An Annotated Bibliography
Of Scientific Publications
On The Risks Associated With Genetic Modification

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Introduction

In order to help facilitate scientific debate on GMO risks a literature search of peer-reviewed science was conducted on GMO risks resulting in the following bibliography. While there is a great deal of published science on genetic modification in general, there is far less that specifically targets the bio-safety issues associated with genetic modification. In order to make scientifically informed decisions relating to the adoption or regulation of this emerging technology, it is important that all of the relevant information is available to decision makers. One of the themes that has coloured the portrayal of the "GE" debate in the popular media is that of science on one side (supporting these innovations) and uninformed emotional arguments on the other. This bibliography is designed to help bring this debate into a scientific arena by providing references to bio-safety concerns that can be obtained by any decision making body. The decision to restrict this bibliography to scientific publications is designed to ensure that the arguments and the information presented has been scrutinised by scientists in the peer review or editorial process and as such should guard against non-scientific contributions to this important scientific debate. This has meant, however, that books written by scientists have been excluded from this bibliography, even though they may provide important contributions to the scientific debate. Of course, the issues surrounding the adoption and regulation of genetic modification are more than scientific, and include ethical, economic, cultural, legal, intellectual property, and liability dimensions. These themes are beyond the scope of this bibliography, which is explicitly focused on biological science.

The primary focus of this collection of references is the risks to human health. Some sections of the bibliography venture slightly beyond this (e.g. the issues surrounding Bt toxin and animal welfare), but are none-the-less related (i.e. risks associated with non-target effects). In general the bibliography is organised according to the nature of the risk in question. The first section involves risks associated with the expression of the gene. The second category of risk is associated with expression of the transgene in non-target organisms. This includes possibilities of genetic transfer across species barriers (horizontal gene transfer) as well as the possibilities of transfer through pollination to wild relatives. These papers in no way imply that transgenes are any more likely to be transferred than any other gene. The third category of risk relates to risks inherent in genetic modification as such, independent of the gene expressed. These studies show that inserting transgenes can alter the interaction of the genome in unpredictable ways, and that trangenes may also be inherently more unstable or easily transferred than other genes.

Risks Associated With Expression Of The Gene

<u>Resistance to Bt toxin</u>

Bacillus thuringiensis is a bacterium that produces a natural insecticide. The insecticide is used in organic agriculture as a non-toxic means of insect pest control. Biotechnologists have engineered plants to express this toxin by transferring a copy of the gene that codes for the toxin from the bacterium to a plant genome. One of the theoretical issues arising from any pesticide (this applies to conventional chemical pesticides as well as bioengineered pesticides) is the effect of a killing substance on the target population. Because a target population is a biological entity, and as such, subject to evolutionary and ecological processes, evolutionary ecologists would predict that a substance designed to kill insects would at the same time act as

a selection pressure for resistance to the same substance. This theoretical concern has been repeatedly demonstrated in conventional agriculture with the evolution of resistance to antibiotics in human medicine and in the evolution of resistance to chemical pesticides in agriculture. There are over 500 species of insect known to be resistant to insecticides (Green et al 1990). Because Bt toxin is an insecticide, and because it is constantly present in the host plant (i.e. 24 hours a day for the life of the plant) the argument from analogy would suggest that bioengineered Bt plants would produce a very high degree of selection pressure selecting for resistance to this toxin. This theoretical concern, expressed by scientists when Bt toxin was being promoted as a target effect in agricultural genetic engineering, has also produced empirical evidence of such selection pressure (as predicted), together with a range of other non-target effects.

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Non-specificity of Bt insecticidal plants (non-target effects) (Monarch butterfly studies)

Some of the non-target risks associated with transgenic insecticidal plants include non-target effects on beneficial organisms (e.g. insects and soil micro-fauna). The toxicity of the pollen of Bt plants on Monarch butterflies has received a lot of attention from both sides of the scientific divide.

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<u>Non-target effects of insecticidal plants (including Bt plants) on other insects</u>

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Debate on Bt cotton in India

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Effects of insecticidal endotoxins on soils and soil microfauna

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Palm, C.J. Seidler, R.J. Donegan, K.K., and Harris, D. (1993). Transgenic plant pesticides: fate and persistence in soil. *Plant Physiology Supplement 102*: 166.

Palm, C.J. Schaller, D., Donegan, K.K., and Seidler, R.J.(1996). Persistence in soil of transgenic plant produced *Bacillus thuringiensis var kurstaki* delta-endotoxin. *Canadian Journal of Microbiology* 42(12): 1258-1262.

Saxena D., Flores S., Stotzky G. (1999). Insecticidal toxin in root exudates from Bt corn. *Nature*, 402: 480.

Saxena, D., Flores, S., and Stotzky, G. (2002). Bt toxin is released in root exudates from 12 transgenic corn hybrids representing three transformation events. *Soil Biology and Biochemistry* 34: 133-137.

Saxena, D., Flores, S., Stotzky, G. 2002. Vertical movement in soil of insecticidal Cry1Ab protein from *Bacillus thuringiensis*. *Soil Biology and Biochemistry*. 34: 111-120.

Stotzky, G. (2000). Persistence and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis* and of bacterial DNA bound on clays and humic acids. *Journal of Environmental Quality* 29: 691-705.

Tapp, H., Calamai, L., and Stotzky, G. (1994). Adsorption and binding of the insecticidal proteins from *Bacillus thuringiensis subspecies kurstaki and tenebrionis* on clay minerals. *Soil Biology and Biochemistry* 26(6): 663-679.

Tapp, H. and Stotzky, G. (1995) Dot blot enzyme-linked immunosorbent assay for monitoring the fate of insecticidal toxins from *Bacillus thuringiensis* in soil. *Applied and Environmental Microbiology* 61, 602-609.

