Ban GMOs *Now*

Health & Environmental Hazards
Especially in the Light of the New Genetics

Dr Mae-Wan Ho & *Dr Eva Sirinathsinghji*

Ban GMOs *Now*

Health & Environmental Hazards
Especially in the Light of the New Genetics

Authors
Dr Mae-Wan Ho
Dr Eva Sirinathsinghji

Production Editor
Julian Haffegee

Institute of Science in Society

www.i-sis.org.uk

Preface

The GM battle intensifies

The industry-funded International Service for the Acquisition of Agri-biotech Applications (ISAAA) claims that the global area of genetically modified (GM) crops reached 170.3 m hectares (420 m acres) in 2012; a 100-fold increase since commercialization began in 1996; and "the fastest adopted crop technology in the history of modern agriculture" [1].

However, GM crops are still confined to 28 countries, with nearly 90 % planted in just five. USA tops the list at 69.5 m ha and 40.8 % of the total area; Brazil and Argentina with 36.6 and 23.9 m ha account for 21.5 % and 14.0 % respectively; and Canada and India with 11.6 and 10.8 m ha account for 6.8 % and 6.3 % respectively. Herbicide (glyphosate) tolerant crops comprise nearly 60 %, Bt crops 15% and stacked traits 25 %. The major crops are just three: herbicide tolerant soybean (47 %) maize (Bt 4%, stacked traits 23 %) and cotton (Bt 11 %, stacked traits 2%).

GM remains limited to two traits in three major crops that are largely kept out of most of the world.

One main reason is its inability to deliver really useful traits. As Geoffrey Lean of the *Telegraph* remarked in reviewing a new book by Prof Sir Gordon Conway, formerly President of the Rockefeller Foundation and Chief Scientific Adviser to the Department for International Development, and a known GM supporter [2]: "But what emerges from his book, *One Billion Hungry*… .is how little – so far, at least – GM technology is contributing to beating hunger." In contrast, conventional breeding assisted by genetic markers has been turning out miracles in the meantime, as described in Conway's book. Scientists at Britain's National Institute of Agricultural Botany (NIAB) have just created new wheat hybrids that could increase yields by 30 %. But it is in Africa that major successes have been tumbling out. Nerica rice varieties up to four times as productive as traditional varieties with much shorter growing season, more protein, resist pests and diseases, thrive on poor soils, and withstand drought; also 30 varieties of drought-tolerant maize are boosting yield 20 to 30 % across 13 countries, climbing beans treble production in Central Africa, wheat varieties thriving on salty soils, plus a host of other wonders: blight-resistant potatoes, crops enriched with vitamin A, iron and other essential nutrients.

The other reason is that resistance to GM crops and GMOs (genetically modified organisms including transgenic trees, fish and livestock) has been growing simultaneously worldwide as the failures and hazards are coming to light behind the corporate propaganda.

GM crops are hardly grown in Europe even though the European Commission has given commercial approval for cultivation, showing every sign of caving in to the GM lobby. But at the end of May 2013, Monsanto, the largest producer of GM seeds, announced it is pulling out from Europe. Monsanto's Europe representative Brandon Mitchener told the press the company would no longer engage in any lobbying in Europe and would not apply for approval of any GM plants [3]. German Agriculture Ministry issued a revealing statement: "The promises of GM industry have not come true for European agriculture, nor have they for the agriculture in developing and emerging economies." Monsanto is the last company to depart Germany, if not Europe, following Bayer CropScience, BASF and Syngenta. On 17 July 2013, Monsanto announced it will withdraw all EU approval requests for new GMO crops [4], to concentrate on growing its conventional seeds business in Europe, and to secure EU approvals to import its GM crop varieties widely grown in the US and South America. So, the company has not given up on pushing GMOs on Europe after all. It was setting up a smokescreen to put us off our guard.

Monsanto has been in the news simultaneously for its unapproved glyphosate tolerant GM wheat that has turned up in a farmer's field in Oregon; and Japan and then South Korea suspended their wheat imports for fear of GM contamination, leading to a 4 % drop in Monsanto's shares [5]. The shipments were eventually cancelled, which could cost US farmers billions [6].

In fact 8 European Union countries have imposed outright bans on crops approved: Austria, France, Germany, Hungary, Luxembourg, Greece, Bulgaria and Poland [7]. Switzerland has had a moratorium on GM crops since 2008, which was set to end in 2013. But in March 2013, the Swiss Parliament voted to prolong the moratorium ignoring the findings of their National Research Programme 59, which [8] "re-confirmed the safety of the commercial use of GM crops and recommended an end to the moratorium." Denmark gave up on GM crops after having allowed Monsanto to carry out field trials of GM maize since 2009 [9]. Italy is the latest to ban cultivation of GM maize (MON 810) citing environmental concerns [10]. In addition, regions and local administrations at every level in 37 European countries have declared themselves GMO-free. As of 2010, this comprises 169 main regions (prefectures, etc.); 123 intermediate regions (provinces, districts, etc.), 4 713 local governments (municipalities and communities up to areas of 1 m ha), and 31 357 individuals [11]; and the movement is growing rapidly.

Within the heartland of GMOs the USA, the failures of GM crops and the problems created are most visible and most acute [12] (GM Crops Facing Meltdown in the USA, *SiS* 46). A new study reveals that the US staple crop system has performed worse than non-GM Europe in yields, pesticide use, genetic diversity and resilience since GM crops were planted [13] (US Staple Crop System Failing from GM and Monoculture, *SiS* 59); with a dangerous downward trend in recent years. Meanwhile, a pitched battle is taking place to get GM crops out through GMO-labelling legislation that would unleash the power of consumers against the might of the biotech industry [14]. Close to 95 % of Americans support GM labelling. In October 2011, the Center for Food Safety filed a legal petition with the FDA to require labelling of all GM food. In 2012, 55 members of Congress wrote a letter to the FDA commissioner in support of the petition. The FDA has received over one million public comments supporting the petition, the largest response ever received by the agency. Meanwhile, 37 GM food labelling bills have been introduced in 21 states in 2013. In the latest move in Washington, Senator Barbara Boxer and Congressman Peter DeFazio have jointly sponsored new federal legislation that requires labelling of all GM food in the US. The Genetically Engineered Food Right-to-Know Act is the first national labelling bill to be introduced in Congress since 2010. The US Green Party has called Monsanto "a top risk to public health and the environment," and has urged a moratorium on GM food crops [15].

In November 2012, Peru imposed a 10 year ban on GMOs in the country, thanks to the effort of farmers from Parque de la Papa in Cusco (see cover picture), a community of 6 000 anxious to protect indigenous biodiversity especially of corn and potatoes on which their livelihood depends [16].

In the same month, Kenya banned import of all GMOs with immediate effect [17]. This followed a decision made by the cabinet on the basis of "inadequate research done on GMOs and scientific evidence provided to prove the safety of the foods".

On 1 June 2013, the new administration in Venezuela announced a new law to protect farmers against GM seeds [18].

On 22 July 2013, the Indian Supreme Court's expert panel of scientists called for a ban on herbicide tolerant crops for India [19].

A critical juncture

The rising opposition to GMOs has done little to diminish the aggressive expansionist agenda of the GM corporate empire. Mexico is a major target. US biotech firms Monsanto, DuPont and Dow have applied for permits to grow more than two million hectares of GM maize in northern Mexico [20]. Mexico is the birthplace of maize and a centre of biodiversity. Since 2009, the Mexican government has granted 177 permits for experimental plots of GM maize covering 2 664 hectares. Large-scale commercial release of GM maize has not yet been authorised; but GM contamination of native maize has already been discovered, as the result of what some regard as "a carefully and perversely planned strategy".

The other major strategy of the GM corporate empire is seed monopoly and escalating seed costs. Conventional non-GM seeds are pushed out at the expense of GM seeds, thereby reducing farmers' choices [21]. The big four biotech seed companies – Monsanto DuPont/ Pioneer Hi-Bred, Syngenta, and Dow AgroSciences – now own 80 % of the US corn market and 70 % of soybean business. The costs of seeds have increased two to three fold since 1995. This is destroying the lives of farmers around the world; the most visible in India, where the introduction of GM cotton has coincided with an escalation of farm suicides ([22] Farmer Suicides and Bt Cotton Nightmare Unfolding in India, *SiS* 45). At the same time, farmers who want to return to conventional non-GM seed after experiencing increased pest resistance and crop failures find themselves unable to do so, on account of the limited availability of non-GM seeds [18].

Ban GMOs Now

This is a dangerous situation for the future of food and farming, one that needs to be reversed as quickly as possible, particularly as GM agriculture is failing on all counts. That can only be achieved by a ban on GMOs, an action already taken by countries and local communities around the world. We need to join forces with them, to put an end to the GM corporate empire.

Ten years ago, 24 scientists from around the world formed an Independent Science Panel and produced a report ([24] *The Case for A GM-Free Sustainable World*, ISIS/TWN publication) summarizing compelling evidence on the hazards of GM crops and the benefits of organic agro-ecological farming, and called for a global ban on environmental releases of GMOs, and a shift to non-GM sustainable agriculture. This report was widely circulated, translated into several languages, and republished in the US a year later. It remains the most succinct and complete account on the subject; but crucial new evidence has come to light within the past decade that strengthens the case considerably.

First of all, decisive evidence has emerged on the unsustainability and destructiveness of conventional industrial agriculture, of which GM is the most extreme; in stark contrast to the proven successes of non-GM ecological farming: its productivity and resilience, environmental and health benefits, and in particular, its enormous potential for saving energy and carbon emissions in mitigating and adapting to climate change. We presented all that in a comprehensive and definitive report published in 2008 ([25] Food Futures Now *Organic *Sustainable *Fossil Fuel Free , ISIS/TWN publication). Our report is completely in line with the International Assessment of Agricultural Knowledge, Science and Technology for Development (IAASTD) report [26], which resulted from a three-year consultative process involving 900 participants and 110 countries around the world; a sure sign of the scientific consensus that has emerged around non-GM ecological farming as the way forward in food and farming.

To complete the case, we need to bring together all the damning evidence against GMOs on health and the environment, especially in the light of new discoveries in molecular genetics within the past ten years. That is the main reason for the present report.

GM agriculture is a recipe for disaster, as this report will make clear. It is also standing in the way of the shift to sustainable agriculture already taking place in local communities all over the world that can truly enable people to feed themselves in times of climate change. Future generations will not forgive us if we do not stop the GM takeover now.

Please use this report, circulate it widely, and send it to your political representatives.

Dr Mae-Wan Ho
12 June 2013
Updated 24 July 2013

Executive Summary

Since the first commercial growing began in 1996, the global area of genetically modified (GM) crops is reported to have increased 100-fold. However, nearly 90 % are confined to 5 countries, with top grower the US accounting for more than 40 %. GM crops have been largely excluded from Europe and most developing countries because opposition has been growing simultaneously as widespread agronomical failures of the GM crops as well the health and environmental impacts are coming to light.

GM remains limited to three major crops – soybean, maize and cotton – and two traits: herbicide (mainly glyphosate) tolerance (HT) at nearly 60 % and insect resistance with toxins from the soil bacterium *Bacillus thuringiensis* (Bt) at 15 %, with the remaining stacked traits (HT and one or more Bt) at 25%.

The failures and hazards of glyphosate and glyphosate tolerant crops and Bt crops are reviewed respectively in Chapter 1 and Chapter 2. Chapter 3 reviews the range of hazards resulting from the uncontrollable, unpredictable process of genetic modification itself in the light of advances in molecular genetics within the past decade, which tells us why the technology cannot be safety applied to grow our crops or produce our food.

Glyphosate & glyphosate tolerant crops

Glyphosate use has gone up sharply worldwide since the introduction of glyphosate-tolerant GM crops. Herbicide use per acre has doubled in the US within the past five years compared with the first five years of commercial GM crops cultivation, the increase almost entirely due to glyphosate herbicides. Glyphosate has contaminated land, water, air, and our food supply. Damning evidence of its serious harm to health and the environment has been piling up, but the maximum permitted levels are set to rise by 100-150 times in the European Union with further hikes of already unacceptably high levels in the US if Monsanto gets its way.

1. Scientific evidence accumulated over three decades documents miscarriages, birth defects, carcinogenesis, endocrine disruption, DNA damage, general toxicity to cells, neurotoxicity, and toxicity to liver and kidney at glyphosate levels well below recommended agricultural use.

2. The major adjuvant POEA in glyphosate Roundup formulations is by far the most toxic for human cells, ahead of glyphosate and its metabolite. It also amplifies the toxicity of glyphosate.

3. A recent review blames glyphosate for practically all modern diseases as its general chelating action affects numerous biological functions that require metal cofactors. It is the most pervasive environmental chemical pollutant that also inhibits enzymes involved in detoxification of xenobiotics, thereby increasing *their* toxicity. In addition, it kills beneficial gut bacteria that prevent pathogens from colonizing the gut and promotes the growth of the pathogenic bacteria, leading to autism and other diseases.

4. Rats fed Roundup contaminated and Roundup tolerant maize beyond the required 90 days showed a startling range of health impacts. Females were 2 to 3 times as likely to die as controls and much more likely to develop mammary tumours. In males, liver congestions and necrosis were 2.5 to 5.5 times as frequent as controls, while kidney diseases were 1.3-2.3 times controls. Males also develop kidney or skin tumours 4 times as often as the controls and up to 600 days earlier. The harmful effects were found in animals fed the GM maize that was not sprayed with Roundup, as well as those that were, indicating that the GM maize has its own toxicities apart from the herbicide.

5. Livestock illnesses from glyphosate tolerant GM feed including reproductive problems, diarrhoea, bloating, spontaneous abortions, reduced live births, inflamed digestive systems and nutrient deficiencies. Evidence has also emerged of chronic botulism in cattle and farmers as the result of glyphosate use.

6. Glyphosate is lethal to frogs and Roundup is worse; it increases toxic blooms, and accelerates the deterioration of water quality. It use also coincides with the demise of monarch butterflies.

7. Glyphosate poisons crops and soils by killing beneficial microbes and encouraging pathogens to flourish. Forty crop diseases are now linked to glyphosate use and the number is increasing.

8. Glyphosate-resistant weeds cover 120 million ha globally (61.8 m acres in the US) and continue to spread; it is a major factor accounting for the enormous increase in pesticide use since herbicide tolerant GM crops were introduced.

9. Contamination of ground water supplies, rain, and air has been documented in Spain and the US. Berlin city residents were found to have glyphosate urine concentrations above permitted EU drinking water levels.

Bt crops

Bt crops were sold on the premise that they would increase yields and reduce pesticide use; instead they have resulted in too many crop failures, and the introduction of Bt cotton is now acknowledged to be responsible for the escalation in farm suicides in India.

1. Bt crops' claim to reduce pesticide use is based on excluding the Bt produced in the crops in total 'pesticides applied'; but the Bt toxins leach from the plants and persist in soil and water, with negative impacts on health and the ecosystem comparable to conventional pesticides.

2. Fungicide use and insecticide treatment of corn and soybean have gone up dramatically since the introduction of Bt crops.

3. The breakdown of Bt traits due to target pest resistance and secondary pests has resulted in increasing use of conventional pesticides; and pesticide companies are reporting 5 to 50% increase in sales for 2012 and the first quarter of 2013.

4. Contrary to industry's claim that Bt is harmless to non-target species, independent studies showed that Bt toxins elicit immune response in mammals in some cases comparable to that due to cholera toxin. This is consistent with farm workers' reports of allergic symptoms affecting the eyes, skin and respiratory tract.

5. A new study found Bt proteins toxic to developing red blood cells as well as bone marrow cells in mice.

6. Toxicity to human kidney cells has been observed *in vitro*, consistent with *in vivo* experiments in lab animals showing toxicity to heart, kidney and liver.

7. Bt crops fail to control target pests due to insufficient expression of Bt toxins, thereby promoting the evolution of resistance.

