incomplete information or analytically flawed comparisons that do not tell the whole story' [1]. And as for RR corn, USDA data suggest that in 2000, the average RR corn acre was treated with about 30% more herbicide than the average non-GM corn acre [19].

Worryingly, research from the University of Alberta has revealed the rapid creation of multiple herbicide resistant canola plants in Canada as a result of pollen flow over significant distances [21]. Cross-hybridizations occurred between a glyphosate-resistant variety and either glufosinate- or imidazolinone-resistant varieties. The evidence pointed to resistant gene movement via pollen flow from one field to another. Unusually, this occurred rapidly and multiple times, such that, through random crossing, certain plants showed triple resistance [22]. One of the triple-resistant plants was found over 550m from the pollen sources, greatly exceeding the 100m buffer mandated for seed producers.

Reduced profits

The greater expense of GM seeds and increased herbicide costs can already hit farmers' pockets. Add to these the costs of yield drag and technology fees, and it is bad news for profitability. For example, the added costs for soya producers can be more than 12% of gross income per acre [1].

The Leopold Center for Sustainable Agriculture, Iowa State University, interviewed 800 Iowa farmers in 1998 to determine if growing GM crops was more profitable [5]. Random surveys of 62 continuous cornfields, 315 rotated cornfields, and 365 soya fields concluded that the difference in profitability was non-significant for both crops. Thus, the farmers who raised GM crops did not gain any competitive edge. A recent survey, using crop data from 2000, yielded similar findings [23]. There was no economic advantage for Iowa farmers to plant herbicide-tolerant soybeans or Bt corn. There was essentially no difference in the return to using herbicide-tolerant versus non-tolerant soybeans. Yield for RR soybeans was lower, averaging 43.4 bushels per acre vs. 45.0 bushels for non-RR. While herbicide costs for RR soybeans was $6.17 less than for non-RR, seed cost for RR soybeans was $5.69 per acre more than non-RR. So while the use of herbicide-tolerant varieties results in lower herbicide and weed management costs, the higher seed costs and lower yields negated any advantage gained. Similarly, Bt corn produced a return essentially equal to non-Bt corn. While average yield for Bt corn was higher (152 bushels per acre vs. 149 bushels for non-Bt), seed and fertilizer costs for Bt corn averaged $4.31 and $4.63 per acre more, respectively, than for non-Bt corn.

The first farm-level economic analysis of Bt corn, in demonstrating less net profit, lower corn prices, and lost corn exports, questions whether planting GM corn is worth the cost [24]. From 1996-2001, American farmers paid at least $659 million in price premiums to plant Bt corn, while boosting their harvest by only 276 million bushels - worth $567 million in economic gain. The bottom line for farmers is a net loss of $92 million - about $1.31 per acre. Furthermore, the US has foregone about 350 million bushels of corn export sales to the European Union since 1996/97 because the EU doesn't want GMOs. This is thus part of a triple negative for farmers - lost corn exports, lower corn prices and less net profit from Bt corn.

Furthermore, while transgenic cotton varieties may make pest control easier, they are not always worth the added expense when it comes to yield and fibre quality. Research by the University of Arkansas shows that many conventionals are the highest yielding varieties [25]. Comparing the economics of a Bollgard/Roundup Ready variety with a conventional variety, "in a year when insect pressure was low… the farmer spent about $10 an acre less for insect control with the conventional variety than he did with the more expensive stacked gene variety". And William Dunavant Jr., chief executive of top U.S. cotton merchant Dunavant Enterprises, has commented that American cotton quality remains a problem, blaming the seed produced by biotech companies [26].

And can we put a price tag on the environment? Research points to the popularity of GM crops with many North American farmers because of their "convenience". A University of Nebraska report shows that farmers are using the technology to needlessly destroy weeds to get a "weed-free" field [2]. The study demonstrates not only reduced profits, but also destruction of biodiversity.

Lessons from the South

We would do well to draw on the experiences of farmers in the South. The viability of non-GM alternatives has been demonstrated in a review of 208 projects/initiatives from 52 countries, adopted by 8.98 million farmers on 29 million hectares of land in Asia, Africa, and Latin America [27]. Using a range of sustainable agriculture technologies - none of which involved GM – farmers have achieved yield increases of 50-100% for rainfed agriculture, and 5-10% for irrigated crops.