Tapp, H. and Stotzky, G. (1995) Insecticidal activity of the toxins from *Bacillus thuringiensis* subspecies *kurstaki* and *tenebrionis* adsorbed and boundon pure and soil clays. *Applied and Environmental Micrbiology* 61, 1786-1790.

Tapp, H. and Stotzky, G. (1998) Persistence of the insecticidal toxin from *Bacillus thuringiensis* subsp. *kurstanki* in soil. *Soil Biology and biochemistry* 30, 471-476.

Tapp, H., Calamai, L. and Stotzky, G. (1994) Adsorbtion and binding of the insecticidal proteins from *Bacillus turingiensis* subsp. *kurstaki* and subsp. *tenebrionis* on clay minerals. *Soil Biology and Biochemistry* 26, 663-679.

Bt plants and human health

A number of concerns have been raised about the effects on humans consuming plants expressing Bt toxin, although it is pointed out by some that the toxin is degraded rapidly in the acidic conditions of the mammalian gut (different to insect digestive biochemistry).

Goldburg, R.J. and Tjaden, G. (1990) Are B.T.K. plants really safe to eat? *Bio/technology* 8, 1011-1015.

GM plant resistance to herbicides may lead to increase in spraying

One of the target effects of using insecticidal plants in agriculture is the consequential reduction in the need for chemical pesticides to control insect pests. Because all agricultural systems exist within an ecosystem, and because agricultural systems are subject to ecological dynamics there is concern among scientists (particularly agri-ecologists) that numerous complex interrelationships can become disrupted by the introduction of bio-engineered toxins.

Johnson, B. and Hope, A. (2000) GM crops and equivocal environmental benefits. *Nature biotechnology* 18, 242.

Vertical And Horizontal Gene Transfer

Survival of transgenic DNA in mammals (Horizontal Gene Transfer)

One of the assumptions in genetic modification is that either transgenes are able to be contained in some way, or that any movement of transgenes is of little or no consequence in terms of risk. Until recently, the transfer of genetic material was assumed to be predominantly restricted to reproductive processes (both sexual and asexual). One of the biggest environmental and human health concerns associated with genetic modification is the ability to contain viable genetic material so that it does not spread to non-target species, crops or organisms. If viable DNA could only be transmitted by means of reproduction then containment would be relatively easy. The movement pathways for viable DNA are now understood to be far more complex. In recent years a significant stream of scientific research has shed light on the transfer of genes horizontally (i.e. outside the reproductive process and across species barriers).

In order to contain genetic material one needs to establish effective barriers to the movement viable DNA into the wider environment. The normal pathways of DNA movement need to be removed or significantly obstructed. There are many pathways through which viable genetic material can travel. These include sexual reproduction and dispersal mechanisms (e.g. pollen, fruit, gametes, embryos, offspring), and non-sexual gene transferring material (e.g. horizontal gene transfer between bacteria and each other and between bacteria and higher organisms). Horizontal gene transfer only requires the exposure of viable genetic material to living bacteria. This genetic material need not be in a living organism, but could be in decaying material, faeces, or in the gut.

This means that pathways for genetic exchange for a genetically modified organism include the passage of DNA:-
From living cells of a GMO to bacteria either in the gut or in the soil
From living cells of a GMO to the gut of parasites that can then disperse and reproduce at some distance of the GMO
From dead or decaying cells of a GMO to bacteria
In the form of naked GMO DNA that is attached to soil particles or contained in dung
From bacteria to other organisms in the food chain.

This passage of genetic material can then include any pathway by which bacteria travel in a viable form, such as by means of inoculation of soils, movement in ground water and surface water flows, in effluent that is carried elsewhere, on dust particles that are wind blown, in the gut of blood feeding insects and other parasites. Fences at the edge of a field trial present no barrier to the movement of DNA. GMO DNA therefore will not be restricted to the fields of field trials. If genetic material is transferred horizontally from bacteria to other organisms and taken up into the genome of those organisms then the pathways for the movement of transgenes (the genetically engineered gene) will include the normal movements and reproductive strategies for those organisms.

NB: The papers presented below, describing horizontal gene transfer, do not imply that transgenic DNA is any more (or less) likely to be absorbed than non-transgenic DNA.

Martin-Orue, SM, O'Donnell, AG, Arino, J, Netherwood, T., Gilbert, HJ. and Mathers, JC (2002) Degradation of transgenic DNA from genetically modified soya and maize in human intestinal simulations. *British Journal of Nutrition* 87, 533-542.

Duggan, P.S., Chambers, P.A., Heritage, J. and Forbes, J.M. (2000) Survival of free DNA encoding antibiotic resistance from transgenic maize and the transformation activity of DNA in ovine saliva, ovine rumen fluid and silage effluent. *FEMS Microbiology Letters* 191, 71-77.

Mercer, D.K., Scott, K.P., Bruce-Johnson, W.A., Glover, L.A. and Flint, H.J. (1999) Fate of free DNA and transformation of the oral bacterium *Streptococcus gorndonii* DL1 by plasmid DNA in human saliva. *Applied and Environmentla Microbiology* 65, 6-10.

Doerfler, W. and Schubbert, R. (1998) Uptake of foreign DN from the environment: the gastrointestinal tract and the placenta as portals of entry. *Wein Klin. Wochenschr* 110/2, 40-44.

Schubbert, R., Hohlweg, U., Renz, D. and Doerfler, W. (1998) On the fate of orally ingested foreign DNA in mice: chromasomal association and placental transmission to the fetus. *Molecular and General Genetics* 259, 569-576.

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. *Proceedings of the National Academy of Sciences USA* 94, 961-966.