8. Bt crops promote the emergence of secondary pests when target pests are killed. Primary and secondary pests are already huge problems in the US, India and China, and are now hitting multiple crops in Brazil since Bt maize was introduced.

9. Stacked varieties containing multiple Bt toxins are predicted to hasten the evolution of multiple toxin resistance, as resistance to one toxin appears to accelerate the acquisition of resistance to further toxins.

10. Bt toxins harm non-target species including water fleas, lacewings, monarch butterflies, peacock butterflies and bees, which are showing worrying signs of population decline across the world.

11. Bt toxins leach into the soil via the root of Bt crops where they can persist for 180 days; this has been linked to the emergence of new plant diseases and reduced crop yields.

12. Bt toxins also persist in aquatic environments, contaminating streams and water columns and harming important aquatic organisms such as the caddisfly.

The new genetics & hazards of genetic modification

The rationale and impetus for genetic engineering and genetic modification was the 'central dogma' of molecular biology that assumed DNA carries all the instructions for making an organism. This is contrary to the reality of the fluid and responsive genome that already came to light since the early 1980s. Instead of linear causal chains leading from DNA to RNA to protein and downstream biological functions, complex feed-forward and feed-back cycles

interconnect organism and environment at all levels, marking and changing RNA and DNA down the generations. In order to survive, the organism needs to engage in natural genetic modification in real time, an exquisitely precise molecular dance of life with RNA and DNA responding to and participating fully in 'downstream' biological functions. That is why organisms and ecosystems are particularly vulnerable to the crude, artificial genetically modified RNA and DNA created by human genetic engineers. It is also why genetic modification can probably never be safe.

1. Genetic modification done by human genetic engineers is anything but precise; it is uncontrollable and unpredictable, introducing many collateral damages to the host genome as well as new transcripts, proteins and metabolites that could be harmful.

2. GM feed with very different transgenes have been shown to be harmful to a wide range of species, by farmers in the field and independent scientists working in the lab, indicating that genetic modification itself is unsafe.

3. Artificial genetic modification done by human genetic engineers is different from natural genetic modification done by organisms themselves. Artificial genetic modification relies on making unnatural GM constructs designed to cross species barriers and jump into genomes; it combines and transfers genes between species that would never have exchanged genes in nature; GM constructs tend to be unstable and hence more prone to further horizontal gene transfer after it has integrated into the genome.

4. Horizontal gene transfer and recombination is a major route for creating new viruses and bacteria that cause diseases and spread drug and antibiotic resistance. Transgenic DNA is especially dangerous because the GM constructs are already combinations of sequences from diverse bacteria and viruses that cause diseases, and contain antibiotic resistance marker genes.

5. There is experimental evidence that transgenes are much more likely to spread and to transfer horizontally.

6. The instability of the GM construct is reflected in the instability of transgenic varieties due to both transgene silencing and the loss of transgenes, for which abundant evidence exists. ***Transgenic instability makes a mockery of 'event-specific' characterization and risk assessment, because any change in transgene expression, or worse, rearrangement or movement of the transgenic DNA insert(s) would create another transgenic plant different from the one that was characterized and risk assessed. And it matters little how thoroughly the original characterization and risk assessment may have been done. Unstable transgenic lines are illegal, they should not be growing commercially, and are not eligible for patent protection.***

7. There is abundant evidence for horizontal transfer of transgenic DNA from plant to bacteria in the lab and it is well known that transgenic DNA can persist in debris and residue in the soil long after the crops have been cultivated. At least 87 species (2 % of all known species) of bacteria can take up foreign DNA and integrate it into their genome; the frequency of that happening being greatly increased when a short homologous anchor sequence is present.

8. The frequency at which transgenic DNA transfers horizontal has been routinely underestimated because the overwhelming majority of natural bacteria cannot be cultured. Using direct detection methods without the need to culture, substantial gene transfers were observed on the surface of intact leaves as well as on rotting damaged leaves.

9. In the only monitoring experiment carried out with appropriate molecular probes so far, China has detected the spread of a GM antibiotic resistance gene to bacteria in all of its major rivers; suggesting that horizontal gene transfer has contributed to the recent rise in antibiotic resistance in animals and humans in the country.

10. GM DNA has been found to survive digestion in the gut of mice, the rumen of sheep and duodenum of cattle and to enter the blood stream.

11. In the only feeding trial carried out on humans, the complete 2 266 bp of the epsps transgene in Roundup Ready soybean flour was recovered from the colostomy bag in 6 out of 7 ileostomy subjects. In 3 out of 7 subjects, bacteria cultured from the contents of the colostomy bag were positive for the GM soya transgene, showing that horizontal transfer of the transgene had occurred; but no bacteria were positive for any natural soybean genes.

12. The gastrointestinal tract of mammals is a hotspot for horizontal gene transfer between bacteria, beginning in the mouth.

13. Evidence is emerging that genomes of higher plants and animals may be even softer targets for horizontal gene transfer than genomes of bacteria.

14. The CaMV 35S promoter, most widely used in commercial GM crops, is known to have a fragmentation hotspot, that makes it prone to horizontal gene transfer; in addition. It is promiscuously active in bacteria, fungi, and human cells. Recent evidence suggests that the promoter may enhance multiplication of disease-associated viruses including HIV and cytomegalovirus through the induction of proteins required for transcription of the viruses. It also overlaps with a viral gene that interferes with gene silencing, an essential function in plants and animals that protects them against viruses.

15. The *Agrobacterium* vector, most widely used for creating GM plants is now known to transfer genes also to fungi and human cells, and to share genetic signals for gene transfer with common bacteria in the environment. In addition, the *Agrobacterium* bacteria as well as its gene transfer vector tend to remain in the GM crops created, thereby constituting a ready route for horizontal gene transfer to all organisms interacting with the GM crops, or come into contact with the soil on which GM crops are growing or have been grown.

16. In 2008, *Agrobacterium* was linked to the outbreak of Morgellons disease. The US Centers for Disease Control launched an investigation, which concluded in 2012 with the finding: "no common underlying medical condition or infection source". But they had failed to investigate the involvement of *Agrobacterium*.

17. New GM crops that produce double-stranded RNA (dsRNA) for specific gene-silencing are hazardous because many off-target effects in RNA interference are now known, and cannot be controlled. Furthermore, small dsRNA in food plants were found to survive digestion in the human gut and to enter the bloodstream where they are transported to different tissues and cells to silence genes.

18. Evidence accumulated over the past 50 years have revealed nucleic acids (both DNA and RNA) circulating in the bloodstream of humans and other animals that are actively secreted by cells for intercommunication. The nucleic acids are taken up by target cells to silence genes in the case of double-stranded microRNA (miRNA), and may be integrated into the cells' genome, in the case of DNA. The profile of the circulating nucleic acids change according to states of health and disease. Cancer cells use the system to spread cancer around the body. This nucleic acid intercom leaves the body very vulnerable to genetically modified nucleic acids that can take over the system to do considerable harm.

Conclusion

The serious harm to health and the ecological and agronomical impacts of glyphosate and glyphosate tolerant crops are the most thoroughly researched, and for which there is little remaining doubt. The same kind of evidence has now emerged for Bt crops and Bt toxins. Evidence that genetic modification *per se* is harmful is also convincing, and can be attributed to the uncontrollable process of genetic modification itself as well as the dangers from the horizontal transfer of the GM constructs, which can spread antibiotic resistance, create new pathogens and trigger 'insertion carcinogenesis', and taking over the body's natural nucleic acid intercom to do harm.

There is a compelling case for banning all environmental releases of GMOs *now*, and with that the glyphosate herbicides. Action can be taken locally in communities, villages, towns, municipalities, regions, as well as nationally and globally. It must be done now; for time is running out. We need to shift comprehensively to non-GM sustainable ecological farming in order to feed ourselves under climate change. We the people need to reclaim our food and seed sovereignty from the corporate empire before they destroy our food and farming irreversibly.

Contents

1
Double Jeopardy of Glyphosate & Glyphosate-Tolerant Crops

Glyphosate tolerant GM crops have greatly increased the use of the herbicide, which has contaminated land, water, air, and our food supply; the maximum permitted levels are set to rise by100-150 times in the European Union if Monsanto gets its way while US EPA has announced new hikes as damning evidence of serious harm to health & the environment piles up

Dr Eva Sirinathsinghji** and **Dr Mae-Wan Ho

1 Introduction

A feeding trial lasting two years on rats showed that females exposed to Monsanto's glyphosate formulation Round-up and/or Roundup-tolerant genetically modified (GM) maize were 2 to 3 times as likely to die as controls and much more likely to develop large mammary tumours [1]. In males, liver congestions and necrosis were 2.5 to 5.5 times as frequent as the controls, while kidney diseases were 1.3-2.3 times controls. Males also presented large kidney or skin tumours four times as often as the controls and up to 600 days earlier. Biochemical data confirmed significant kidney chronic deficiencies for all treatments and both sexes.

The research team, led by Giles-Eric Séralini of Caen University in France, suggested that the results can be explained by "non-linear endocrine-disrupting effects of Roundup" and "the overexpression of the transgene in the GMO and its metabolic consequences."

The results were dynamite, and the repercussions are still to be played out (see [2] Excess Cancers and Deaths with GM Feed: the Stats Stand Up, *SiS* 56). Predictably, the pro-GM brigade around the world launched a concerted campaign to discredit the scientists and their findings (see commentary by John Vidal on the *Guardian* website [3].

But contrary to the impression given in the popular media, this is not an isolated study suddenly to reveal that GM feed and the most widely used herbicide in the world may be toxic. It is the latest in a series of laboratory experiments backed up by experience of farmers and farm workers around the world that have found toxicity both for GM crops and for the herbicide. It is also the most thorough study to be carried out for the longest duration of two years. Currently, European regulators require companies to do feeding trials for only 90 days.

Note that the new study found toxicity not just for Roundup herbicide, but also for the Roundup-tolerant GM maize (NK603) that had *not* been sprayed with herbicide. In other words, GM maize has toxicity independently of the herbicide (see Chapter 3 for a list of all possible sources of hazards from genetic modification). As most Roundup tolerant GM crops have been sprayed and contain substantial amounts of herbicide and herbicide residues, they may also mask the toxicity of the GM crops themselves.

We review existing evidence on the health and environmental impacts of glyphosate herbicides and glyphosate-tolerant GM crops as the maximum permitted levels of the herbicide and herbicide residues in food are set to rise 100-150 times in the European Union if Monsanto's new proposal is approved [4]. Meanwhile, in the United States, Monsanto has petitioned the Environmental Protection Agency (EPA) for further hikes in what are already unacceptably high permitted levels of the herbicide [5].

2 Regulators and industry both culpable

Healthy food and clean water are fundamental needs and basic human rights, but these are being compromised by the ever increasing use of synthetic chemicals in agriculture. Glyphosate-based herbicides, originally developed by Monsanto, are the most widely used in the world and increasing numbers of studies are documenting its link to serious illnesses and environmental damage. Most disturbingly, both Monsanto and the European Commission knew that the chemical could lead to cancer and birth defects prior to its approval for Europe in the 1980s; despite that, glyphosate continues to be touted as a 'safe' chemical [6, 7] (see [8] EU Regulators and Monsanto Exposed for Hiding Glyphosate Toxicity, *SiS* 51).

Both Monsanto and the European Commission knew that the chemical could lead to cancer and birth defects prior to its approval for Europe in the 1980s; despite that, glyphosate continues to be touted as a 'safe' chemical

The first glyphosate-based herbicide, Roundup®, was launched by Monsanto in 1974 and its use has risen sharply since the introduction of glyphosate-tolerant GM crops in 1996. Following the expiry of the glyphosate patent in the US in 1991 and outside the US in 2000, many commercial formulations are available. An estimated 180-185 million pounds (or 81-84 million kilograms) of glyphosate was used in the US in 2007. Following the expiry of the glyphosate patent in the US in 1991 and outside the US in 2000, many commercial formulations are available. Based on US data, GM crops have been directly responsible for a 7 % increase in overall pesticide use from 1996 to 2011 [9] (see [10] Study Confirms GM crops lead to increased Pesticide , *SiS* 56). This is predicted to increase further with the emergence and spread of herbicide resistant weeds (see section 5.1), and insects resistant to Monsanto's Bt toxin insecticides, as well as the introduction of GM crops with tolerance to multiple herbicides. Comparing the past five years (2008-2012) with the first five years of commercial GM crops (1996-2000), herbicide use per acre in the US has doubled, with glyphosate/Roundup accounting essentially for all the increase [11].

Proponents of industrial chemical agriculture and GM crops

Brief history of Monsanto – chemical company turned biotech giant

This review focuses primarily on the scientific effects of glyphosate, but the context of its production is important when considering Monsanto's recent move from chemical production to agriculture. Can we really trust a chemical company to produce healthy food?

Founded in 1901 by John Francis Queeny in St Louis, Missouri, Monsanto's first product was saccharin, an artificial sweetener. By the 1920s, the company was producing basic industrial chemicals, including sulphuric acid. During the 1940s they were involved in uranium research for the Manhattan Project that developed the first nuclear bomb; they continued running a nuclear facility until the 1980s. In addition, they became a large manufacturer of synthetic plastics including polychlorinated biphenols (PCBs) used as a chemical insulator and banned in 1979 in the US due to carcinogenicity. Lawsuits have been filed against Monsanto for contaminating residential areas with PCBs that have left whole towns crippled with cancers and other illnesses. Following the Second World War, Monsanto expanded into large-scale production of chemical pesticides, including DDT and Agent Orange, the latter notoriously used as a defoliant during the Vietnam War. One of the components, dioxin, has now been classified as a probable carcinogen by the US Environmental Protection Agency (EPA). It is estimated that Agent Orange killed hundreds of thousands of Vietnamese civilians and American soldiers. In addition, it caused cancers and other illness in 2 million people, and birth defects affecting hundreds of thousands. Monsanto was later sued and forced to pay out $180 million to sick US war veterans. DDT was also banned in 1972 (although its use was permitted under certain circumstances) mainly due to effects on wildlife, but it was still exported to foreign countries until 1985. It is now classified by the EPA as a 'probable carcinogen', and has been associated with diabetes, Parkinson's disease and endocrine disruption linked to developmental defects. Lasso, another herbicide manufactured by Monsanto was banned in the EU in 2006. Monsanto was recently found guilty of chemical poisoning a French farmer who suffered neurological problems including memory loss, headaches and stammering after inhaling Lasso in 2004 [14].

The commercialisation of Roundup® in 1974 turned Monsanto into the largest pesticide manufacturer in the world. They later turned to biotechnology and the production of GM crops, generating the first GM plant cell in 1982. By 1996, the first GM crop tolerant to glyphosate – Roundup Ready (RR) soybean - was on the market. Today, there are many glyphosate-tolerant crops, including corn, canola, sugar beet, cotton, wheat and alfalfa. Similar varieties made by Bayer CropScience, Pioneer Hi-Bred and Syngenta AG are termed Gly-Tol TM, Optimum ® GAT ® and Agrisure ® GT, respectively. The generation of plants tolerant to glyphosate allows farmers to apply glyphosate while crops are growing, theoretically killing every plant but the crop. The consequence is that crops now contain residual levels, directly exposing consumers and livestock to glyphosate. Not only that, glyphosate tolerant crops accumulate the herbicide and transport it to the roots, excreting it into the root zone (rhizosphere) of the soil, harming the next crop to be planted in the same field (see main text).

argue glyphosate increases crop yields, providing a more efficient, cost-effective and safe method of agriculture necessary to tackle hunger and food insecurity across the world. The US officially recognises glyphosate as a safe chemical with regards to human health [12], currently defined as a Toxicity Class III herbicide (slightly toxic) with no carcinogenic activity. The EU classifies it as an irritant that can also cause severe ocular damage [13].