Low-tech innovations by Southern farmers have boosted production [28]. For example, in East Africa, maize faces two major pests – stem borer and *Striga*, a parasitic plant. By planting a local weed (napier grass) that the stem borer prefers, pests are lured away from the maize into a honey trap – the grass produces a sticky substance that kills stem borer larvae. By planting another weed, *Desmodium*, between rows of maize, *Striga* won't grow, as it is adverse to *Desmodium*. Pesticides are replaced with natural predators, and fertilisers by natural dung, crop wastes and plants that fix nitrogen from the air.

Further, going organic, entailing a restriction in the use of synthetic fertilisers and pesticides while excluding GM technology, could be more beneficial for the economies of developing countries. The FAO has recently urged poor nations to boost exports of organic produce to take advantage of booming markets in developed countries [29].

Sustainable agriculture and organic farming are not a panacea. They however offer alternative approaches to GM technology that have been demonstrated to provide increased yields and more income, while remaining environmentally friendly. No myths about that.

1. Benbrook, C.M. (1999) 'Evidence of the magnitude and consequences of the Roundup Ready soybean yield drag from university-based varietal trials in 1998', Ag BioTech InfoNet Technical Paper Number 1, www.biotech-info.net/RR_yield_drag_98.pdf
2. University of Nebraska (2000) 'Research shows Roundup Ready soybeans yield less', IANR News Service, www.biotech-info.net/Roundup_soybeans_yield_less.html
3. See Griffiths, M. (1999) 'The emperor's transgenic clothes', Are GMO lemmings in the US leading all of us over the biotechnology cliff?
www.btinternet.com/~nlpwessex/Documents/gmlemmings.htm
4. See www.btinternet.com/~nlpwessex/Documents/wisconsinRRsoyatrials98.htm
5. Duffy, M. (1999) '1998 crop survey shows equal returns for GMO, non-GMO crops', www.leopold.iastate.edu/news/9-22-99gmorel.html
6. Oplinger, E.S., M.J. Martinka, and K.A. Schmitz (1999) 'Performance of transgenetic soybeans - Northern US', presented to the ASTA Meetings, Chicago, cited in [8].
7. Reported in Farmers Weekly (UK), 4th December 1998.
8. Clark, E.A. (1999) '10 reasons why farmers should think twice before growing GE crops', www.plant.uoguelph.ca/faculty/eclark/10reasons.htm
9. Benbrook, C.M. (2001) 'Troubled times amid commercial success for Roundup Ready soybeans: glyphosate efficacy is slipping and unstable transgene expression erodes plant defenses and yields', Ag BioTech InfoNet Technical Paper Number 4, www.biotech-info.net/troubledtimes.html
10. 'Economist: Biotech has not made impact yet', Farm Progress, 21 November 2000.
11. Coghlan, A. (1999) 'Monsanto's modified soya beans are cracking up in the heat', *New Scientist*, 20 November 1999, http://www.biotech-info.net/cracking.pdf
12. Rose, F. (2001) 'Yellowing of soybean leaves may be result of several factors', 22 August 2001, http://agebb.missouri.edu/news/queries/showcur.idc?story_num=1298&iln=390
13. See Krishna PJ, 'Environmental influences on gene silencing', ISB News Report, 1 August 2001, http://www.bio-scope.org./disp_doc.cfm?id=21362B4DAA704E0FAB587666BF6CB833
14. See 'Glyphosate weed killer benefits soil fungus', Progressive Farmer, 3 January 2001, http://www.biotech-info.net/soil_fungus.html).
15. See the Jakarta Post.com, 'Pests attack genetically modified cotton', 29 June 2001, www.thejakartapost.com/yesterdaydetail.asp?fileid=20010629.A06
16. See www.biotech-info.net/Aussie_bt_cotton_problems.html
17. 'Resistance management plan for INGARD® Cotton 2001-2002', Transgenic and Insect Management Strategy (TIMS) Committee of the Australian Cotton Growers Research Association, www.cotton.pi.csiro.au/Publicat/Pest/IRMS/irms0102.htm
18. See Thalmann, P. & V. Kung (2000) 'No reduction of pesticides use with genetically engineered cotton', for WWF International, www.biotech-info.net/WWF_inter_update.pdf; and Thalmann, P. & V. Kung (2000) 'Transgenic cotton: Are there benefits for conservation? A case study of GMOs in agriculture, with special emphasis on freshwater', www.panda.org/resources/publications/water/cotton/transgenic.html
19. Benbrook, C.M. (2001) 'Do GM crops mean less pesticide use?' Pesticide Outlook, October 2001.
20. Dailey, D. (2001) '"Fantastic year" for waterhemp, an aggressive weed, is bad news for soybean farms, say MU agronomists', University of Missouri, 20 June 2001, see http://www.btinternet.com/~nlpwessex/Documents/Missourisoybeans.htm
21. Hall, L.M., J. Huffman, and K. Topinka (2000), 'Pollen flow between herbicide tolerant canola (*Brassica napus*), *Weed Science Society of America Abstracts* 40: 48, http://www.mindfully.org/GE/Multiple-Resistant-Volunteers.htm
22. Westwood, J. (2001) 'Cross-pollination leads to triple herbicide resistance', ISB News Report [extract only] March 2001, covering Agricultural and Environmental Biotechnology Developments, www.biotech-info.net/cross_pollination2.html
23. Duffy, M. (2001) 'Who benefits from biotechnology?' Presented at the American Seed Trade Association meeting, 5-7 December 2001, Chicago, http://www.leopold.iastate.edu/pubinfo/papersspeeches/biotech.html
24. Benbrook, C.M. (2001) 'When does it pay to plant Bt corn: farm-level economic impacts of Bt corn, 1996-2001',
www.gefoodalert.org/library/admin/uploadedfiles/When_Does_It_Pay_To_Plant_Bt_Corn.pdf or www.biotech-info.net/Bt_corn_FF_final.pdf; press release from the Institute of Agriculture and Trade Policy (IATP), www.gefoodalert.org/library/admin/uploadedfiles/Benbrook_Bt_Press_Release.doc