Schubbert, R., Lettmann, C. and Doerfler, W. (1994) Ingested foreign (phage M13) DNA survives transiently in the gastrointestinal tract and enters the bloodstream of mice. *Molecular and General Genetics* 242, 495-504.

Willerslev, E., Hansen, A.J., Binladen, J., Brand, T.B., Thomas, M., Gilbert, P., Shapiro, B., Bunce, M., Wiuf, C., Gilichinsky, D.A., and Cooper, A. 2003. Diverse Plant and Animal Genetic Records from Holocene and Pleistocene Sediments. *Science* 300: 791-795.

<u>Other papers on horizontal gene transfer</u>

Forano, E. and Flint, HJ (2000) Genetically modified organisms: consequences for ruminant health and nutrition. *Ann. Zootech* 49, 255-271.

Nielsen, K.M., Bones, A.M., Smalla, K. and van Elsas, J.D. (1998) Horizontal gene transfer from transgenic plants to terrestrial bacteria – a rare event? *FEMS Microbiology Reviews* 22, 79-103.

Daane, I.L., Molina, J.A.E., Berry, E.C. and Sadowsky, M.J. (1996) Influence of earthworm activity on gene transfer from *Pseudomonas fluorescens* to indigenous soil bacteria. *Applied and Environmental Microbiology* 62, 515-521.

Nikolich, M.P., Hong, G., Shoemaker, N.B. and Salyers, A.A. (1994) Evidence for natural horizontal transfer of *tetQ* between bacteria that normally colonize humans and bacteria that normally colonize livestock. *Applied and Environmental Microbiology* 60, 3255-3260.

Risks of transgene flow from GM plants to their wild relatives (super-weeds)

Serious concerns have been raised in the scientific community about the risks of transgene escape into the wild relatives of crop plants (particularly for herbicidal resistant engineered plants) leading to super-weeds. Such weeds will not respond to the chemicals normally used to control them, necessitating an increase in the volume and toxicity of chemicals required to control such weeds. There is also a concern that insect resistance genes (in plants) may escape into the wild (particularly in a less toxic form than in a crop plant) leading to the more rapid development of resistance in insect populations. The science of gene flow influences on weediness is still in its relatively early stages and much of the research so far has focused on the mechanisms for gene flow (between crops and their wild relatives) as a natural phenomenon and the effects of this in the case of herbicide resistant or insect resistant transgenic plants. A number of the papers listed below have shown the existence of gene flow from a transgenic crop to a wild relative. The implications of this for biosecurity are yet to be fully understood among scientists, although theoretical concerns combined with empirical evidence that such concerns are valid seems to define the current state of knowledge. There will be debate as to the ability to manage this situation, and whether the external costs of such gene flow outweighs the risks. (It is not known whether the scientific papers marked with '*'were peer reviewed.)

Bartsch, D., S. Driessen, A. Gathmann, A. Hoffmann, M. Lehnen, T. Muecher, C. Saeglitz, U. Wehres, and Schuphan, I. (2002). Monitoring the Environmental Consequences of Gene Flow from Transgenic Sugar Beet. Pp. 78-93. Proceedings of the Gene Flow Workshop, The Ohio State University, March 5 and 6, 2002.

Brown, J., Thill, D.C., Brown, A.P., Brammer, T.A., and Nair, H. (1995). Gene transfer between canola (B*rassica napus*) and related weed species**.** Proceedings and Papers from the 1996 Risk Assessment Research Symposium. http://www.nbiap.vt.edu/brarg/brasym96/brown96.htm *

Chèvre, A-M., Eber, F., Baranger, A. and Renard, M. (1997) Gene flow from transgenic crops. *Nature* 389, 924.

Dalton, R. (2002) Superweed study falters as seed firms deny access to transgene. *Nature* 419, 655. (News and views section: not an original or review paper).

Ellstrand, N.C. (1992). Gene flow by pollen – implications for plant conservation genetics. *Oikos* 63(1): 77-86.

Ellstrand, N.C., Prentice, H.C., Hancock, J.F. (1999). Gene flow and introgression from domesticated plants into their wild relatives. *Annual Review of Ecology and Systematics* 30: 539-563.

Ellstrand, N.C., and Schierenbeck, K.A. (2000). Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences* 97(13): 7043-7050.

Ellstrand, N.C. (2002). Gene Flow from Transgenic Crops to Wild Relatives: What Have We Learned, What Do We Know, What Do We Need to know? Pp. 39-46. Proceedings of the Gene Flow Workshop, The Ohio State University, March 5 and 6, 2002.

Giddings, G.D., Hammilton, N.R.S., and Hayward, M.D. (1997). The release of genetically modified grasses. Part 2: The influence of wind direction on pollen dispersal. *Theoretical and Applied Genetics* 94: 1007-1014.

Halfhill, M.D., Millwood, R.J. Raymer, P.L. and Stewart, Jr. C.N. 2002. *Bt*-transgenic oilseed rape hybridization with its weedy relative, *Brassica rapa. Environmental Biosafety Research* 1: 19-28.

Holt, J. (2002). Prevalence and Management of Herbicide-Resistant Weeds. Pp. 47-57. Proceedings of the Gene Flow Workshop, The Ohio State University, March 5 and 6, 2002.

Jergensen, R., Hauser, T., Mikkelsen, T., and Ostergard, H. (1996). Transfer of engineered genes from crop to wild plants. *Trends in Plant Science* 1(10): 356-358.

Jørgensen, R.B., Anderton, B., Snow, A. and Hauser, T.P. (1999) Ecological risks of growing genetically modified crops. *Plant Biotechnology* 16, 69-71.

Kaiser, J. (2001). Breeding a hardier weed. *Science* 293: 1425-1426.

Klinger, T., and Ellstrand, N.C. (1994). Engineered genes in wild populations: fitness of weed-crop hybrids of *Raphanus sativus. Ecological Applications* 4: 117-120.