The accumulation of scientific peer-reviewed publications, clinical observations and witness reports from farmers and residents living in glyphosate-treated areas however, refutes the official line. Over a hundred peer-reviewed publications show detrimental effects, proving to the scientific community what farmers in the global South have known for a long time. Not acknowledging those studies goes against fundamental scientific and medical principles as well as the basic human right to a healthy environment, not least because the evidence challenges the naïve assumption that governments' primary concern is to protect our health and not the pockets of multinational corporations.

3 How glyphosate works

Glyphosate or N-(phoshonomethyl) glycine (molecular formula - $C_3H_8NO_5P$) acts through inhibiting the plant enzyme – EPSPS (enolpyruvylshikimate-3-phosphate synthase) in the shikimate pathway [15] (see [16] Glyphosate Tolerant Crops Bring Diseases and Death, *SiS* 47). It catalyses the transformation of phosphoenol pyruvate (PEP) to shikimate-3-phosphate, required for making essential aromatic amino acids phenylalanine, tyrosine and tryptophan. Amino acids are essential building blocks for all proteins. This metabolic pathway exists in all plants, fungi, and some bacteria, especially gut bacteria that protect mammals from pathogenic bacteria (see later). Animals do not have the shikimate pathway, and depend on getting the essential amino acids from their diet. Inhibition of protein synthesis leads to rapid necrosis (premature cell death) in the plant. As the EPSPS enzyme is present in all plants, glyphosate can effectively kill all plant species. The high solubility of glyphosate formulations allows it to be taken up by the plant where it acts systematically from roots to leaves.

Glyphosate-tolerant crops are either engineered to carry extra copies of the EPSPS gene isolated from the soil bacterium *Agrobacterium tumefaciens*, or glyphosate intolerant versions of EPSPS. These GM crops are therefore tolerant to the herbicide, but are not engineered to metabolise or get rid of it, resulting in GM crops

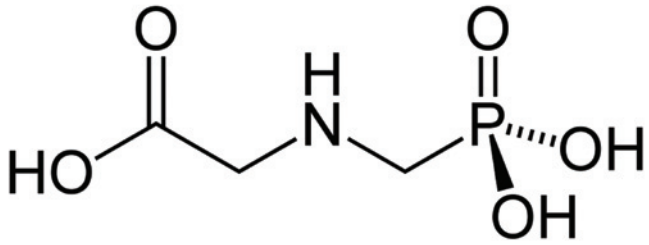

Figure 1 Chemical Structure of Glyphosate

with the herbicide and its residues throughout the plant destined to become food or animal feed.

In addition to inhibition of EPSPS, glyphosate disrupts many biochemical and physiological functions of plants. Glyphosate was first patented as a general metal chelator and strongly chelates micronutrients such as manganese, which is an important co-factor of the EPSPS enzyme (see [16]). This is suggested to be the mechanism by which glyphosate kills plants. Manganese is a co-factor in over 25 plant enzymes. Other macro and micronutrients are also chelated by glyphosate including Ca^{2+}, Mg^{2+}, Cu^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+} and Zn^{2+}. This interference with biochemical pathways goes on to compromise biological functions including the immune system as well as crop productivity (see [17] USDA scientist reveals All, *SiS* 53).

4 Health impacts

There is a wealth of evidence on the health hazards of glyphosate. Its approval, along with other hazardous chemicals, relies on systematic flaws in the EU and US regulatory processes, which to this day, do not require evaluation by independent research, and instead rely solely on the industry's own studies. Approval is therefore often based on data not available to the public or independent research scientists. Nevertheless, raw data have been obtained from the industry through the law courts, which, when re-analysed by independent scientists, also provide evidence of toxicity.

Taken together, glyphosate is implicated in *birth and reproductive defects, endocrine disruption, cancers, genotoxicity, neurotoxicity, cytotoxicity, respiratory problems, nausea, fever, allergies* and skin problems.

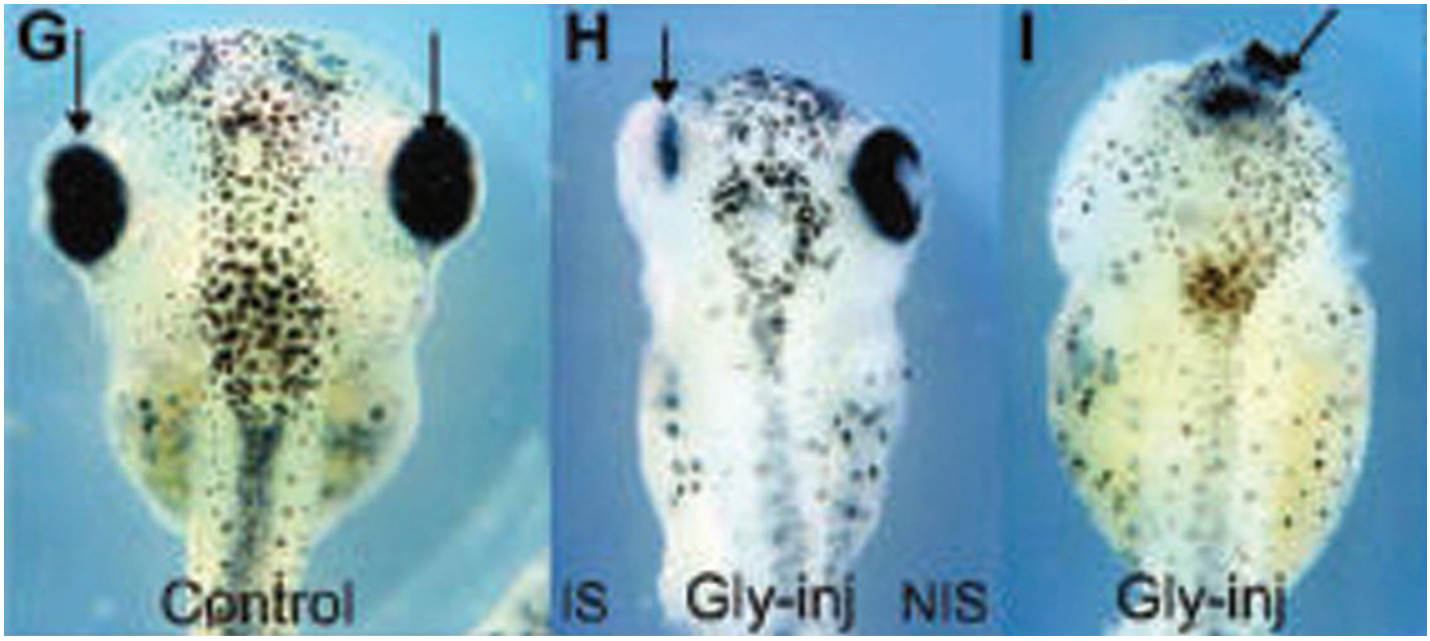

Figure 2 Effect of glyphosate injection; left to right: control embryo not injected with glyphosate; embryo injected in one cells only; and embryo injected in both cells. Note the reduction of the eye, adapted from [21]

4.1 Teratogenicity and reproductive effects

Evidence of teratogenicity (birth defects) and reproductive problems stretches back to the 1980s [6]. Observations made by Monsanto were acknowledged by the German government (and its agencies), acting as the "rapporteur" state on risk assessment to the European Commission. The German bodies concluded that high doses (500 mg/kg) led to significant skeletal and/or visceral (internal organ) abnormalities in rats and rabbits including the development of an extra 13th rib, reduced viability, and increased spontaneous abortions. Low doses (20 mg/kg) were later shown to cause dilated hearts. The questionable analysis and interpretation of the data by Germany (including claims that dilated hearts had unknown consequences and sample sizes were too small and lacking dose-dependent results) meant that the findings were not considered relevant to human risk assessment. This argument has been comprehensively rebutted in a report by Open Earth Source [6]. Most importantly, the findings have been corroborated subsequently.

Independent studies confirmed birth defects in laboratory animals. Defects in frog development were first observed with lethal doses of Roundup® (10mg/L, roughly equivalent to 0.003% dilution of Roundup®) that were still below agricultural concentrations. Effects were 700 times more pronounced with Roundup® compared to another formulation lacking the surfactant polyoxyethyleneamine (POEA), which is added to maximise glyphosate's leaf penetration, and is thought to increase glyphosate penetration of animal cells as well [18]. POEA may also have independent toxic properties (see later).

It is important to note that regulatory approval does not require assessment of the risk of commercial formulations, and instead relies on testing glyphosate alone. Sub-lethal doses also led to a 15-20 % increase in gonad size and reduced egg viability in Leopard frogs and catfish respectively [19, 20].

A definitive study conducted by Andrés Carrasco and his colleagues in Argentina found neural and craniofacial defects in frogs exposed to sub-lethal doses (1/5,000 dilutions) of glyphosate and Roundup® [21] (see [22] Lab Study Establishes Glyphosate Link to Birth Defects, *SiS* 48). These effects correlated with over-active retinoic acid (RA), a well-known regulator of the posterior-anterior axis during development (Figure 2). RA is an oxidised form of vitamin A and women are already advised against taking excess vitamin A during pregnancy. It also regulates the expression of genes essential for the development of the nervous system during embryogenesis (*shh*, *slug*, *otx2*), which were inhibited following glyphosate exposure. Inhibition of RA signalling prevented the teratogenic effects of glyphosate, further confirming its involvement in the observed abnormalities.

The craniofacial defects in frogs are similar to human birth defects linked to retinoic acid signalling such as anencephaly (neural tube defect), microcephaly (small head), facial defects, myelomeningocele (a form of spina bifida), cleft palate, synotia (union or approximation of the ears in front of the neck, often accompanied by the absence or defective development of the lower jaw), polydactily (extra digit), and syndactily (fusion of digits); these diseases are on the rise in pesticide-treated areas such as Paraguay [23].

Findings in mammals are consistent with those in amphibians. According to the World Health Organisation (WHO), the administration of high doses of glyphosate (3 500 mg/kg per day) to pregnant rats resulted in an increased incidence of soft stools, diarrhoea, breathing rattles, red nasal discharge, reduced activity, increased maternal mortality (24 % during the treatment period), growth retardation, increased incidence of early resorptions, decrease of total number of implantation and viable foetuses, and increased number of foetuses with reduced ossification of sternebra [24]. Rats orally treated with sub-lethal doses of Roundup® also showed dose-dependent reductions in craniofacial ossification (bone development), caudal vertebrae loss, and increased mortality [25], consistent with amphibian data and RA signalling defects. Prepubescent exposure led to disruption in the onset of puberty in a dose-dependent manner, reduced testosterone production, and abnormal testicular morphology [26]. Reproductive effects were transgenerational, with male offspring of exposed pregnant rats suffering from abnormal sexual behaviour, increased sperm count, early puberty as well as endocrine disruption (see below) [27].

In a feeding trial, senior scientist of the Russian Academy of Sciences Irina Ermakova found that female rats fed rat chow plus Roundup Ready soybean gave birth to an excess of stunted pups: 55.6 % compared with 6.8 % in litters from control rats fed rat chow only and 9.1 % of litters from control rats fed rat chow supplemented with non-GM soybean. The stunted rats were dead by three weeks, but the surviving rats in the exposed litters were sterile [28] (see [29] GM Soya Fed Rats: Stunted, Dead, or Sterile, *SiS* 33). The experiment was repeated with very similar results. Unfortunately, Irmakova did not succeed in her attempt to get the Roundup Ready soybean analysed for herbicide and herbicide residues, so the effects could be due to a mixture of the GM soya and herbicide/herbicide residues. The second experiment included a group of females fed rat chow plus GM soya protein did not do as badly as those exposed to GM soybean; the mortality rate of pups at three weeks was 15.1 % compared with 8.1 % for controls fed rat chow only, 10 % for controls fed rat chow plus non-GM soybean, and 51.6 % for litters of females

Dr Irina Ermakov with the Occupy Monsanto demonstration 17 September 2012

fed rat chow plus Roundup Ready soybean. This suggests that extra deaths and stunting were due to the GM soybean; as consistent with the new findings by Séralini and colleagues [1].

Irmakova too, was fiercely attacked, and attempts to discredit her continued for years afterwards, orchestrated by the journal *Nature Biotechnology* (see [30] Science and Scientist Abused, *SiS* 36).

Cell culture models offer insight into a possible mechanism of glyphosate reproductive toxicity. Death of testicular cells [31, 32] (see [33] Glyphosate Kills Rat Testes Cells, *SiS* 54) as well as embryonic, placental and umbilical cells occurs at levels 10 times below agricultural dilutions and is exacerbated by the presence of POEA in commercial formulations. Endocrine disruption was also noted at lower concentrations (see below).

Clinical and epidemiological data gathered by The Network Of Physicians Of Drop-Sprayed Towns in Argentina show a 2- and 3-fold increase in congenital and musculoskeletal defects respectively between 1971 and 2003, while another doctor noted an increase in birth defects of around 50 % among his patients. Argentina dedicates vast areas of land to RR soybean production, and as a result, an estimated 12 million people in rural/semi-urban areas are exposed to glyphosate. Increases in miscarriages, difficulty in conceiving as well as spontaneous abortions were documented. Many other illnesses were also suspected to have arisen as a result of pesticide spraying (see [34] Pesticide Illnesses and GM Soybeans. Ban on Aerial Spraying Demanded in Argentina, *SiS* 53). The local physicians confirmed that Carrasco's laboratory results on amphibians (see earlier) were consistent with the illnesses of their patients.

4.2 Endocrine disruption

The endocrine system consists of various glands that release hormones into the bloodstream, acting as chemical messengers affecting many functions including metabolism, growth and development, tissue function, behaviour and mood. Disruption of the endocrine system does not commonly result in cell death, or acute toxicity. Instead, endocrine disruption can have serious health effects through interference in cell signalling and physiology, resulting in a range of developmental impacts including sexual and other cell differentiation, bone metabolism, liver metabolism, reproduction, pregnancy, behaviour, and hormone-dependent diseases such as breast or prostate cancer. Endocrine disruption may well underlie many of the reproductive, teratogenic, and carcinogenic effects of glyphosate.

The synthesis of sex hormones is disrupted by glyphosate and Roundup® in both males and females. Mouse and rat testicular Leydig cells (testosterone producing cells) have reduced testosterone levels as well as increased levels of aromatase, an enzyme complex that converts testosterone into oestrogen [31, 32]. Human placental cells, on the other hand, showed *decreased* aromatase expression [35]. All these imbalances were observed with concentrations well below agricultural dilutions, and effects were more pronounced with commercial formulations containing adjuvants.

Abnormal expression of testosterone and/or oestrogen receptors as well as oestrogen regulated genes has been documented in human liver cells exposed to both glyphosate alone or four commercial formulations, and breast cancer cells exposed to glyphosate [36, 37].

Other hormones were shown to be dysregulated in the presence of glyphosate, including increased expression and serum concentration of leutinising hormone and increased expression of follicle-stimulating hormone. These are both gonadotropin hormones secreted by the pituitary glands that regulate growth, sexual development and reproduction [27].

Rats exposed to Roundup and/or Roundup-tolerant maize over two years exhibited a range of endocrine disruption effects that, typically, differ between the sexes [1]. Thus mammary tumours were rife in exposed females while liver pathologies predominated in exposed males. Similarly, pathology of the pituitary was more significantly increased in exposed females; and big kidney and skin tumours were confined to males.

4.3 Carcinogenicity

Epidemiological studies found that glyphosate exposure increased the risk of developing non-Hodgkin's lymphoma, a blood cancer of the lymphocytes [38, 39], with one study showing a dose-dependent correlation with exposure to commercial formulations [40]. A rise in plasma cell proliferation associated with multiple myeloma was documented in exposed agricultural workers [41]. The Network of Physicians of Aerial Sprayed Towns in Argentina has implicated glyphosate (see Figure 3), along with other pesticides, in the startling increase in both childhood and adult cancers in pesticide-treated regions, particularly in the vicinity of GM soybean plantations [34]. Increased incidence of interstitial testicular cell tumours at low doses of 32 mg/kg was documented in a two- year rat feeding study [24]. Mouse experiments also showed that glyphosate promotes skin cancer, although not sufficient to initiate tumours by itself [42]. These findings make the latest results from Séralini's team [1] all the more significant, as the mammary cancers in herbicide-exposed females and kidney and skin cancers in males are further corroboration of glyphosate's carcinogenic potential suggested by the earlier findings.