25. See 'Conventional vs. transgenic cotton', edited by AgWeb.com Editors, 12/3/2001, www.agweb.com/news_show_news_article.asp?articleID=81926&newscat=GN
26. See 'Dunavant says U.S. cotton quality still lags, blames seeds', 10 January 2002, Reuters, http://www.plant.uoguelph.ca/safefood/archives/agnet/2002/1-2002/agnet_january_11.htm
27. Pretty, J. and R. Hine (2001) 'Reducing food poverty with sustainable agriculture: a summary of new evidence', Occasional Paper 2001-2, Centre for Environment and Society, University of Essex,

www2.essex.ac.uk/ces/ResearchProgrammes/CESOccasionalPapers/SAFErepSUBHEADS.htm
28. Pearce, F. (2001) 'An ordinary miracle', *New Scientist*, Vol. 169, Issue 2276, p. 16, 3 February 2001.
29. Brough, D. (2001) 'FAO urges poor nations to boost organic food sales', Reuters, 4 December 2001, www.planetark.org/dailynewsstory.cfm/newsid/13562/story.htm

Transgenic Pollution by Horizontal Gene Transfer?

Landraces of indigenous maize growing in remote regions in Mexico have been found contaminated with transgenic DNA. Molecular analysis suggests horizontal gene transfer mediated by CaMV 35S promoter. ***Dr. Mae-Wan Ho*** *reports.*

Researchers in University of California Berkeley reported in the journal *Nature* [1] that indigenous landraces of corn, growing in remote regions of Mexico, have become contaminated by transgenic corn.

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

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

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

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

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

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

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

What is horizontal gene transfer?

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

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

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

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

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

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

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

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

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

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

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

1. Quist D and Chapela IH. Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature* 2001, 414, 541-3, 2001.
2. "Doubts linger over Mexican corn analysis", by John Hodgson, *Nature biotech* 2002, 20, 3-4.
3. Ho MW, Ryan A and Cummins J. Cauliflower mosaic viral promoter – a recipe for Disaster? *Microbial Ecology in Health and Disease* 1999: 11: 194-197.
4. Ho MW, Ryan A and Cummins J. Hazards of transgenic plants with the cauliflower mosaic viral promoter. *Microbial Ecology in Health and Disease* 2000: 12: 6-11.
5. Ho MW, Ryan A and Cummins J. CaMV35S promoter fragmentation hotspot confirmed and it is active in animals. *Microbial Ecology in Health and Disease* 2000: 12: 189.