Lefol, E., Danielou, V., Darmency, H., Boucher, F., Maillet, J., and Renard, M. (1995). Gene dispersal from transgenic crops: growth of interspecific hybrids between oilseed rape and the wild hoary mustard. *Journal of Applied Ecology* 32: 803-808.

Lefol, E., Fleury, A., and Darmency, H. (1996). Gene dispersal from transgenic crops: hybridization between oilseed rape and the wild hoary mustard. *Sexual Plant Reproduction* 9: 189-196.

Lefol, E., Seguin-Swartz, G, and Downey, R.K. (1997). Sexual hybridization on crosses of cultigated Brassica species with the crucifers *Erucastrum gallicucm* and *Raphanus raphanistrum*: potential for gene introgression. *Euphytica* 95: 127-139.

Linder, C.R., Schmitt, J. (1995). Potential persistence of escaped transgenes: performance of transgenic oil-modified Brassica seeds and seedlings. *Ecological Applications* 5: 1056-1068.

Linder, C.R. (1998). Potential persistence of transgenes: seed performance of transgenic canola and wild x canola hybrids. *Ecological Applications* 894: 1180-1195.

Linder, C.R., Taha, I., Seiler, G.J., Snow, A.A. and Rieseberg, L.H. (1998) Long term introgression of crop genes into wild sunflower populations. *Theoretical and Applied Genetics* 96, 339-347.

Metz, P.L.J., Jacobsen, E., Nap, J.P., Pereira, A., and Stiekema, W.J. (1997). The impact on biosafety of the phosphinothricin-tolerance transgene in inter-specific *B. rapa* x *B. napus* hybrids and their successive backcrosses. *Theoretical and Applied Genetics*. 95: 442-450.

Mikkelsen, T.R., Andersen, B. and Jørgensen, R.B. (1996) The risk of crop transgene spread. *Nature* 380, 31.

Parker, I.M., and Kareiva, P. (1996). Assessing the risks of invasion for genetically engineered plants: acceptable evidence and reasonable doubt. *Biological Conservation* 78: 193-203.

Pilson, D., Snow, A., Rieseberg, L. and Alexander, H. (2003) Fitness and population effects of gene flow from transgenic sunflower to wild *Helianthus annuus*. *Ecologicla Applications* (in press, Jun 2003).

Pohl-Orf, M., Brand, U., Drießen, S., Hesse, P.R., Lehnen, M., Morak, C., Mücher, T., Saeglitz, C., von Soosten, C. and Bartsch, D. (1999) Overwintering of genetically modified sugar beet, *Beta vulgaris* L. subsp. *vulgaris*, as a source for dispersal of transgenic plants. *Euphytica* 108, 181-186.

Quemada, H., and Strehlow, L. (2002). Case Study: Gene flow from commercial transgenic *Cucurbita pepo* to "free-living" *C. pepo* populations. Pp 71-77, Proceedings of the Gene Flow Workshop, The Ohio State University, March 5 and 6, 2002.*

Raybould, A. and Gray, A. (1993). Genetically modified crops and hybridization with wild relatives: a UK perspective. *Journal of Applied Ecology* 30: 199-219.

Raybould, A. and Gray, A. (1994). Will hybrids of genetically modified crops invade natural communities? *Trends in Ecology and Evolution* 9(3): 85-89.

Rieger, M.A., Potter, T.D., Preston, C., and Powles, S.B. (2001). Hybridization between *Brassica napus* L. and *Raphanus raphanistrum* L. under agronomic field conditions. *Theoretical and Applied Genetics* 104(4): 555-560.

Rieger, M.A., Lamond., M., Preston, C., Powles, S., and Rouch, R.T. 2002. Pollen-mediated movement of herbicide resistance between commercial canola fields. *Science* 296: 2386-2388.

Sagers, C. L., Nigemann S., and Novak, S. (2002). Ecological Risk Assessment for the Release of Transgenic Rice in Southeastern Arkansas. Pp. 94-105, Proceedings of the Gene Flow Workshop, The Ohio State University, March 5 and 6, 2002.*

Slavov, G. T., DiFazio, S. P., and Strauss, S. H. (2002). Gene flow in forest trees: From empirical estimates to transgenic risk assessment. Pp. 94-105, Proceedings of the Gene Flow Workshop, The Ohio State University, March 5 and 6, 2002.*

Snow, A.A., Andersen, B. and Jørgensen, R.B. (1999) Costs of transgenic herbicide resistance introgressed from *Brassica napus* into weedy *B. rapa*. *Molecular Ecology* 8, 605-615.

Snow, A.A., Uthus, K.L. and Culley, T.M. (2001) Fitness of hybrids between weedy and cultivated radish: implications for weed evolution. *Ecological Applications* 11, 934-943.

Somers, D.A., Rines, H.W., Gu, W., Kaeppler, H.F., and Bushnell, W.R. (1992). Fertile, transgenic oat plants. *Bio/Technology* 10: 1589-1594.

Spencer, L.J. and Snow, A.A. (2001) Fecundity of transgenic wild-crop hybrids of *Cucurbita pepo* (Cucurbitaceae): implications for crop-to-wild gene flow. *Heredity* 86, 694-702.

Stewart, Jr. C.N., All, J.N., Raymer, P.L., and Ramachandran, S. (1995). Increased fitness of transgenic insecticidal canola under insect selection pressure**.** Proceedings and Papers from the 1996 Risk Assessment Research Symposium. http://www.nbiap.vt.edu/brarg/brasym96/stewart96.htm *

Stewart, Jr. N.C., Halfhill, M.D. and Warwick, S. (2002). Gene flow and its consequences: *Brassica napus* (canola, oilseed rape) to wild relatives. Pp. 106-112, Proceedings of the Gene Flow Workshop, The Ohio State University, March 5 and 6, 2002.*

Watkinson, A.R., Freckleton, R.P., Robinson, R.A. and Suterland, W.J. (2000) Predictions of biodiversity response to genetically modified herbicide-tolerant crops. *Science I289,* 1554-1557.