Further epidemiological and clinical studies are urgently needed to assess glyphosate's carcinogenic activity considering the growing evidence of its genotoxic properties.

4.4 Genotoxicity

Genotoxicity refers to damage of DNA. DNA damage can result in mutations that lead to adverse health effects including cancer, reproductive problems, and developmental defects. Evidence of genotoxicity not only relates to glyphosate, but also to its principle metabolite 2-amino-3-(5-methyl-3-oxo-1,2- oxazol-4-yl)propanoic acid (AMPA). Epidemiological data gathered in both Argentina [34] (exposure to glyphosate among other pesticides) and Ecuador [43] (exposure only to glyphosate) showed DNA damage in blood samples taken from exposed people.

Unpublished industry studies from the 1980s showed that Roundup® causes chromosomal aberrations and gene mutations in mouse lymphoid cells [6]. Increased frequency of DNA adducts (covalently bound chemicals on DNA) in the presence of glyphosate has been documented in the liver and kidney of mice in a dose-dependent manner [44]. This was consistent with the research team's previous study showing increased frequency of DNA adducts in Italian floriculturist workers exposed to pesticides [45]. Chromosomal and DNA damage was noted in bone marrow, liver, and kidney of mice acutely exposed to sub-lethal doses of Roundup®. Significant effects with glyphosate alone were also observed in the kidney and bone marrow [46]. Human epithelial cells derived from the buccal cavity suffer DNA damage at levels well below agricultural dilutions (20 mg/L) [47], these are the cells likely to be affected by exposure through inhalation (see [48] Glyphosate Toxic to Mouth Cells & Damages DNA, Roundup Much Worse, *SiS* 54).

Among non-mammals, glyphosate caused cell division dysfunction and alterations in cell cycle checkpoints in sea urchins by disrupting the DNA damage repair machinery [49, 50]. The failure of cell cycle checkpoints can lead to genomic instability and cancer in humans. Glyphosate is also genotoxic in goldfish, European eels, and Nile tilapia [51- 53]. Moreover, fruit flies showed increased susceptibility to gender-linked lethal recessive mutations as a result of exposure to glyphosate [54].

Not much is known regarding glyphosate's main breakdown product AMPA; one study suggested it has acute genotoxic effects [55] and should be investigated further.

4.5 Cytotoxicity of glyphosate and adjuvant

Toxicity to cells in general was demonstrated in an experiment [56] in which four different Roundup (R) formulations (R 7.2, R360, R400, R450, the numbers representing g/L glyphosate with various adjuvants) were compared with glyphosate (G), AMPA (metabolite of glyphosate) and the main adjuvants POEA, a surfactant (see

Some species have already evolved resistance to two or even three types of herbicides. In some cases, these "superweeds" are so resilient that the only method of destroying them is to pull them out by hand

Figure 3 Aerial spraying of herbicides, Eugene Daily News

[57] Death by Multiple Poisoning, Glyphosate and Roundup, *SiS* 42). Three human cell lines - primary cell line HUVEC from umbilical cord vein epithelium, embryonic cell line 293 derived from kidney, and placenta cell line JEG3 – were monitored for three effects that could kill the cell - damage to the cell membrane, poisoning of the mitochondria (site of energy metabolism), and programmed cell death – after 24 h exposure to the toxic substances/formulations at different concentrations.

All cells died within 24 hours of exposure to the Roundup formulations; but the toxicities were not proportional to the amount of glyphosate they contained. POEA by itself was much more toxic than the Roundup formulations, while AMPA is more toxic than glyphosate. The presence of POEA amplified glyphosate's toxic effects.

The most toxic Roundup formulation was R400; it killed all cells at a dilution of 20 ppm (parts per million) equivalent to 8 ppm of G. However, 4-10 ppm G alone was non-toxic, its toxicity began around 1 % (10 000 ppm). While the R formulations damaged the cell membrane and also poisoned the mitochondria, G poisons the mitochondria without damaging the cell membrane. R400 was more toxic than R450, which was in turn more toxic than R360 and R7.2, the latter two had approximately the same killing power; this was consistent among all the cell lines, indicating that unknown substances in the formulations were involved in the toxic effects.

AMPA and POEA also killed the cells by poisoning the mitochondria and damaging the cell membrane. POEA was so potent that it began to damage the cell membrane in HUVEC and poison the mitochondria in the other cells lines at 1 ppm. Roundup formulations were more toxic than either G or AMPA. AMPA by itself destroyed the cell membrane. While G did not destroy the cell membrane, it was 3-8 times more toxic for the mitochondria than AMPA. But as cell membrane damage is more serious for the cell, AMPA is more toxic than G, while POEA is the most toxic of all.

Not surprisingly, for HUVEC and 293 cells, combinations of G and POEA, G and AMPA, AMPA and POEA were all more toxic than the same concentration of the single ingredients.

For programmed cell death, the action was quicker. The marker enzymes were activated from 6 h of exposure with a maximum at 12 h in all cases. HUVEC was 60-160 times more sensitive than the other cell lines; G and R350 were effective at exactly the same concentration, from 50 ppm. The adjuvants did not seem necessary. G alone is 30 % more potent than the R formulations in programmed cell death; it acted rapidly at concentrations 500 – 1 000 times lower than agricultural use.

In a second study, human hepatic cell line (HepG2), embryonic cell line 293 and placental cell line JEC3 were tested with 9 glyphosate formulations varying in adjuvant content (Roundup Ultra, Roundup GT, Roundup GE+, Roundup Bioforce, Roundup 3plus, Glyphogan, Topglypho 360, Clinic EF and Bayer GC) compared with the most commonly used major adjuvant POEA-15, glyphosate, and a total formulation without glyphosate (Genamin) [58]. All formulations were more toxic than glyphosate. The formulations fell into three groups in toxicity according to their concentrations in POEA. POEA-15 was clearly the most toxic against human cells. It began to be toxic on mitochondrial activity and membrane integrity between 1 and 3 ppm, which is also the critical concentration at which it forms micelles (aggregates) that could be especially damaging to cell membranes.

Bearing in mind that glyphosate has its own toxicity and may also have long-term toxicity in oxidative stress (see below) and endocrine disruption and induces birth defects (see above), we are dealing with multiple toxicities in formulations where only the active principle glyphosate is regulated and tested. For example values such as the acceptable daily intake (ADI) of glyphosate are calculated with pure glyphosate in toxicological tests. The authors conclude that [58] "pesticide formulations should be studied as mixtures for toxic effects."

4.6 Neurotoxicity

Emerging evidence suggests that glyphosate is neurotoxic, including two published cases of Parkinsonism in humans. A 54 year old man in Brazil was diagnosed with Parkinsonism following accidental spraying; he developed skin lesions six hours after being exposed to spraying, and a month later he developed Parkinson's disease symptoms [59]. The other case involved a woman in Serbia who ingested 500 millilitres of glyphosate solution and developed Parkinsonism along with lesions of the brain's white matter and pons (part of brain stem), and altered mental status. The woman suffered additional non-neurological symptoms (see acute toxicity section) and eventually died [60]. Consistently, increased oxidative stress, mitochondrial dysfunction and loss of cell death markers were found in the substantia nigra, the brain region most affected in Parkinson's disease, of rats exposed chronically to glyphosate at sub-lethal levels [61, 62]. Oxidative stress represents an imbalance between the production of reactive oxygen species (ROS), also known as free radicals, and the body's ability to detoxify these reactive intermediates or repair the damage caused by them. ROS are a natural by-product of oxygen metabolism such as mitochondrial respiration, and have important roles in signalling and metabolism. Excess amounts however, can have damaging effects on many components of the cell including lipids in cellular membranes, DNA and proteins. Excess ROS has been implicated in the aetiology of a wide array of diseases including Alzheimer's disease, Parkinson's disease (PD), atherosclerosis, heart failure, myocardial infarction and cancer (see [63] Cancer a Redox Disease, *SiS* 54). Activation of the tightly regulated apoptotic and autophagic cell death pathways is also implicated in neurodegenerative diseases and has been observed in rat neuronal cell lines exposed to glyphosate in a dose-dependent manner [64].

Other mechanisms of neurotoxicity include the inhibition of acetylcholine esterase (AChE), an enzyme that metabolises the excitatory neurotransmitter acetylcholine. AChE inhibitors such as organophosphate pesticides are potent nerve agents. Symptoms of AChE inhibition include miosis (closing of the eyes), sweating, lacrimation, gastrointestinal symptoms, respiratory difficulties, dyspnea, bradycardia, cyanosis, vomiting, diarrhoea, personality changes, aggressive events, psychotic episodes, disturbances and deficits in memory and attention, as well as coma and death. Further, increased risk of neurodevelopmental, cognitive and behavioural problems such as Attention-Deficit Hyperactive disorder (ADHD), deficits in short-term memory, mental and emotional problems have been associated with exposure to glyphosate-based herbicides in children and the newborn [65]. Although glyphosate is an organophosphate, it is not an organophosphate ester but a phosphanoglycine, and therefore not been assumed to inhibit AChE. New studies suggest otherwise. Catfish and another fish species, *C. decemmaculatus*, showed AChE inhibition at environmentally relevant concentrations of Roundup® and glyphosate respectively [66, 67]. Furthermore, these effects were seen following acute exposure of up to 96 hours. A tentative association between glyphosate and ADHD in children has been made in an epidemiological study [68].

Further studies need to be done by independent scientists as original neurotoxicology data presented by Monsanto was ruled invalid by the EPA [69].

4.7 Internal organ toxicity

As in the brain (see above), increases in reactive oxygen species (ROS) have been found in the liver, kidney and plasma of rats exposed to acute doses of glyphosate. Concomitant decreases in enzymes that act as powerful antioxidants such as superoxide dismutase occur in the liver (see [70] *The Case for A GM-Free Sustainable World*, ISIS publication). Liver cells exposed to four glyphosate formulations at low concentrations showed decreases in oestrogen and testosterone receptor levels, DNA damage and decreases in aromatase enzyme activity (see [71] Ban Glyphosate Herbicides *Now*, *SiS* 43). Other studies suggest mitochondrial damage to rat and carp liver cells *in vitro* and *in vivo* respectively at sub-lethal concentrations [72, 73].

A meta-analysis of 19 feeding studies originally conducted by Monsanto, but later re-analysed by a group of French scientists led by Séralini, found kidney pathology in animals fed RR soybean, including significant ionic disturbances resulting from renal leakage

(see [74] GM Feed Toxic, Meta-analysis Reveals, *SiS* 52). This is consistent with previous results from cell cultures treated with glyphosate (see [57]), suggesting that glyphosate present in the GM food was responsible. Liver pathology in animals fed RR soybean included the development of irregular hepatocyte nuclei, more nuclear pores, numerous small fibrillar centres, and abundant dense fibrillar components, indicating increased metabolic rates.

4.8 Acute toxicity

Acute toxicity of glyphosate has been classified 'low' based on rat studies performed by industry that only showed effects at concentrations of 5 000 mg/kg. However, agricultural workers exposed at much lower concentrations have documented various symptoms, highlighted in Argentina (see [75] Argentina's Roundup Human Tragedy, *SiS* 48). Acute toxicity of glyphosate through skin contact and inhalation includes skin irritation, skin lesions, eye irritation, allergies, respiratory problems and vomiting. In cases of ingestion, severe systemic toxicity and even death has occurred. Ingestion of small amounts can lead to oral ulceration, oesophageal problems, hypersalivation, nausea, vomiting and diarrhoea. Ingestion of larger amounts (usually >85 ml) causes significant toxicity including renal and hepatic impairment, acid–base disturbance, hypotension and pulmonary oedema, impaired consciousness and seizures, coma, hyperkalemia, encephalopathy (global brain dysfunction), Parkinsonism, respiratory and renal failure. Suicide attempts have been noted as 10-20 % successful with as little as 100 ml ingested.

4.9 Glyphosate & modern diseases

An extensive review [76] published in 2013 blames glyphosate for "most of the diseases and conditions associated with a Western diet, which include gastrointestinal disorders, obesity, diabetes, heart disease, depression, autism, infertility, cancer and Alzheimer's disease." The authors argue that glyphosate residues are pervasive in the environment, much more so than any toxic chemical, and it is in the main foods of the Western diet, sugar, corn, soy and wheat. Apart from its action as a chelator, which would affect numerous biological functions, glyphosate's inhibition of cytochrome P450 (CYP) enzymes, known since the late 1980s, has been overlooked, and these enzymes play crucial roles in biology, among which is to detoxify xenobiotics (chemicals foreign to organisms). Glyphosate therefore enhances the damaging effects of other chemical residues contaminating food and environmental toxins. Another impact of glyphosate is its extreme toxicity to beneficial gut bacteria, such as *Enterococcus*, *Bacillus* and *Lactobacillus* which protect the host from pathogenic bacteria. These bacteria also have the shikimate pathway that is inhibited by glyphosate. Disruption of gut bacteria leaves the host gut open to colonization by *Clostridium* and other pathogens that produce toxins, resulting in gut inflammation, neurotoxicity and autism associated with leaky gut. *Pseudomonas*, for example, can metabolize glyphosate for its growth, and produce formaldehyde as a by-product [77]. Formaldehyde is neurotoxic, and at 100 ppm was found to induce misfolding of tau protein in neurons, resulting in protein aggregates similar to those found in Alzheimer's disease, and programmed cell death of the neurons [78].

Within the past 10-15 years, an increase in diseases associated with *Clostridium botulinum* in cattle has been observed in Germany, and also in France and the UK. Since the 1990s, several cattle farms reported excess mortality of cows with the highest incidence during the perinatal period [79]. At the same time, chronic botulism was detected in farmers taking care of the afflicted cattle. However, there was no transmission of the bacteria between humans and the animals, as the bacteria in cows and humans belong to different types. The role of glyphosate was investigated in an experiment in which *Enterococcus* bacteria were isolated from cattle, horses and the alga *Chlorella vulgaris*, and added at different concentrations to a culture medium seeded with *Clostridium botulinum* and incubated for 5 days. *Clostridium botulinum* produces neurotoxins, which are typed and measured with the ELISA (enzyme-linked immunosorbent assay) method [80]. The researchers found that all *Enterococcus* iso-

Figure 4 Field infested with Palmer amaranth 'superweed', Agweb

Figure 5 Effects of long-term glyphosate on crop (wheat) health; left not treated with glysphosate, right, treated with glyphosate; adapted from Huber's presentation [17]

lates inhibited neurotoxin production by *C. botulinum* and reduced its growth even at the lowest concentration of *Enterococcus* bacteria added. While glyphosate suppressed the growth of *Enterococcus* at the lowest concentration tested of 0.1 mg/ml, *C. botulinum* was suppressed only at concentrations 10-100 fold higher. The authors concluded that the ingestion of glyphosate "could be a significant predisposing factor that is associated with the increase in *C. botulinum* mediated diseases in cattle."

We shall come back to livestock diseases from glyphosate tolerant GM feed later.

5 Environmental and agronomic effects

Agribusiness claims that glyphosate and glyphosate-tolerant crops will improve crop yields, increase farmers' profits and benefit the environment by reducing pesticide use. Exactly the opposite is the case. Pesticide use has actually increased in successive surveys (see [81] GM Crops Increase Herbicide Use in the United States , *SiS* 45) and has been recently confirmed in a peer-reviewed study. Overall pesticide use was found to have increased by 7 % from 1996 to 2011 in the US as a direct result of cultivating GM crops, particularly glyphosate-tolerant varieties [9, 10]. In the past five years (2008-2012), herbicide use per acre has doubled in the US compared with the first five years of commercial planting of GM crops (1996-2000), with glyphosate/Roundup accounting essentially for all the increase [11]. Glyphosate herbicides and glyphosate-tolerant crops have had wide-ranging detrimental effects, including glyphosate resistant super weeds, virulent plant (and livestock) pathogens, reduced crop health and yield, harm to off-target species from insects to amphibians and livestock, as well as reduced soil fertility.