6. Christou P, Kohli A, Stoger E, Twyman RM, Agrawal P, Gu X. Xiong J, Wegel E, Keen D, Tuck H, Wright M, Abranches R and Shaw P. Transgenic plants: a tool for fundamental genomics research. John Innes Centre & Sainsbury Laboratory Annual Report 1999/2000, p. 29. See "Top research centre admits GM failure" *ISIS News* 7/8, February 2001, ISSN: 1474-1547 (print) ISSN: 1474-1814 (online) www.i-sis.org

Bt Risk Negligible?

The United States Environment Protection Agency has renewed the registration of Bt corn based on new studies that claim the impact of Bt-corn pollen on monarch butterfly is "negligible". But ***Dr. Mae-Wan Ho*** *and* ***Prof. Joe Cummins*** *say that is playing fast and loose with scientific evidence.*

When John Losey and colleagues in Cornell University published their findings that Bt-corn pollen harmed monarch butterflies, the concern raised was not just over monarch butterflies, but on all other (non-target) species in the environment. (Bt-corn is transgenic corn expressing a protein, Bt, isolated from the soil bacterium *Bacillus thuringiensis*, which kills specific insect pests.) Unfortunately, amid the enormous publicity generated, the broader issue became reduced to the impact of Bt-corn on monarch butterflies.

So, when the US Environment Protection Agency (EPA) issued a data call-in on December 1999 to consider re-registration of Bt corn, the Department of Agriculture's Agricultural Research Service (USDA-ARS) responded by sponsoring a Monarch Research Workshop in Feb. 2000 to identify research priorities regarding Bt-corn and monarch butterflies. A request for proposals was announced in April, after which a steering committee selected projects to be funded from a grant pool provided by the USDA-ARS and the Agricultural Biotechnology Stewardship Technical Committee.

The results of those projects were published in the 9 October 2001 issue of the *Proceedings of the National Academy of Science USA*, giving clearance to the EPA's re-registration of Bt-corn for an additional seven years, announced 16 October [1].

The first paper in the series is on risk assessment [2] based on data collected in all the studies. Here, we are immediately reassured by the statement, "This 2-year study suggests that the impact of *Bt* corn pollen from current commercial hybrids on monarch butterfly populations is negligible."

On reading the fine print, one discovers that,

- "Toxicity of purified Bt proteins to larval stages of butterflies and moths is well known" from previous studies, and confirmed in the present ones.
- A transgenic corn expressing high levels of one of the Bt proteins, Cry1Ab, not only killed the butterfly larvae, but also inhibited growth with small numbers of pollen grains.

But, these observations are not considered sufficient to cause concern, why not?

What matters in the first instance, we are told, is the probability of exposure, P_e, which is equal to three numbers I, o, and a, all less than one, multiplied together,

$P_e = I\ o\ a$

I is the proportion of monarchs that visit cornfields, o is the overlap of pollen-shed with susceptible larval stages and a, the adoption rate of Bt corn.

Furthermore, the actual risk R depends, not just on the probability of exposure, P_e, but also on P_t, the proportion of monarchs that would be exposed to pollen levels that exceed the lethal threshold, yet another number less than one.

$R = P_e\ P_t$

By such means, the risk becomes reduced to less than 1% or, better still, less than 0.1%.

Should we be impressed with this kind of special pleading in the light of clear evidence that the Bt protein *is* harmful, and admitted to be so?

The authors devoted several pages of unconvincing arguments in order to come up with estimates of the numbers, the only reliable one is perhaps the adoption rate, a, of Bt corn. All estimates are for the year under study, and could drastically change in future in any case.

The studies on toxicity are on acute effects, ignoring both long-term cumulative and non-linear effects that are well known in the ecological literature, especially with regard to extinction and population density. These short-term studies on monarch butterflies do not address impacts on other non-target species, nor on the multiplier ecological consequences of all the impacts interacting with each other.