Westman, A.L., Levy, B.M., Miller, M.B., Gilles, G.J., Spira, T.P., Rajapakse, S., Tonkyn, D.W. and Abbott, A.G. (2001). The potential for gene flow from transgenic crops to related wild species: a case study in strawberry. *Acta Horticulture* (ISHS) 560: 527-530

Westman, A., Miller, B., Spira, T., Tonkyn, D. and Abbott, A. (2002). Molecular Genetic Assessment of the Risk of Gene Escape in Strawberry, a Model Perennial Study Crop. Pp. 6-24, Proceedings of the Gene Flow Workshop, The Ohio State University, March 5 and 6, 2002.*

Wipff, J.K. (2002). Gene flow in turf and forage grasses (Poaceae). Pp. 143-161, Proceedings of the Gene Flow Workshop, The Ohio State University, March 5 and 6, 2002.*

Zemetra, R.S., Mallory-Smith, C.A., Hansen, J., Wang, Z., Snyder, J., Hang, A., Kroiss, L., Riera-Lizarazu, O., and Vales, I. (2002) The Evolution of a Biological Risk Program: Gene flow between Wheat (*Triticum aestivum* L.) and Jointed Goatgrass (*Aegilops cylindrica* Host). Pp. 178-187, Proceedings of the Gene Flow Workshop, The Ohio State University, March 5 and 6, 2002.*

Risks Inherent In The System Of Genetic Modification Itself

One of the most significant issues associated with the risks of genetic engineering focuses on whether assessments should be restricted to risks associated with particular applications of the technology or whether there are any risks associated with the technology itself. If there are problems with the technology itself then the scope of risks are much greater than case-by-case concerns. One of the key issues here centres around the inability to control the location of the transgene (millions of copies) in the host genome. The consequent potential disruptions to the host genome (with the potential to produce unpredictable effects) are one of the most significant and fundamental criticisms of transgenics among scientific commentators.

Gene therapy trials and unpredictability of transgene

The articles below provide evidence that the viral vector used to insert the transgene has a higher chance of inserting itself into a position on the genome where its expression would cause cancer than can be accounted for by chance effects. This is not a criticism of gene therapy itself, it can still be argued, quite reasonably, that the benefits of this therapy outweigh the risks. It does however highlight the unpredictable nature of gene insertion.

Buckley, RH. (2002) Gene therapy for SCID – a complication after remarkable progress. *The Lancet* 360, 1185-1186.

Check, E. (2002) A tragic setback. *Nature* 420, 116-118. (News and views).

Check, E. (2002) Regulators split on gene therapy as patient shows signs of cancer. *Nature* 419, 545-546. (News and views).

Dixon, N. (2002) Cancer scare hits gene cures. *New Scientist* 12 Oct. 2002, 4-5.

Hargreaves, S. (2002) Rules on gene therapy are tightened after leukemia report. *British Medical Journal* 325, 791.

Li, Z, Dullmann, J, Schiedlmeier, B, Schmidt, A, von Kalle, C, Meyer, J, Forster, M, Stocking, C, Wahlers, A, Frank, O, Sotertag, W, Kuhlcke, K, Eckert, H-G, Fehse, B and Baum, C. (2002) Murine leukemia induced by retroviral gene marking. *Science* 296, 497.

Smaglik, P. (2000) Germline therapy needs tight control, says US panel. *Nature* 407, 278 (news and views).

Inherent instability of transposons and vectors

Bergelson, J., Purrington, C.B. and Wichmann, G. (1998) Promiscuity in transgenic plants. *Nature* 395, 25.

Koga, A., Shimada, A., Shima, A., Sakaizumi, M., Tachida, H. and Hori, H. (2000) Evidence for recent invasion of the medusa fish genome by the *Tol2* transposable element. *Genetics* 155, 273-281.

Sengeløv, G., Kristensen, K.J., Sørensen, A.H., Kroer, N. and Sørensen, S.J. (2001) Effect of genomic location on horizontal transfer of a recombinant gene cassette between *Pseudomonas* strains in the rhizosphere and spermosphere of barley seedlings. *Current Microbiology* 42, 160-167.

Note: This short paper has major significance. It provides experimental evidence that transgenes from genetically modified plants are more likely to spread among wild weedy relatives than non-transgenes.

Ho, M-W., Traavik, T., Olsvik, O., Tappeser, B., Howard, C.V., von Weizsacker, C. and McGavin, G.C. (1998) Gene technology and gene ecology of infectious diseases. *Microbial Ecology n Health and Disease* 10, 33-59.

Animal Welfare Concerns

The intrusive nature of cloning and transgenic experimentation on animals is inherent in the system of animal transgenics. The control of animal cellular development depends on a complex interaction between genetic and epigenetic effects. Disrupting these through cloning and gene insertion can lead to abnormalities in the developing embryo which causes suffering both to the new born animal and to the surrogate mothers. Many of the papers here are by researchers who support the technology, but all clearly show the suffering involved in the processes. Whilst these papers do not necessarily address the animal welfare concerns as such (i.e. in terms of an ethical discourse), they do provide important contributions to any ethical debate that wanted to be scientifically informed.

Cohen, P. (2001) Bad copies. *New Scientist* 3 Feb. 2001, 7.

Oback, B. and Wells, D. (2002) Donor cells for nuclear cloning: many are called but few are chosen. *Cloning and Stem Cells* 4, 147-168.