5.1 Glyphosate resistant weeds

Critics have long predicted the evolution of weeds resistant to glyphosate, consistent with all previous herbicides used in the past; and they are right. This is causing huge agronomic and ecological concern as farmers are forced to abandon whole fields of crops (see [82] GM Crops Facing Meltdown in the USA, *SiS* 46). So much so that Monsanto has issued a statement saying it is no longer responsible for the rising costs of weeds under the Roundup® warranty. The Weed Society of America has since launched free resistance-management courses for farmers, although the solutions are clearly towing the agribusiness line of dousing crops in additional pesticides, a terribly flawed solution that will only lead to more of the same, or worse – weeds resistant to multiple herbicides. Indeed, some species have already evolved resistance to two or even three types of herbicides. In some cases, these "superweeds" are so resilient that the only method of destroying them is to pull them out by hand. Palmer amaranth grows at up to 3 inches a day causing an imaginable headache for farmers (see Figure 4).

First documented in ryegrass in 1996 in Australia, glyphosate-resistance has since been observed in 23 separate species across 16 countries by 2010, covering an estimated 120 million hectares worldwide and continuing to spread [83].

Up until 2003, 5 resistant populations had been documented worldwide. Since 2007, there has been a 5-fold increase in the spread of resistant weeds (See [84] Monsanto Defeated By Roundup Resistant Weeds, *SiS* 53). So far, resistant species listed by the WeedScience database include: Palmer Amaranth, Common Waterhemp, Common Ragweed, Giant Ragweed, Ripgut Brome, Australian Fingergrass, Hairy Fleabane, Horseweed, Sumatran Fleabane, Sourgrass, Junglerice, Goosegrass, Kochia, Tropical Sprangletop, Italian Ryegrass, Perennial Ryegrass, Rigid Ryegrass, Ragweed Parthenium, Buckhorn Plantain, Annual Bluegrass, Johnsongrass, Gramilla mansa and Liverseedgrass.

Of all the resistant species, Palmer Amaranth and Common waterhemp have received the most attention. Waterhemp produces up to a million seeds per plant, making it difficult to prevent spreading of resistant populations. It also has a long emergence pattern, which means that multiple rounds of herbicide treatments are required. Resistant common waterhemp was first documented in fields in Missouri, US, in 2004 after at least 6 consecutive years of growing soybeans. The suggested mechanism of resistance in this population was the amplification of EPSPS genes in the plant, allowing it to compensate for glyphosate's inhibition of the enzyme. According to Bill Johnson, an entomologist from Perdue University in Indiana US, waterhemp is a serious threat to soybean farming with the capacity to reduce yields by 30-50 % [85]. Palmer amaranth is estimated to have infested at least a million separate sites in the US alone. It is a particular hardy plant, and is considered one of the most destructive weed species in the south-eastern US. Field experiments have shown its potential to reduce cotton yields by 17-68 %, having important implications for RR cotton farmers [86].

The problem is rapidly worsening. In January 2013, a survey

conducted by Stratus Agri-Marketing indicated that the area of US cropland infested with glyphosate resistant weeds has expanded to 61.2 million acres in 2012 [87]. Nearly half of all farmers interviewed reported glyphosate-resistant weeds on their farm, up from 34 % of farmers in 2011. The rate at which glyphosate-resistant weeds are spreading is gaining momentum, rising by 25 % in 2011 and 51 % in 2012. Increases were reported in most states, but especially in the Midwest. Not only are glyphosate-resistant weeds spreading geographically, but multiple species are now resistant on an increasing number of farms. In 2012, 27 % of farmers reported multiple glyphosate-resistant weeds up from 15 % in 2011 and 12 % in 2010. In the southern states like Georgia, 92 % of farmers reported glyphosate-resistant weeds. Marestail was the most commonly reported glyphosate-resistant weed followed by Palmer amaranth.

In order to prolong the utility of herbicide-tolerant GM crops, agribusinesses are now developing crops with multiple tolerance traits, or tolerance to old herbicides like 2,4-Dichlorophenoxyacetic acid (2,4-D). Dow Agrosciences are ready to roll out 2,4-D-tolerant corn, soy and cotton even though this year saw the discovery of 2,4-D resistant waterhemp in Nebraska, making it the sixth mechanism-of-action group to which waterhemp has developed resistance [88].

The emergence of resistant weeds explains the increases in pesticide use over the last few years, as farmers apply more and more in an attempt to rid their farms of hardy weeds. As noted by the Network of Argentinian Physicians of Crop Sprayed Towns, repeated glyphosate use on the same plots of land rose from 2 litres per hectare in 1996, to almost 20 litres in 2011 [89], most likely due to the emergence of resistant weeds.

The extent of damage wreaked by glyphosate-resistant weeds has been further exacerbated by the severe US drought of 2012, which dried out weeds and made them less sensitive to herbicides [90]. Global warming and herbicide resistant weeds may therefore have synergistic effects on crop yield losses, again highlighting the unsustainable approach of intensive chemical agriculture.

5.2 Effects on crop and plant health

Glyphosate use has been associated with the increased incidence and/or severity of many plant diseases and the overall deterioration of plant functions such as water and nutrient uptake [16, 17, 91, 92].

As mentioned above, glyphosate's mechanism of action is the systemic chelation of metals, including manganese, magnesium, iron, nickel, zinc and calcium, many of which are important micronutrients. They act as co-factors for numerous plant enzymes including those involved in the plants' immune system [91, 93]. While non-transgenic varieties are killed by glyphosate, glyphosate-tolerant crops do not die; but their physiology can be compromised. Manganese is a co-factor for 25 known enzymes involved in processes including photosynthesis, chlorophyll synthesis and nitrate assimilation, and enzymes of the shikimate pathway to which EPSPS belongs. The shikimate pathway is responsible for plant responses to stress and the synthesis of defence molecules against pathogens, such as amino acids, lignins, hormones, phytoalexins, flavenoids and phenols. The virulence mechanism of some pathogens, including *Gaeumannomyces* and *Magnaporthe* (which lead to 'take-all' and root rot respectively) involves the oxidisation of manganese at the site of infection, compromising the plant's defence against the pathogen. Glyphosate-tolerant crops were found to have reduced mineral content, confirming glyphosates' metal chelating activity [94-97]. Changes in physiology including reduced water uptake [94] and photosynthetic parameters (chlorophyll a degradation and chlorosis) were documented *in vivo* with glyphosate-tolerant soybeans even at recommended spraying concentrations [98].

Various plant diseases have reached epidemic proportions in the US, in its fourth year of epidemics of Goss' wilt and sudden death syndrome and eighteenth year of epidemic of *Fusarium* fungal colonisation resulting in root rot and Fusarium wilt. Not only does glyphosate affect disease susceptibility, there is also evidence of increased disease severity. Examples include 'take all', *Corynespora* root rot in soybean, *Fusarium spp* diseases, including those caused by *Fusarium* species that are ordinarily non-pathogenic. Head-scab caused by *Fusarium spp* of cereals increases following glyphosate application, and is now prevalent also in cooler climates when previously it was limited to warmer climates. Nine plant pathogens have been suggested to increase in severity as a result of glyphosate treatment of crops, while some 40 diseases are known to be increased in weed control programmes with glyphosate and the list is growing, affecting a wide range of species: apples, bananas, bar-

Figure 6 Interactions of glyphosate with plant and soil biology; adapted from Huber's presentation [17]

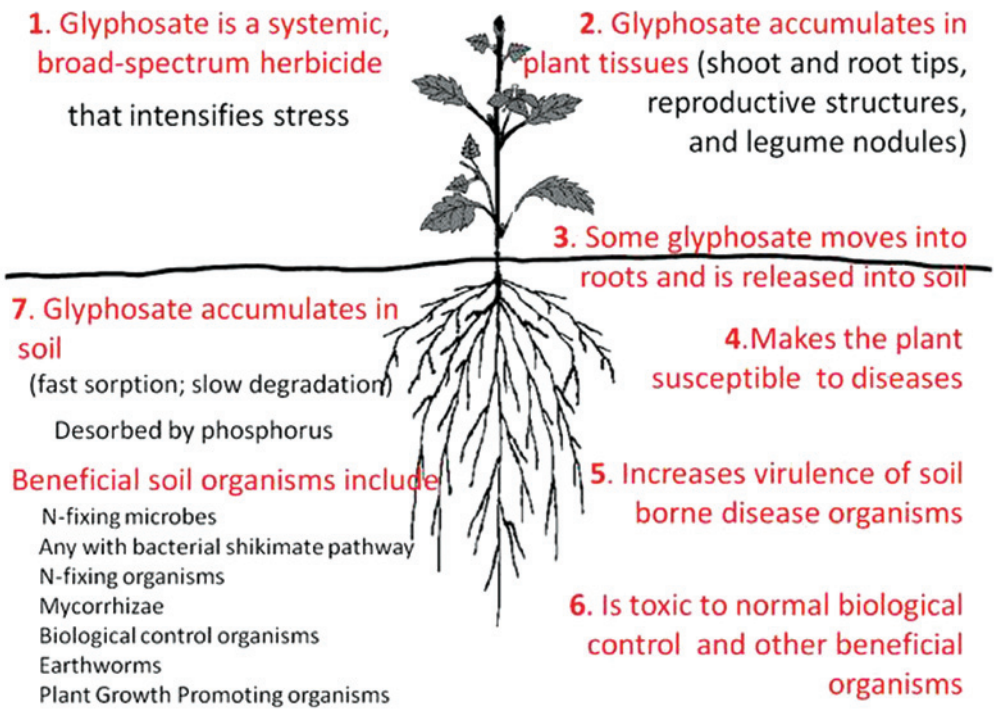

Figure 7 Monarch butterflies, University of Arkansas System

ley, bean, canola, citrus, cotton, grape, melon, soybean, sugar beet, sugarcane, tomato and wheat [99].

USDA scientist Professor Emeritus Don Huber presented detailed evidence including a photograph (Figure 5) to the UK Parliament that glyphosate-tolerant crops are less healthy and yield less. They have a compromised immune system and require extra water, which are major problems as climate change is likely to increase infectious diseases and exacerbate water scarcity [17].

With regard to non-GM crops, pre-application of glyphosate has been shown to damage wheat varieties. This effect was exacerbated by additional factors including long-term non-tillage farming, which increases the glyphosate residues in the soil and high weed densities; and the application of phosphorus fertilizers that actually remobilise glyphosate in the soil. Weed density increases glyphosate toxicity through accumulation in the roots of weeds [100].

As consistent with previous findings, GM crops are suffering heavy yield losses in drought-stricken US in 2012 (see [101] GM Crops Destroyed by US Drought but non-GM Varieties Flourish, *SiS* 56). A farmer who has grown both GM and non-GM varieties of corn and soybean side by side reported an average of 100-120 bushels per acre harvested from non-GM corn compared to 8-12 bushels to 30-50 bushels per acre from GM corn.

According to a report published by the Union of Concerned Scientists, GM crops have certainly not succeeded in increasing yields [102]. Field experiments from the University of Wisconsin found that glyphosate-tolerant maize reduced yield by 5.98 bushels per acre compared to conventional non-GM maize [103].

As with animal species, endocrine dysfunction has been suggested in plants exposed to glyphosate (see above), potentially affecting plant health as well as crop yields. Inhibition of auxins involved in plant growth and development, as well as reduced methionine levels have been observed; methionine is a principle substrate for fruit, flower opening and shedding of leaves [104].

Various aquatic species including microalgae, protozoa and crustaceans are susceptible to glyphosate, but more so to the surfactant POEA [105] in Roundup formulations, in common with human cells (see Section 4.5).

5.3 Effects on soil ecology

Soil fertility is fundamental in maintaining plant health and yields. However, along with the rise in industrial agrochemical farming practices, there has been a general increase in the number of plant diseases in the past 15 to 18 years.

Glyphosate has been shown to stimulate the growth of fungi and increase the virulence of soil pathogens such as *Xylella fastidi-*

osa which causes citrus variegated chlorosis, while also decreasing the presence of beneficial soil organisms [92, 106] Scientists Reveal Glyphosate Poisons Crops and Soil (*SiS* 47). Four primary soil fungi, *Fusarium*, *Phythium*, *Rhizocccccctonia*, and *Phytophthora*, have become more active with the use of glyphosate; and concomitantly diseases caused by these fungi have increased, such as head scab in corn, or root rot in soybeans, crown rot in sugar beets. Fusarium head blight, which affects cereal crops, is a disease that produces a mycotoxin that could enter the food chain.

Beneficial micro- and macro-organisms damaged by glyphosate include earthworms, microbes producing indole-acetic acid (a growth-promoting auxin), mycorrhizae associations, phosphorus & zinc uptake microbes such as Pseudomonads and Bacillus that convert insoluble soil oxides to plant-available forms of manganese and iron, nitrogen-fixing bacteria *Bradyrhizobium*, *Rhizobium*, and organisms involved in the biological control of soil-borne diseases that reduce root uptake of nutrients (see [17] (see Figure 6).

In addition to soil microorganisms, Roundup® but not glyphosate alone, kills three beneficial food microrganisms (*Geotrichum candidum*, *Lactococcus lactis* subsp. *cremoris* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) widely used as starter cultures in the dairy industry [107]. This may explain the loss of microbiodiversity in raw milk observed in recent years.

It has been assumed that glyphosate is short-lived, degrading in two weeks, and has low accumulation and drift. However, this conventional view may only be applicable, if at all, in certain environments. Studies in northern regions of the globe have demonstrated that glyphosate and its main metabolite AMPA can remain in the soil even years after the last spraying [108]. That means the herbicide and its residues can remain active and accumulate in soils with increasingly devastating effects on soil ecology.

5.4 Effects on ecosystems

Glyphosate use impacts animal biodiversity and health either directly or indirectly through destruction of habitats. It is considered to be particularly toxic to aquatic and amphibian species, due to its high water solubility.

Amphibians are the most endangered animal class on Earth. Recent studies have highlighted glyphosate's toxicity to frog species, with exposure killing 78 % of animals in laboratory conditions (see [109] Roundup Kills Frogs, *SiS* 26). A 2012 study found enlarged tails in exposed tadpoles, similar to the adaptive changes seen in response to the presence of predators. Tadpoles adapt their body shape to suit environmental conditions, so any changes not suited to the environment could put the animals at a distinct disadvantage [110]. Currently unpublished data from The Department of Herpetology at the Society of Sciences, Aranzad, Spain suggests that glyphosate concentrations below agricultural levels are sufficient to kill 10 species of amphibians in the Basque region of Spain [111]. As mentioned earlier, birth defects in frogs have also been detailed in laboratory conditions [21, 22].

The model species for testing toxicity to aquatic organisms is *Daphnia magna*. It has been shown to suffer from both acute and chronic toxicity at levels orders of magnitude lower than officially recognised by government agencies. Concentrations of 10 mg/L in 48 h acute experiments; and chronic exposure, particularly to formulated Roundup, causes serious reproduction damage at levels close to (1.35 mg/L) or even below (0.45 mg/L) accepted threshold values for glyphosate in surface waters in the United States in general (0.7 mg/l) and in the state of California specifically (1.0 mg/l). Roundup was more toxic in all the measurements of chronic toxicity that included survival, growth, fecundity, abortion rates and juvenile body size [112].

Studies in aquatic microcosms and mesocosms found that Roundup at 8 mg glyphosate/L inhibited the growth of green algae at the expense of toxic bloom-forming cyanobacteria, with potentially drastic impacts on freshwater aquatic ecosystems [113, 114]. It also accelerates the deterioration of water quality, that is already jeopardising global water supply (see [115,116] (World Water Supply in Jeopardy, *SiS* 56, GM Crops and Water - A recipe for Disaster, *SiS* 58).