Bt has already been shown to have the following additional negative impacts, as reviewed elsewhere by the two research teams who found clear evidence that Bt-corn pollen harmed the larvae of Monarch butterflies [3]:

- Increased mortality of lacewing larvae fed on artificial diet containing Bt-toxin or on corn-borer larvae that had eaten Bt-corn.
- Bt sprays used to reduce caterpillars in forests led to fewer black-throated blue warbler nests.
- A parasite of corn-borers, *Macrocentris cingulum*, was found to be reduced in Bt-cornfields compared with non-Bt corn fields.
- One preparation of Bt (*var. tenebrionis*), reported to be specific for Coleoptera, caused significant mortality in domestic bees.
- Soil-dwelling collembola, *Folsomia candida*, an important decomposer, suffered significant mortality from transgenic corn with Cry1 Ab.
- Bt not only remains in the soil with Bt-plant debris, it is actively exuded from the plant roots where it binds to soil particles and persists for 180 days or more [4], so its effects on soil decomposers and other beneficial arthropods may be extensive.
- Bt-crops have speeded up the evolution of Bt-resistance in pest populations.

In addition, Bt-toxins are actual and potential allergens for human beings. Field workers exposed to Bt spray experienced allergic skin sensitization and induction of IgE and IgG antibodies to the spray [5]. A Bt strain that caused severe human necrosis (tissue death) killed mice infected through the nose within 8 hours, from clinical toxic-shock syndrome [6]. Both Bt protein and Bt-potato harmed mice in feeding experiments [7].

Of course, proponents of Bt-crops can easily reduce all of those impacts, one at a time, to "negligible" levels by the same kind of exercise carried out in the 'risk assessment' described above; ignoring the complex interactions between the individual impacts which can have catastrophic long-term consequences.

The proponents will still claim that the benefit of Bt-crops in reducing the use of broad-spectrum insecticides outweighs the risks. Unfortunately, that claim is not borne out by the evidence [3]. During the past five years, the percentage of field corn treated with insecticides in the US has remained at approximately 30%, despite a significant increase in the hectares of Bt corn planted. Corn borer is not a serious pest, and only 1% to 2% of the crops in Iowa was treated with insecticide for corn borer between 1995 and 1998. Most farmers in Iowa and Minnesota had never used insecticides for the corn borer. Only during years when corn borer densities are high do transgenic crops out-perform the non-transgenic.

1. EPA press release Biotechnology Corn approved for Continued Use Oct.2001 http://yosemite1.epa.gov/opa/admpress.nsf/b1ab9f485b098972852562e7004dc686/8db7a83e66e0f7d085256ae7005d6ec2?
2. Sears MK, Hellmich RL, Stanley-Horn DE, Oberhauser KS, Pleasants JM, Mattila HR, Siegfried BD and Dively GP. Impact of Bt corn pollen on monarch butterfly populations: A risk assessment. *PNAS* 2001, 98, 11937-42.
3. Obrycki JJ, Losey JE, Taylor OR and Jesse LCH. Transgenic insecticidal corn: beyond insecticidal toxicity to ecological complexity. *BioScience* 2001, 51, 353-61.
4. Saxena D and Stotzky G. Bt toxin uptake from soil by plants. *Nature Biotech*.2001, 19, 199.
5. Bernstein I, Bernstein J, Miller M, Tiewzieva S, Bernstein D, Lummus Z, Selgrade M, Doerfler D and Seligy V. Immune responses in farm workers after exposure to *Bacillus thuringiensis* pesticides. *Environ Health Perspect* 1999, 107,575-82
6. Hernandez E, Ramisse F, Cruel T, le Vagueresse R and Cavallo JD. *Bacillus thuringiensis* serotype H34 isolated from human and insecticidal strains serotypes 3a3b and H14 can lead to death of immunocompetent mice after pulmonary infection. *FEMS Immunol Med Microbiol* 1999, 24,43-7
7. Fares NH and El-Sayed AK. Fine structural changes in the ileum of mice fed on endotoxin-treated potatotes and transgenic potatoes. *Natural Toxins*:1998: 6: 219-33.