Oback, B. and Wells, D. (2002) Practical aspects of donor cell selection for nuclear cloning. *Cloning and Stem Cells* 4, 169-174.

Wells, D.M., Misica, P.M., Tervit, H.R. and Vivanco, W.H. (1998) Adult somatic cell nuclear transfer is used to preserve the last surviving cow of the Enderby Island breed. *Reproductive Fertility and Development* 10, 369-378.

Wells, D.N., Misica, P.M., and Tervit, H.R. (1999) Production of cloned calves following nuclear transfer with cultured adult mural granulosa cells. *Biology of Reproduction* 60, 996-1005.

Wells, D.N., Misica, P.M., Day, A.M. and Tervit, H.R. (1997) Production of cloned lambs from an established embryonic cell line: a comparison between in vivo- and in vitro-matured cytoplasts. *Biology of Reproduction* 57, 385-393.

Wells, D.N., Misica, P.M., Day, A.M., Peterson, A.J. and Tervit, H.R. (1998) Cloning sheep from cultured embryonic cells. *Reproductive Fertility and Development* 10, 615-626.

Wilmut, I., Beaujean, N., de Sousa, P.A., Dinnyes, A., King, T.J., Paterson, L.A., Wells, D.N. and Young, L.E. (2002) Somatic cell nuclear transfer. *Nature* 419, 583-586.

Wilmut, I., Schnieke, A.E., McWhir, J., Kind, A.J. and Campbell, K.H.S. (1997) Viable offspring derived from fetal and adult mammalian cells. *Nature* 385, 810-813.

Compositional Difference Between Transgenic And Non-Transgenic Plants

One of the principles of GM food safety put forward by organisations promoting GM foods is that genetically modified food crops are substantially equivalent to their conventional counterparts derived from conventional breeding. This assumption has been challenged by scientists on theoretical grounds, and the papers below provide empirical evidence that in fact, GM plants are often substantially different from their conventional counterparts. Indeed, the expression of novel proteins is one of the target effects in GM crop production, and for such a protein to be truly novel (and the gene sequence patentable) it would have to be substantially different from the original.

One way of testing the equivalence of GM and non-GM plants is to test their compositional characteristics. When the compositional differences are unrelated to the product expressed by the transgene, this is evidence for an interaction between the transgene and the rest of the genome, producing unpredictable effects. The authors cited below often play down any compositional differences, to the extent that the abstract and even the title can be misleading.

Abidi, S.L., Llist, G.R. and Rennick, K.A. (1999) Effect of genetic modification on the distribution of minor constituents in canola oil. *Journal of the American Oil Chemist's Society* 76, 463-467.

Berberich, S.A., Ream, J.E., Jackson, T.L., Wood, R., Stipanovic, R., Harvey, P., Patzer, S. and Fuchs, R.L. (1996) The composition of insect-protected cottonseed is equivalent to that of conventional cottonseed. *Journal of Agricultural Food Chemistry* 44, 365-371.

Brake, J. and Vlachos, D. (1998) Evaluation of transgenic event 176 "Bt" corn in broiler chickens. *Poultry Science* 77, 648-653.

Esposito, F., Fogliano, V., Cardi, T., Carputo, D. and Filippone, E. (2002) Glycoalkaloid content and chemical composition of potatoes improved with nonconventional breeding approaches. *Journal of Agricultural and Food Chemistry* 50, 1553-1561.

Fox, J.L. (1997) Farmers say Monsanto's engineered cotton drops bolls. *Nature Biotechnology* 15, 1233. (News section).

Hashimoto, W., Momma, K., Katsube, T., Ohkawa, Y., Ishige, T., Kito, M., Utsumi, S. and Murata, K. (1999) Safety assessment of genetically engineered potatoes with designed soybean glycinin: compositional analyses of the potato tubers and digestibility of the newly expressed protein in transgenic potatoes. *Journal of the Science of Food and Agriculture* 79, 1607-1612.

Inose, T. and Murata, K. (1995) Enhanced accumulation of toxic compound in yeast cells having high glycolytic activity: a case study on the safety of genetically engineered yeast. *International Journal of Food Science and Technology* 30, 141-146.

Lappé, M.A., Bailey, E.B., Childress, C. and Setcell, K.D.R. (1998/99) Alterations in clinically important phytoestrogens in genetically modified, herbicide-tolerant soybeans. *Journal of Medicinal Food* 1, 241-245.

Masoero, F., Moschini, M., Rossi, F., Prandini, A. and Pietri, A. (1999) Nutritive value, mycotoxin contamination and *in vitro* rumen fermentation of normal and genetically modified corn (CRY1A(B)) grown in Northern Italy. *Maydica* 44, 205-209.

Momma, K., Hashimoto, W., Ozawa, S., Kawai, S., Katsube, T., Takaiwa, F., Kito, M., Utsumi, S. and Murata, K. (1999) Quality and safety evaluation of genetically engineered rice with soybean glycinin: analyses of the grain composition and digestibility o glycinin in transgenic rice. *Biosci. Biotechnol. Biochem.* 63, 314-318.

Mutsuga, M., Ohta, H., Toyoda, M. and Goda, Y. (2001) Comparison of caretenoid components between GM and non-GM papaya. *Shokuhin Eiseigaku Zasshi* 42, 367-373 [In Japanese].

Nair, R.S., Fuchs, R.L. and Schuette, S.A. (2002) Current methods for assessing safety of genetically modified crops as exemplified by data on roundup ready soybeans. *Toxicologic Pathology* 30, 117-125.

Novak, W.K. and Haslberger, A.G. (2000) Substantial equivalence of antinutrients and inherent plant toxins in genetically modified novel foods. *Food and Chemical Toxicology* 38, 473-483.