The indirect effect of habitat destruction is exemplified by the decline of Monarch butterfly numbers (see [117] Glyphosate and Monarch Butterfly Decline, *SiS* 52) (Figure 7). The larvae of this species feed almost exclusively on milkweed plants, which are being destroyed through glyphosate treatment of GM crops. In the Midwest of the US, there has been a 58 % decline in milkweed plants and a resulting 17-year decline in Monarch butterfly [118]. A decline in their winter migration to Mexico has been observed stretching back 15 years.

5.5 Diseases of livestock

The rise of certain diseases in livestock populations has been linked to glyphosate ingestion from feeding on RR crops. Huber claims that livestock are suffering a triple whammy of reproductive toxicity caused by endocrine dysfunction (as described above with regards to human health), nutrient deficiency, and a novel unknown pathogenic 'entity' found in many reproductive tissues and dead foetuses as well as other body parts [17].

With regards to nutrient deficiency, manganese deficiencies have been associated with various animal diseases and reproductive failures, which are becoming increasingly common in livestock. In Australia, following two seasons of high levels of stillbirths in cattle, it was found that all dead calves were manganese deficient [119]. Moreover, 63 % of newborn with birth defects were also deficient. Manganese is known to be important for mobilising calcium into bones, correlating with abnormal bone formation in these calves.

A Danish farmer recently reversed illnesses in his pigs through reverting back to a non-GM feed. Illnesses included birth defects, reduced live births, diarrhoea, bloating and poor appetite disappeared, resulting in increased profit for his farm (see [120] GM Soy Linked to Illnesses in Farm Pigs, *SiS* 55). The farmer attributed the illnesses and deaths to chronic botulism in his pigs fed GM feed. This was consistent with research in Germany mentioned earlier, linking the rise of chronic botulism in cattle and in farmers to an increase in glyphosate use in Europe within the past 10 to 15 years [79, 80]. Glyphosate destroys beneficial bacteria that inhibit *Clostridium botulinum* in the gut, allowing the pathogen to flourish.

5.6 Widespread contamination of water supplies

With all the described toxic effects of glyphosate, it becomes imperative to assess the level of contamination of the water supplies, our source of drinking water (see [121] GM Crops and Water - A Recipe for Disaster, *SiS* 58). Recent research in Catalonia, Spain, revealed widespread contamination of their groundwater [122]. In the US, glyphosate has been detected in rain and air samples [123].

In Germany, glyphosate was detected in the urine of all tested Berlin city residents, including one person who had been eating organic food for over 10 years [124]. Levels reached 5-20 times the established permitted level in drinking water in the EU. Even those who live away from farming areas are not protected. Glyphosate was previously found in urine samples of farm workers at concentrations shown to have cause endocrine disruption.

To conclude

Glyphosate toxicity can no longer be ignored. While evidence of its harm to health and the environment grows, Monsanto is proposing to raise permitted residual levels in lentils by 100 fold in the EU [4], and petitioned for further hikes in already unacceptable high permitted levels in the US [5]. Brazil has recently proposed a new bill that will ban many environmental toxins including glyphosate [125]. A global ban or phase-out of glyphosate and glyphosate-tolerant crops is a matter of urgency, and with that, widespread adoption of non-GM sustainable agriculture [126] (Food Futures Now *Organic *Sustainable *Fossil Fuel Free , ISIS Report).

2
Bt Crops Failing & Harmful to Health and Environment

The claim that genetically modified organisms are the most promising way of increasing crop yields is falsified by many independent scientific studies as well as direct experience with GM crops in India, China, Argentina and the United States; at the same time evidence of harm to health and the ecosystem accumulates
Dr Eva Sirinathsinghji

Protestors at rally in India that stopped Bt brinjal (eggplant)

1. Crop failures, farm suicides, & false accounting

One of the two major types of genetically modified (GM) crops grown commercially carries insecticidal toxins from the *Bacillus thuringiensis* (Bt) bacterium. Since the first planting of Bt crops in 1996, they now represent 65 % and 75 % of all corn and cotton varieties in the US respectively [1], and cover an estimated 66 million hectares of land globally in 2011. Approximately 90 % of all cotton in India is also GM; a devastating example of the GM crop system, where monopoly of the seed market by agritech giants has forced Indian farmers into a cycle of debt caused by expensive GM seeds that are not only failing to control pests, but also failing to yield (see [2] Transgenic Cotton Offers No Advantage, SiS 38). The Indian government admitted that 2012 yields in the state of Maharashtra was likely to be down by 40%; that, coupled with continually rising costs, forced 5 million cotton farmers to demand compensation from the government [3, 4]. The huge numbers of farmers committing suicide in cotton-growing is now acknowledged by the Indian Ministry of Agriculture to be directly linked to Monsanto's Bt cotton varieties [5], as we have reported earlier ([6] Farmer Suicides and Bt Cotton Nightmare Unfolding in India, SiS 45) (Figure 1).

The selling point of Bt crops was to reduce pesticide applications. It did initially, largely because Bt toxins from GM crops, claimed to be harmless to both humans and non-target species, are not included when pesticide applications are quantified. It turns out that the health and environmental burdens from Bt 'biopesticides' incorporated into Bt crops are not too different from conventional pesticides. The Bt toxins are more likely to be ingested by humans and other animals as they are present inside the plant cells and cannot be washed off, making it all the more important to include the toxins in pesticide counts. The Wall Street Journal has just reported that conventional pesticides have made a dramatic comeback nevertheless, as pests acquired rapid resistance to the Bt toxins. Pesticide companies have seen their sales go up 5 to 50 % in 2012 and the first quarter of 2013 [7]. One company, American Vanguard, acquired a

series of insecticides firms and technologies during the past decade, betting that insecticide demand would return as Bt loses effectiveness, which was just what many scientists have predicted. Corn rootworm resistance is a particularly big problem currently (see Figure 2)

Risk assessment to-date for Bt crops (even more so than other GM crops) is widely regarded as inadequate (see [8] Bt Toxins in Genetically Modified Crops: Regulation by Deceit, *SiS* 22). The alleged efficacy and safety of these products cannot be established when exposure levels have not been reliably determined. In particular, reports of declining concentrations in the food chain and soils are unreliable and need to be re-evaluated. Despite these inadequacies in risk assessments so far, evidence of Bt toxicity to health and the environment is steadily accumulating.

Increases in crop yields have also been used to sell Bt crops. Available data show no clear yield increases with Bt crop cultivation. At best, there is some evidence of a small advantage during high infestations of the European cornborer to Bt maize crops, but during low to moderate infestations, there appears to be little advantage even without the use of insecticides on non-GM crops [9]. Certain single transgene Bt varieties as well as stacked varieties were shown to reduce crop yields, particularly Bt varieties targeting the corn rootworm, with yield losses of 12 bushels per acre compared with conventional varieties. Stacked traits also reduced yields through negative genetic interaction of the multiple transgenes, which varied with different combinations of transgenes [10].

Charles Benbrook at the Centre for Sustaining Agriculture and Natural Resources, Washington State University, Washington USA, highlights the "dramatic changes" in GM corn and soybeans over the past five years compared with the first five years of commercial use (1996-2000); among them, two to six Bt toxins needed in corn to deal with the European cornborer and the corn rootworm complex; the use of delayed release, systemic seed treatments including two insecticides and two fungicides, one of which a nicotinoid implicated in honey bee colony collapse, an unprecedented increase in fungicide use on corn: 11 % crop acres in latest 2010 USDA survey compared with no more than 1% treated previously [11]. This completely gives the lie to the claim that Bt crops reduce pesticide use, and this is in addition to about double the herbicides used per acre, with glyphosate/Roundup accounting essentially for all the growth.

Bt toxin has been shown to cause damage to multiple organs including the heart, kidney and liver of lab animals in industry studies that were re-analysed by independent scientists

2. Risks to human health

Bt toxin has been shown to cause damage to multiple organs including the heart, kidney and liver of lab animals in industry studies that were re-analysed by independent scientists [12] (see [13] GM Feed Toxic, New Meta-analysis Confirms, SiS 52). Toxicity to human kidney cells has been further confirmed *in vitro*, where exposure to Cry1Ab toxin caused necrotic cell death [14] (see [15] Bt Toxin Kills Human Kidney Cells, SiS 54). Moreover, adverse immune responses were detected in lab animals as well as humans. One study found immune responses to the Bt toxin similar to that seen with the cholera toxin

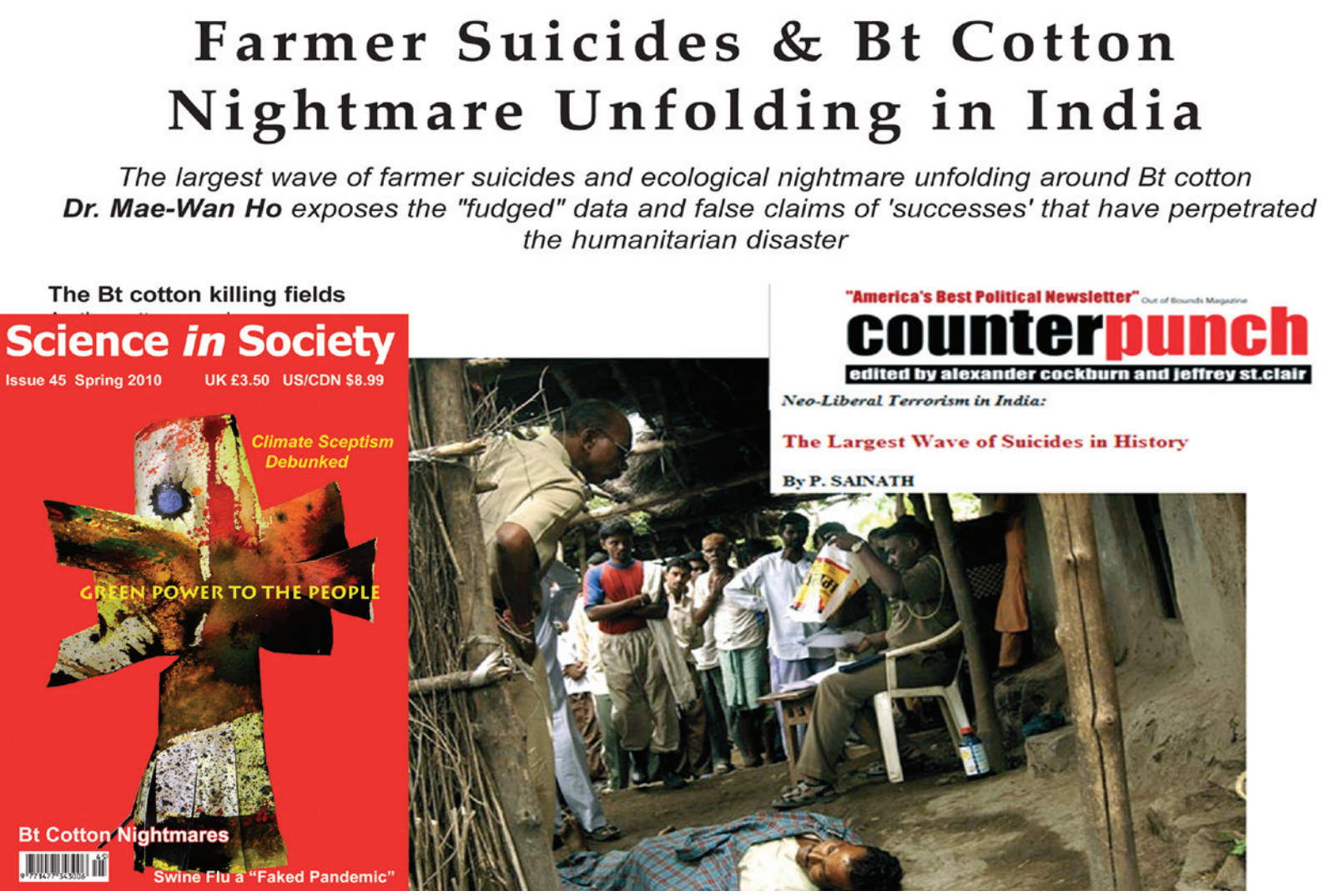

Figure 1 Escalation of farm suicides in India from Bt cotton

Figure 2 Field with Bt corn plants lodged from rootworm attack, Aaron Gassmann/Iowa State University

Maize crops expressing Cry1Ab have been infested with the western bean cutworm (Striacosta albicosta) since 2000, and so severely that it is termed 'pest replacement'

[16], while Cry1Ab has been shown in feeding studies of Monsanto's MON810 maize to induce intestinal and peripheral immune responses with a rise in T and B cells, $CD4^+$, $C84^+$, γδT, and αβT subpopulations of immune cells in both the gut and peripheral sites after 30 and 90 days of feeding in mice [17].

Most recently, immune responses were observed in mice administered Cry1Aa, Cry1Ab, Cry1Ac or Cry2A by gavage directly to the stomach. Combinations of the proteins also elicited immune responses that included haematotoxicity (toxicity to red blood cells), particularly for cells developing into red blood corpuscles, as well as a significant reduction in bone marrow cell proliferation [18]. The immune responses showed a more pronounced effect from 72 hours onwards after a single exposure.

Allergenic responses had been reported by farmers and factory workers handling Bt crops for years, with effects on eyes, skin and the respiratory tract (see [19] (More illnesses linked to Bt crops, SiS 30). Contrary to industry's claims, neither the Bt gene nor the toxin protein is degraded in the gut, but circulate in the blood stream. A recent study in Canada found that over 90 % of women and their unborn babies had the toxin in their blood streams, just from eating a typical Canadian diet [20]. The toxin crossing the placental barrier is of obvious concern. Reduced fertility in mice fed Bt maize has been reported in a lab study (see [21] GM Maize Reduces Fertility & Deregulates Genes in Mice, SiS 41).

3. Breakdown of pest control

The breakdown of pest control can occur at three levels: the expression of the toxin not reliably sufficient to kill all target pests; secondary pests that are not susceptible to the Bt toxins emerging as a result of reduced use of other pesticides, bad agricultural practices such as monoculture farming, and the reduction of food or niche competitors (target pests); and pests developing resistance to Bt toxins, rendering them completely ineffective. All such breakdown of Bt pest control have occurred.

3.1 Bt toxin levels insufficient to kill pests

Genetic modification of plants is unpredictable by nature (see Chapter 3). Bt toxins were inserted in plants so that they can be expressed consistently across the whole plant. However, studies have found inconsistent expression both across the whole plant and during its life-span, resulting in insufficient toxin to kill target pests [22, 23]. Farmers have reported crop failures from target pests. In the US, 25 farmers filed a law suit against Monsanto for the failure of their Bt cotton to protect from bollworm infestation [24]. A 2005 survey of over 100 Indian farmers in Andhra Pradesh found that 32.5 % of farms had infestations of American bollworm (see [25] Organic Cotton Beats Bt Cotton in India, SiS 27). At the same time, organic farmers reported a low 4.1 % incidence of infestation.

3.2 Secondary pest and disease infestations

A study published in *Science* in 2011 found that over a period of 10 years, the mirid bug, previously considered an occasional or minor pest, acquired pest status, with increasing population sizes that corresponded with decreased pesticide use on Bt cotton fields in Northern China. Not only is this a problem for Bt crops, but many other important food crops as well, and conventional pesticides are brought back in increasing amounts [26] (see [27] GM-spin Meltdown in China, SiS 47). With expensive GM seeds and additional pesticide costs, farmers are left worse off than before. Bt cotton fields in India are also showing infestations of new pests such as the

Figure 3 Mealy bugs on dead plant leaf (left), close-up of single giant bug on cotton plant (right), from [28]

mealy bug (Figure 3), gall midges, mosquitos and safflower caterpillars that were not previously a problem (see [28] Mealy Bug Plagues Bt Cotton in India and Pakistan, SiS 45). One mechanism behind secondary pest infestation has been suggested in a 2013 study showing that cottons' innate insecticidal defensive strategy is reduced when target pests are killed by Bt toxins. In natural situations, plant damage caused by pests induces the release of the insecticidal proteins called terpenoids. But, with Bt killing target pests, the plant damage and consequently, terpenoid release is reduced, leading to higher than normal infestation by other pests like cotton aphids [29].

Although initially, Bt cotton had partial success in reducing bollworm infestations, cotton can be targeted by 165 different species of pests that are not all susceptible to the Bt toxin, and secondary pest infestations as well as new illnesses such as leaf 'streak' virus and lalya are on the rise (see [6]). The new mealy bug infestations seen across India and Pakistan have considerably reduced crops yields (45-50 % in 2007-2008). The two predominant species of mealy bugs originated from the US and arrived since the introduction of Bt cotton. They have now been found on other crops including brinjal, okra, tomato, chilli, potato, cluster bean, green gram, papaya and sunflower [30]. Independent studies in India show significant reductions in crop yields that correlate with reduced profits as well as devastating numbers of farmer suicides due to indebtedness from expensive Bt seed varieties, combined with the low yielding crops [5]. A study comparing organically grown cotton and Bt cotton over 200 farms in Andhra Pradesh in India highlighted the propensity of Bt cotton to accumulate diseases and pests, along with reduced yields [25].

Brazil is suffering the same fate; 2-4 % of their cotton and soybean crops in Bahia are lost to corn earworm caterpillars. Since the Bt toxins killed off the *Spodoptera* species that eat the corn earworm, a plague of caterpillars (*Helicoverpa zea*) is now costing over 2 billion Brazilian Reals to cover losses and alternative insecticides [31].

The US has been seeing huge rises in secondary pest infestations cotton crops since Bt cotton was introduced [32]. Data from 2010 compiled by Mississippi State University entomologist Mark Williams identified the top cotton pests: in the south of the cotton belt (Arkansas), the tarnished plant bug; in Tennessee, the stink bug followed by plant bugs and the spider mites; in the Southwest

Figure 4 The tarnished plant bug, from [34]

Tarnished plant bugs can reduce yields by 50 %

(Texas, Oklahoma and Kansas) fleahopper especially in the eastern part of Texas including the coastal and central areas followed by thrips and bollworms; In the Southeast (Virginia, North Carolina, South Carolina, Georgia, Alabama and Florida), stink bugs followed by thrips and bollworms; in the West (California, Arizona and New Mexico), tarnished plant bug *Lygus*, followed by mites, thrips, whiteflies and aphids [33]. Tarnished plant bugs can reduce yields by 50 %. Research showed that the yields of an experiment where insecticide applications to control the tarnished plant bugs were initiated from

Figure 5 The stink bugs top to bottom: brown, green and southern green stink bugs (left) and their immature nymphs (right), rearranged from [35]

the second week of flowering and continued throughout the season yielded twice as much as the untreated controls [34]. The tarnished plant bug *Lygus lineolaris* (Figure 4) belongs to a group that includes also the clouded plant bug and the cotton fleahopper.

Stink bug pests across the southeastern cotton belt consist of three main species, the brown stink bug, *Euschistus servus* (Say); the green stink bug *Acrosternum hilare* (Say), and the southern green stink bug *Nezara viridula* (L.), with population levels of the three species varying widely across seasons, states and fields [35]. Stink bugs primarily feed on a wide range of developing fruit and seed hosts (more than 200 cultivated and noncultivated) including cotton, corn soybeans, peanuts, fruits, grains, vegetables, grasses, shrubs and trees. Adults overwinter in protected areas such as leaf litter, straw, under tree bark and at the base of native grasses. As the season progresses, adults fly to find a sequence of host plants with overlapping reproductive stages. Populations continue to increase before moving onto late-season crops like cotton, peanuts, soybeans, and fall vegetables and pecans. The largest populations are generally in the late summer and fall. Depending on the species and latitude, one to five generations develop annually.

Maize crops expressing Cry1Ab have been infested with the western bean cutworm (*Striacosta albicosta*) since 2000, and so severely that it is termed 'pest replacement', a phenomenon usually associated with intensive farming and the heavy use of pesticides [36]. Prior to 2000, this species was not considered a maize pest but instead was confined to narrow regions. It has since affected at least 8 US states. The development of CryF maize that targets the cutworm with 80-90 % efficiency, along with the SmartStax crops that also carry Cry1F as well as 5 other Bt toxins are predicted to exacerbate the problem, as large-scale cultivation of these crops could encourage selection of the 10-20 % not killed by Cry1F which could then spread throughout the population. This could also provide big business to pesticide produces when the Cry1F maize is not effective.

3.3 Bt resistance in target pests

Rising insect resistance to Monsanto's biggest selling Bt corn is threatening its utility and profitability. Insect resistance prompted an investigation by the Environmental Protection Agency (EPA). According to documents in the docket (Docket No: EPA-HQ-OPP-2011-0922) [37], "severe" damage to corn by rootworm has oc-

Studies also suggested a link between Bt toxicity and global bee population decline. Bt crops expressing Cry1Ab exacerbated the damage caused by microsporidia, a fungal parasite

curred in four states in the US (see Fig. 2). Further, the EPA describes Monsanto's insect resistance monitoring program as "inadequate".

As predicted by many scientists, as the cultivation of Bt crops expanded, Bt resistance has emerged and is spreading. So far, 8 populations of Bt resistant pests have been documented, 2 resistant to Bt sprays, with the rest resistant to Bt crops (see [38] Bt Resistant Rootworm Spreads, SiS 52). Resistance appears to be a dominant trait at least in Iowa, so only one copy of a resistant gene is necessary for the pest to survive, instead of two copies. This means resistance can spread much more rapidly through pest populations.

To counteract Bt resistant pests, agritech businesses are busy making next generation crops that carry more and more Bt toxins. For example, the original Bolgard cotton contained one Bt toxin Cry1Ac, Bollgard II cotton contains 2 toxins, while Bollgard III contains 3. The latest Smartstax has 8 genes, 6 for insecticide resistance and two for herbicide tolerance. Thus, the failure of first generation transgenic crops can actually prove profitable for Industry. Farmers become locked in a cycle of dependency, having to buy stronger or more expensive products. It is a well-known business model of 'planned obsolescence' [39]. This strategy is already being called into question as insects seem to be developing resistance to multiple toxins. The EPA and researchers who created these pyramid crops predicted that insects exposed to multiple toxins are controlled by "redundant killing", where two or more toxins act in different way to kill a pest. But in laboratory settings, this prediction was not borne out. On the contrary, pests already resistant to one Bt toxin more rapidly develop resistance to other toxins. These experiments also revealed a decrease in Cry1Ac and Cry2Ab expression over the growing season, which is likely to exacerbate the problem [40].

4. Environmental and ecological damage

Besides hazards to human health, Bt toxins also impact negatively on ecosystems. A common model organism for eco-toxicity studies is the water flea *Daphnia magna*. When fed a diet of 100 % Bt maize, it showed increased mortality, reduced numbers of females reaching sexual maturity, and reduced overall egg production [41].

An independent study exposed industry toxicology tests to be completely inadequate. It showed clear toxicity of the Cry1Ab toxin to the target pest European cornborer, and most importantly, also to the non-target 2-spotted ladybird [42] (see [43] Bt Toxicity Confirmed: Flawed Studies Exposed, SiS 55).

Pollen from Bt maize was found to increase mortality of monarch butterfly larvae. This, along with glyphosate destruction of their habitats, may be at least partially responsible for declines in Monarch butterfly numbers [44, 45] (see [46] Glyphosate and Monarch Butterfly Decline, SiS 52). Peacock butterflies, a protected species in Europe, is also predicted to be under threat from Bt Maize in Southern Europe. Simulation models of both maize and butterflies were combined with actual dose-response data of peacock butterfly exposure to MON810 maize showed that maize pollen is likely to harm the butterfly larvae. The species is bivoltine (has two broods per year), the second brood coinciding with maize pollination. Furthermore, maize pollen is wind-borne, and Bt toxins inside pollen are not UV degradable, unlike the Bt sprays used in organic agriculture, making exposure more likely. This study contradicts the predictions made by the EFSA (European Food Safety Authority), which was based more on assumptions than empirical data as in this study [47].

Studies also suggested a link between Bt toxicity and global bee population decline. Bt crops expressing Cry1Ab exacerbated the damage caused by microsporidia, a fungal parasite (see [48] The Mystery of the Disappearing Bees, SiS 34). Honey bees exposed to the Cry1Ab protein, took longer to imbibe the contaminated syrup. The same study also found learning in bees significantly affected following Cry1Ab oral exposure [49]. Lacewings, an important predator of wheat pests, suffered significantly reduced survival and delayed development when fed an insect pest (lepidopteran) that has eaten GM maize containing the Bt toxin Cry1Ab, but not when fed the same pest treated with much higher levels of the natural toxin (see [50] GM Food & Feed Not Fit for "Man or Beast", ISIS report) [51, 52].

As the toxin is expressed also in the roots of the plants, it seeps into the soil where it was found to persist for 180 days. This affects soil fertility by harming soil organisms, thereby depleting the land and reducing crop yields. A study conducted in India found that soil bacteria in Bt cotton fields were reduced by 14 %, while total microbial biomass was reduced by 8.9 % (see [53] Monsanto's Bt Cotton Kills the Soil as Well as Farmers, ISIS Report). This has implications for yields of crops as well as illnesses, with a new disease termed lalya emerging as a result of nutrient deficiencies in the soil. This causes the plants to redden and wilt. Cross contamination of GM varieties with non-GM varieties also poses big risks for biodiversity, as has been documented with GM corn in Mexico [54].

By-products of Bt crops were found in field studies to reduce the growth rate of aquatic insects (caddisflies) by 50 % and increase mortality rates [55] (see [56] Bt Crops Threaten Aquatic Ecosystems, SiS 36). Half the caddisflies living near Bt maize fields had Bt maize pollen in their gut. Potential off-target effects are possible in soil as well as streams and rivers. Bt toxin contamination of streams and waterways has been documented in and around Bt maize plantations. One study found 13 % of stream sites and 26 % of water column sites in the US contaminated with Cry1Ab [57].

5. To conclude

Bt crops are at best useless in pest control, and at worst, an exacerbating factor for pest infestation and reducing crop yields. They are also proving hazardous to non-target species in the ecosystem and to human health. All the evidence favours non-GM integrated pest control as a far superior strategy.

3
The New Genetics and Dangers of GMOs

The original rationale and impetus for genetic modification was the 'central dogma' of molecular biology that assumed DNA carries all the instructions for making an organism, which are transmitted via RNA to protein to biological function in linear causal chains. This is contrary to the reality of the 'fluid genome' that has emerged since the mid-1970s. In order to survive, the organism needs to engage in natural genetic modification in real time, an exquisitely precise molecular dance of life with RNA and DNA responding to and participating fully in 'downstream' biological functions. That is why organisms and ecosystems are particularly vulnerable to the crude, artificial genetically modified RNA and DNA created by human genetic engineers; and why genetic modification can probably never be safe

Dr Mae-Wan Ho

1. What's a GMO?

A *genetically modified organism* (GMO) is an organism with synthetic genetic material inserted into its genome. It is made in the laboratory with sterile techniques, which also means without sex. The genome is practically all of the genetic material of an organism, a copy of which is present (with few exceptions) in every cell of its body.

Every organism, as for example, a plant, is made of tissues, tissues of cells, and as you go down the scale with an increasingly powerful microscope, you can see that each cell has a nucleus containing a copy of all its genetic material - the genome – packaged in structures called chromosomes. Each chromosome, when unwound is a very long thread, called chromatin, and when you strip away the special proteins from the chromatin, you end up with the double helix DNA (deoxyribosenucleic acid), the genetic material (Figure 1). The DNA is what gets chopped and changed in genetic engineering and genetic modification.

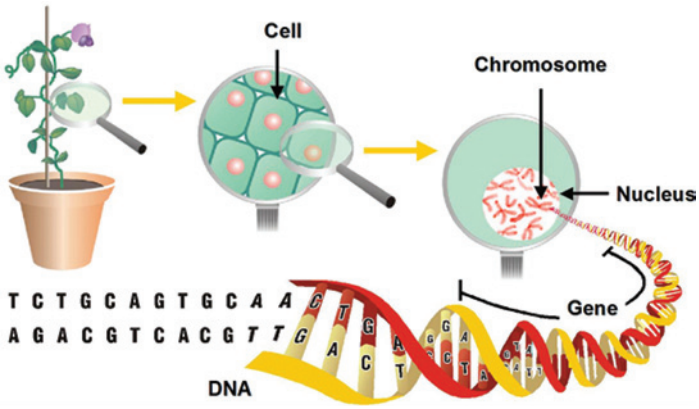

Figure 1 Genetic engineering involves chopping and changing DNA in organisms, CSIRO

In genetic modification, foreign DNA is introduced into the genome of plant cells maintained in culture using a *vector*, a piece of DNA carrying the foreign DNA and designed to jump into the host cell genome. The foreign DNA - *transgenic DNA* or *GM construct* - typically contains more than one *gene*, a sequence of DNA that expresses a protein. Each transferred gene - a *transgene* - consists of a *coding sequence* that specifies the distinctive amino acid sequence of a protein, with a *promoter*, the signal for starting in front of it and behind, a *terminator*, the signal for stopping (see Figure 2). In addition, the GM construct usually includes one or more *marker genes* that enable the *transformed cells* (cells that have taken up and integrated the transgenic DNA) to be identified and selected. Antibiotic resistance marker genes express antibiotic resistance, so transformed cells can be selected with antibiotics, which kill untransformed cells. Each transformed cell is then induced to grow into a plantlet that develops into a distinct transgenic plant. Because there is no control over where and in what form the transgenic DNA lands in the host genome, each resulting transgenic plant is essentially a different transgenic line. (For more, see [1] (FAQ on Genetic Engineering, ISIS Tutorial).

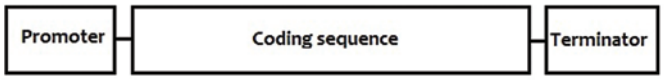

Figure 2 A transgene

The rationale and impetus for genetic engineering and genetic modification is the 'central dogma' of molecular biology due to Francis Crick [2, 3], who shared the Nobel Prize with James Watson for the DNA double helix structure [4]. In its original form, the central dogma supposed that DNA carries all the instructions for making an organism. Individual 'genetic messages' in DNA are faithfully copied or transcribed into RNA (ribosenucleic acid), which is then translated into a protein via a genetic code; the protein determining a particular trait, such as herbicide tolerance, or insect resistance; one gene, one character (Figure3). If it were really as simple as that, genetic modification would work perfectly every time. Unfortunately things are vastly more complicated.

2. The fluid genome and natural genetic engineering

I first warned of the hazards of genetic modification more than 15 years ago. In [5] Genetic Engineering Dream or Nightmare (ISIS publication, first edition 1997), I described the new genetics of the 'fluid genome ' - a term coined by molecular geneticists in the early 1980s - and said the greatest danger of genetic modification is that it is misguided by the ideology of genetic determinism (the 'central dogma' of the old genetics). Genetic determinism made genetic engineering seem compelling, but was totally contrary to reality.