Outwater, J.L., Nicholson, A. and Barnard, N. (1997) Dairy products and breast cancer: the IGF-I, estrogen, and bGH hypothesis. *Medical Hypotheses* 48, 453-461.

Taylor, N.B., Fuchs, R.L., MacDonald, J., Shariff, A.R. and Padgette, S.R. (1999) Compositional analysis of glyphosate-tolerant soybeans treated with glyphosate. *Journal of Agricultural Food Chemistry* 47, 4469-4473.

Miscellaneous Environmental Effects Caused By The Transgene Or Its Insertion

Experimental evidence showing that the transgene produces unpredictable consequences unrelated to the expression of that particular gene. In other words, the unpredictable consequences predicted by gene ecologists (resulting from the disruption of the host genome by means of uncontrolled transgene insertion) are borne out with empirical evidence.

Allison, R.F., Greene, A.E., and Schneider, W.L. (1995). RNA recombination in virus resistant transgenic plants. Proceedings and Papers from the 1996 Risk Assessment Research Symposium. http://www.nbiap.vt.edu/brarg/brasym96/allison96.htm *

Donegan, K.K., Palm, C.J., Fieland, V.J., Porteous, L.A., Ganio, L.M., Schaller, D.L., Bucao, L.Q. and Seidler, R.J. (1995). Changes in levels, species and DNA fingerprints of soil

microorganisms associated with cotton expressing the *Bacillus thuringiensis* var. *kurstaki* endotoxin. *Applied Soil Ecology* 2, 111-124.

Donegan, K.K., Seidler, R.J., Doyle, J.D., Porteous, L.A., Digiovanni, G., Widmer, F. and Watrud, L.S. (1999) A field study with genetically engineered alfalfa inoculated with recombinant *Sinorhizobium meliloti*: effects on the soil ecosystem. *Journal of Applied Ecology* 36, 920-936.

Holmes, B. (2002) Genetically engineered fungus bites back at the crops it's meant to save. *New Scientist,* 28 September 2002, 7.

Jackson, R.L., Ramsay, A.J., Christensen, C.D., Beaton, S., Hall, D.F. and Ramshaw, I.A. (2001) Expression of mouse interleukin-4 by a recombinant ectromelia virus suppresses cytolytic lymphocyte responses and overcomes genetic resistance to mousepox. *Journal of Virology* 75, 1205-1210.

The following paper is a reply to the two above, and makes the argument that the effect of the transgene should have been predictable. Whether or not this is true however, the fact is that competent scientists were not able to predict the effect.

Müllbacher, A. and Lobigs, M. (2001) Creation of killer poxvirus could have been predicted. *Journal of Virology* 75, 8353-8355.

Nowak, R. (2001) Disaster in the making. *New Scientist* 13 January 2001, 4.

Siciliano, S.D. and Germida, J.J. (1999) Taxonomic diversity of bacteria associated with the roots of field-grown transgenic *Brassica napus* cv. Parkland. *FEMS Microbiology Ecology* 29, 263-272.

Siciliano, S.D., Theoret, C.M., de Freitas, J.R., Hucl, P.J. and Germida, J.J. (1998) Differences in the microbial communities associated with the roots of different cultivars of canola and wheat. *Canadian Journal of Microbiology* 44, 844-851.

Feeding Studies Showing Differences In Effects Of GM And Non-GM Foods

As is the case for substantial equivalence, the abstracts and titles can be misleading. Authors often assume that if a difference is not harmful it is not significant. Even non-harmful differences however, highlight the ways in which transgenes may modify the characteristics of the plant in unpredictable ways.

Ewen, S.W.B. and Pusztai, A. (1999) Effect of diets containing genetically modified potatoes expressing *Galanthus nivalis* lectin on rat small intestine. *The Lancet* 354, 1353-1354.

Fares, N.G. and El-Sayed, A.K. (1998) Fine structural changes in the ileum of mice fed on [delta] endotoxin-treated potatoes and transgenic potatoes. *Natural Toxins* 6, 219-233.

Hashimoto, W., Momma, K., Yoon, H-J., Ozawa, S., Ohkawa, Y., Ishige, T., Kito, M., Utsumi, S. and Murata, K. (1999) Safety assessment of transgenic potatoes with

soybean glycinin by feeding studies in rats. *Bioscience Biotechnology Biochemistry* 63, 1942-1946.

Tutelyan, V.A., Dravchenko, L.V., Lashneva, N.V., Avrenieva, L.A., Guseva, G.V., Sorokina, E.Y. and Chernysheva, O.N. (1999) Medical and biological evaluation of safety of the albuminous concentrate from genetically modified soybean. *Biochemical data 5/6*, 9-12. [in Russian].

The Possible Link Between GM Tryptophan And Eosinophilia-Myalgia Syndrome

One of the more well known cases of a non-target effect of a genetic modification process and product associated with human health is the case of L-tryptophan – a genetically engineered dietary supplement that, although sold as a health product, caused Eosinophilia-myalgia Syndrome (a multi-systemic, chronic, autoimmune disease), which by 1992 killed 37 people and left approximately 1,500 permanently disabled. The product was contaminated with a novel amino acid not present in conventional (non-GM) tryptophan.

Belongia, E.A., Hedberg, C.W., Gleich, G.J., White, K.E., Mayeno, A.N., Loegering, D.A., Dunnette, S.L., Pirie, P.L., MacDonald, K.L. and Osterholm, M.T. (1990) An investigation f the cause of the eosinophilia-myalgia syndrome associated with tryptophan use. *New England Journal of Medicine* 323, 357-365.

Mayeno, A.N. and Gleich, G.J. (1994) Eosinophilia-myalgia syndrome and tryptophan production: a cautionary tale. *Tibtech* 12, 346-352.

Mayeno, A.N., Lin, F., Foote, C.S., Loegering, D.A., Ames, M.M., Hedberg, C.W. and Gleich, G.J. (1990) Characterization of "peak E," a novel amino acid associated with eosinophilia-myalgia syndrome. *Science* 250, 1707-1708.

Nightingale, S.L. (1992) Update on EMS and L-tryptophan. *The Journal of the American Medical Association* 268, 1828.

Slutsker, L., Hoesly, F.C., Miller, L., Williams, L.P., Watson, J.C. and Fleming, D.W. (1990) Eosinophilia-myalgia syndrome associated with exposure to tryptophan from a single manufacturer. *Journal of the American Medical Association* 264, 213-217.

Transgenic Animals And Prion Diseases

Prions are proteins that can influence the folding (shape) of other proteins in a way that is inheritable (and hence gene-like), a form of which is responsible for mad cow disease and associated CJD (the human form). There is concern among some scientists that the engineering of transgenic animals may create conditions that foster the development of new prion borne diseases. Prion diseases are sometimes called spongiform encephalopathies due to the appearance of the brain tissue following post mortem examinations. Prion diseases in animals include scrapie (sheep), TME or transmissible mink encephalopathy (mink), CWD or chronic wasting disease (muledeer, elk), BSE or bovine spongiform encephalopathy (cows). In humans prions cause CJD (Creutzfeld-Jacob Disease), GSS (Gerstmann-Straussler-Scheinker syndrome), FFI (Fatal familial Insomnia), Kuru, and Alpers Syndrome.

Wills, P.R. (1996) Transgenic animals and prion diseases: hypotheses, risks, regulations and policies. *New Zealand Veterinary Journal* 44, 33-36.

(Responding to the paper below)

O'Neil, B.D. (1995) Transgenic animals and prion diseases. *New Zealand Veterinary Journal* 43, 88.

Wills, P.R. (1995) Transgenic animals and prion diseases. *New Zealand Veterinary Journal* 43, 86-87.

General Warnings/Admonitions

A number of studies mention general warnings about the safety of genetic modification, in terms of food safety and in particular potential allergenic effects. Genetic modification is often about introducing gene sequences into organisms that enable them to produce proteins that they were not otherwise able to produce. Proteins are fundamental building blocks in living organisms and play a wide variety of physiological roles. Sometimes novel proteins comprise a desired (i.e. target) effect in a food crop, where that crop (or any crop) has never expressed that protein (e.g. it may be from an animal). Such novel proteins may also have never been in the human diet and as such are novel not only to the plant but novel to those that consume such plants. Allergens are commonly proteins, and novel proteins are potential allergens. Some allergens are fatal (e.g. peanuts are fatal to some people) where they produce an anaphylactic reaction (as with bee stings). Given that novel proteins are potential allergens, and given that novel allergens are potentially fatal, it stands to reason that any product that was produced for human consumption (whether as a food or a drug) should be subject to safety testing that included testing for potential allergenicity. Such testing is required for pharmaceuticals but not for GM foods – hence the concern.

The papers listed below are not restricted to those concerned about potential allergenicity of GM foods, but also include other concerns as well, including the application of the precautionary principle to genetic modification, genetic pollution, and general health risks worth consideration according to these scientists.

Antoniou, M. (1996) Genetic pollution. *Nutritional Therapy Today* 6, 8-11.

Christie, B. (1999) Scientists call for moratorium on genetically modified foods. *British Medical Journal* 318, 483.

Domingo, J.L. (2000) Health risks of GM foods: many opinions but few data. *Science* 288, 1748-1749.

Domingo, J.L. and Arnáis, M.G. (2000) Riesgos sobre la salud de los alimentos modificados genéticamente: una revision bibliografica. *Rev. Esp. Salud Pública* 74, 255-261.

Eubanks, M. (2002) Allergies à la carte: is there a problem with genetically modified foods? *Environmental Health Perspectives* 110, A130-131.

Helm, R.M. (2002) Biotechnology and food allergy. *Current Allergy and Asthma Reports* 2, 55-62.

Millstone, E., Brunner, E. and Mayer, S. (1999) Beyond 'substantial equivalence' *Nature* 401, 525-526.

Myhr, A.I. and Traavik, T. (1999) The precautionary principle applied to deliberate release of genetically modified organisms (GMOs). *Microbial Ecology in Health and Disease* 11, 65-74.

Myhr, A.I. and Traavik, T. (2002) the precautionary principle: scientific uncertainty and omitted research in the context of GMO use and release. *Journal of Agricultural and Environmental Ethics* 15, 73-86.

Nielsen, K.M., Bones, A.M., Smalla, K. and van Elsas, J.D. (1998) Horizontal gene transfer from transgenic plants to terrestrial bacteria – a rare event? *FEMS Microbiology Reviews* 22, 79-103.

Ryder, M. (1994) Key issues in the deliberate release of genetically-manipulated bacteria. *FEMS Microbiology Ecology* 15, 139-146.

Schubert, D. (2002) A different perspective on GM food. *Nature Biotechnology* 20, 969.

Warner, J.O. (2002) Editorial: genetically modified food and the pediatric allergist. *Pediatric Allergy and Immunology* 13, 73-74.

Wolfenbarger, L.L. and Phifer, P.R. (2000) The ecological risks and benefits of genetically engineered plants. *Science* 290, 2088-2093.

Conclusion

It should be clear from the above collection of scientific publications (which is not exhaustive by any means), that concern about the risks of genetic modification is not merely a debate between scientists on the one hand and well meaning but mis-informed lay people on the other. It is a genuine scientific and philosophical debate. Given that studies such as those listed above exist in the published literature, and are therefore available to regulators and decision-makers (and their advisory staff) world wide, there is no reason why they cannot be taken into account when regulatory decisions are made on the use and control of GM technologies and their applications.