Instead of the linear, one-way information flow envisaged in the central dogma from DNA to RNA to protein and 'downstream' biological function, there is intricate cross-talk between the organism and its environment at all levels, with feed-forward and feed-back cycles swirling through the epigenetic and metabolic networks of molecular interactions that mark and change genes as the organism goes about its business of living, their effects reverberating down the generations [6, 7] (Death of the Central Dogma and other arti-

It is the fluid and adaptable genome that defeats all mechanistic attempts at genetic modification, which is why genetic modification can almost never be safe. It is a clash of ideology with reality

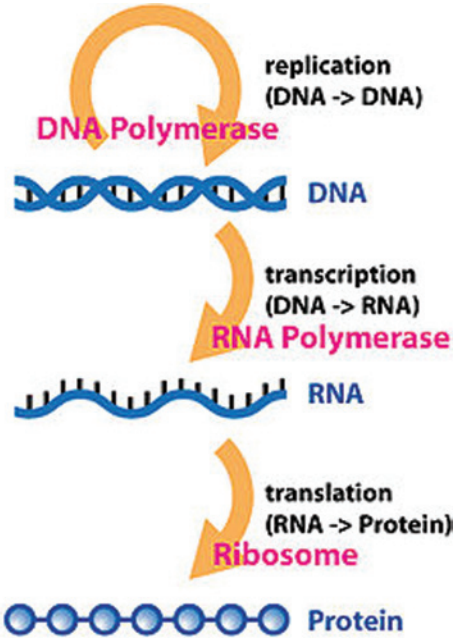

Figure 3 The central dogma of molecular biology, Wikimedia

cles in the series, *SiS* 24; Living with the Fluid Genome, ISIS publication).

The organism is doing its own natural genetic modification with great finesse, a molecular dance of life that's necessary for survival. Unfortunately, genetic engineers don't know the dance; they are only now tracing its footprints in the genome. It is clearly impossible to modify one gene or one function at a time without affecting other functions, ultimately the entire organism. It is also this molecular dance of life that makes organisms and ecosystems so vulnerable to the unintended effects of genetic modification. Furthermore, the insults and injuries to organisms and ecosystems exposed to the GMOs can be passed on to future generations to influence the course of evolution (see Figure 4) ([8]Development and Evolution Revisited, ISIS scientific publication). The human organism shapes its own development and evolutionary future; that is why it is so important for us to take responsible action.

Indeed, new findings on the fluidity and responsiveness of the genome have made the hazards of genetically modified DNA and RNA even greater than I had envisaged, and I shall update the big picture in this chapter.

3. GM inherently hazardous

Reliable evidence obtained by scientist independent of the biotech industry going back to the 1990s and evidence obtained by farmers in the field both show that GM feed invariably causes harm, regardless of the animal species or the food crops that were genetically modified or the genes and constructs inserted into the genome. A full list is presented in Box 1, partly drawn from [9] GM Food Nightmare Unfolding in the Regulatory Sham (ISIS scientific publication) and updated with studies done and cases uncovered since. It presents a consistent picture of GM-linked deaths and illnesses, with scientists confirming what farmers have experienced for years. This is particularly significant as independent scientific studies are by very meagrely supported, and scientists find it very difficult to obtain the GM material from the companies for their research.

The inevitable conclusion one comes to is that GM is inherently hazardous.

3. What are the hazards of GMOs?

There are many possible hazards of GMOs associated with the fluid and responsive genome; I have put them into four categories in Table 1 [5, 36] (*The Case for A GM-Free Sustainable World*, ISIS/TWN publication).

Despite the lack of dedicated research, there is now a wealth of evidence that GM food and feed is unsafe, both from lab studies by independent scientists, and from farmers' experiences in the field all over the world. The list of health impacts from GM feed includes birth defects, miscarriages, infertility, cancers, and mysterious new pathogens. There is also evidence that GM crops yield less, poison crops and soil, and cause the emergence and re-emergence of many crop diseases as described in the first two chapters of this report.

Although the weight of evidence against the safety of GMOs is overwhelming, we are still largely in the dark as to the precise nature of the hazard(s) associated with different GMOs. Toxicity has been found for transgene products such as the Bt proteins from

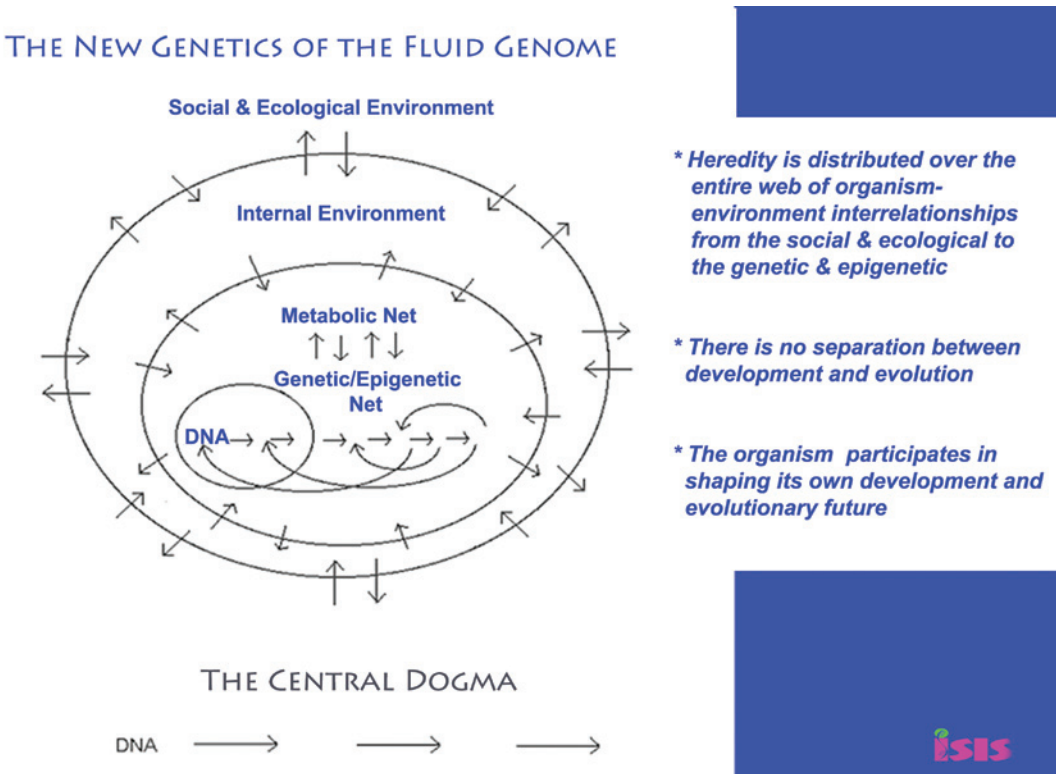

Figure 4 The new genetics of the fluid genome versus the old genetics of the central dogma [8]

Box 1
Accumulating evidence on the health hazards of GM food and feed

1. A 2-year lab feeding trial reported in 2012 found rats of both sexes exposed to Roundup and/or Roundup-tolerant maize *not* sprayed with herbicide were 2 to 3 times as likely to die as controls and to develop large tumours, of mammary glands in females and of kidney and skin in males [10] (see [11, 12] (GM Cancer Warning Can No Longer Be Ignored and Excess Cancers and Deaths with GM Feed: the Stats Stand Up, *SiS* 56). In other words, the GMO without the herbicide was also harmful in every respect. Pituitary disease was up more than 2 fold in females and liver and kidney diseases up 1.5 to 2 fold in males on GM maize alone.
2. A Danish farmer found excessive illnesses and deaths in his pigs fed GM soy meal including chronic diarrhoea, birth defects, reproductive problems, bloating, stomach ulcers, weak and smaller piglets, and reduced litter size. These were entirely reversed when he put them on a GM-free diet [13] (GM Soy Linked to Illnesses in Farm Pigs, *SiS* 55).
3. A meta-analysis pooling all available data on 19 feeding trials carried out for 90 days on GM soybean and maize, both glyphosate tolerant and Bt crops representing 83 % of commercialized GMOs, found significant disruption of liver and kidney functions [14, 15] (GM Feed Toxic, New Meta-Analysis Confirms, *SiS* 52).
4. Professor emeritus Don Huber at Purdue University warned of "pathogen new to science" associated with glyphosate tolerant GM crops and livestock fed on them, causing unprecedented deaths and infertility [16, 17] (Emergency! Pathogen New to Science Found in Roundup Ready GM Crops and Scientist Defends Claim of New Pathogen Linked to GM Crops, *SiS* 50).
5. Between 2005 and 2006, senior scientist Irina Ermakova at the Russian Academy of Sciences reported that female rats fed glyphosate-tolerant GM soybeans produced excessive numbers of severely stunted pups and more than half of the litter dying within three weeks, while the surviving pups were completely sterile [18, 19] (GM Soya Fed Rats: Stunted, Dead, or Sterile, *SiS* 33).
6. Between 2004 and 2005, hundreds of farm workers and cotton handlers in Madhya Pradesh, India, reported allergy symptoms from exposure to Bt cotton containing Cry1Ac or both Cry1Ac and Cry1Ab proteins [20] (More Illnesses Linked to Bt Crops, *SiS* 30).
7. Between 2005 and 2006, thousands of sheep died after grazing on Bt cotton crop residues in four villages in the Warangal district of Andhra Pradesh in India [21] (Mass Deaths in Sheep Grazing on Bt Cotton, *SiS* 30).
8. In 2005, scientists at the Commonwealth Scientific and Industrial Research Organization in Canberra Australia tested a transgenic pea containing a normally harmless protein in bean (alpha-amylase inhibitor 1), and found it caused inflammation in the lungs of mice and provoked sensitivities to other proteins in the diet [22, 23] (Transgenic Pea that Made Mice Ill, *SiS* 29).
9. From 2002 to 2005, scientists at the Universities of Urbino, Perugia and Pavia in Italy published reports indicating that GM-soya fed to young mice affected cells in the pancreas, liver and testes [24-28].
10. In 2003, villagers in the south of the Philippines suffered mysterious illnesses when a Monsanto Bt maize hybrid containing Cry1Ab protein came into flower; antibodies to the Cry1Ab protein were found in the villagers, there have been at least five unexplained deaths and some remain ill to this day [29] (GM Ban Long Overdue, *SiS* 29).
11. In 2004, Monsanto's secret research dossier showed that rats fed MON863 GM maize containing Cry3Bb protein developed serious kidney and blood abnormalities [30].
12. Between 2001 and 2002, a dozen cows died in Hesse Germany after eating Syngenta GM maize Bt176 containing Cry1Ab/Cry1Ac plus glufosinate-tolerance; and more in the herd had to be slaughtered from illnesses [31] (Cows Ate GM Maize & Died, *SiS* 21). In 2012, biotech giant Syngenta was criminally charged with denying knowledge it had since 1996 that its GM maize kills livestock during a civil court case brought by the farmer that ended in 2007 [32] (Syngenta Charged for Covering up Livestock Deaths from GM Corn, *SiS* 55) (see Figure 5).
13. In 1998, senior scientist Arpad Pusztai and colleagues formerly of the Rowett Institute in Scotland reported damage in every organ system of young rats fed GM potatoes containing snowdrop lectin, including a stomach lining twice as thick as controls [33].
14. Also in 1998, scientists in Egypt found similar effects in the gut of mice fed Bt potato containing a Cry1A protein [34].
15. In 2002, Aventis company (later Bayer Cropscience) submitted data to UK regulators showing that chickens fed glufosinate-tolerant GM maize Chardon LL were twice as likely to die compared with controls [35] (Animals Avoid GM Food, for Good Reasons, *SiS* 21).

different strains of the soil bacteria *Bacillus thuringiensis* expressed in many GM crops, (reviewed in Chapter 2), while the multiple toxicities and carcinogenicity of glyphosate herbicides, heavily used with glyphosate tolerant GM crops, are no longer in doubt (reviewed in Chapter 1). There remains a range of hazards that are not so easily identified, even though evidence exists for most, if not all of them in the scientific literature. These are due to the unpredictability and uncontrollable nature of the genetic modification process itself (Table 1, category 1), which can activate or inactivate genes, scramble genomes, create new proteins, new nucleic acids, new metabolites, and others due to the transgenic DNA and its instability (Table 1 category 3), of horizontal gene transfer - the direct transfer of DNA into the genomes of cells - from the GMO to all other species interacting with the GMO.

4. Transgene instability & the illegality of GMOs

Since the 1990s, some of us have raised the possibility of unintended secondary horizontal gene transfer from GMOs released into the environment with detailed reviews and reports, many of which were sent to our regulators [37-40] (Gene Technology and Gene Ecology of Infectious Diseases, ISIS scientific publication; Horizontal Gene Transfer - The Hidden Hazards of Genetic Engineering, ISIS/TWN report; GM DNA Does Jump Species, *SiS* 47; Scientists Discover New Route for GM-gene 'Escape', *SiS* 50). At first the regulators and GM proponents denied that horizontal gene transfer could happen at all, or the probability is so tiny as to be practically zero [5]. Later, when it became clear from molecular genetic analyses that rampant horizontal gene transfer has taken place in the course of evolution and in recent times, they said horizontal gene transfer is a natural process, and therefore no need to worry; and anti-GM is just anti-science (see for example [41]).

But there is nothing natural about genetic modification done in the laboratory. First, it is nothing if not facilitated, greatly enhanced horizontal gene transfer, but without the precision and finesse of the natural process. It relies on making unnatural GM constructs that are *designed* to cross species barriers and to jump into genomes, and aggressive promoters to force the GMO to express the transgene. Second, genetic modification enables genes to be transferred between species that would *never* have exchanged genes otherwise: thus, DNA from species living on opposite sides of the globe can be recombined; and genetic material from species that have been extinct for tens or hundreds of thousand years can nevertheless be transferred to living species. Third GM constructs tend to be unstable, and hence, more prone to further horizontal gene transfer

This latest incident in a German farm raises tough questions for our government's scientific advisors who have persisted in ignoring scientific evidence that GM food is far from safe.

Dr. Mae-Wan Ho and Sam Burcher call for a public enquiry.

Cows Ate GM Maize and Died

GM maize and dead cows

Twelve diary cows died after being fed GM maize and silage. This happened on a farm in Woelfersheim in the state of Hesse, Germany.

According to the report by Greenpeace Germany, "common errors in feeding and infections had by and large been ruled out as the cause of death", and the farmer involved, Gottfried Glöckner, a supporter of GM crops, now suspects that Syngenta's GM maize Bt 176 is to be blamed.

Bt 176 contains multiple complex traits, including insect resistance - conferred by a toxin from the soil bacterium *Bacillus thuringiensis* - and tolerance to the herbicide glufosinate. It was produced initially by the company Ciba-Giegy in 1994, and acquired subsequently by biotech giant Syngenta.

Glöckner has been growing Bt 176 increasingly in his fields since 1997, and in 2000 and 2001, switched over entirely to GM maize. Shortly thereafter, five of his cows died within four months in 2001, and another seven in 2002. The rate of milk production decreased in some of the remaining cows and others had to be slaughtered because of unknown illnesses.

Syngenta obtained a European license to market GM maize Bt 176 in 1997 and is currently growing 20 000 hectares commercially in Spain. The US license for the crop expired in 2001 and was not renewed. Austria, Luxembourg and Italy have banned its cultivation.

In Germany, safety concerns were raised in early 2000, causing the German Robert Koch Institute to announce "the suspension of the authorisation for putting the maize line 00256-176 and its derivatives on the market, unless [illegible] research or trial pu[illegible]

In Novembe[illegible] reported the den[illegible] Robert Koch Inst[illegible] were regulating th[illegible] of Syngenta Corp[illegible] was awarded com[illegible] euros by Syngenta[illegible] February 2002 he[illegible] ing his cattle GM [illegible] by October 2002 [illegible] had died. The distr[illegible] this time was over[illegible] pocket, called upo[illegible] Robert Koch Ins[illegible] proper investigatio[illegible]

Cause of death u[illegible]

The Robert Koch [illegible] neither the dead c[illegible] from the farm and [illegible] from the [illegible] tigations [illegible] eed from [illegible] 002 with [illegible] to what [illegible] This was [illegible] ouncil in [illegible] ment in [illegible] the inci[illegible] e deter[illegible] ad cows [illegible] athology [illegible] al tissue [illegible] versity of [illegible] ed in un[illegible] ording to [illegible] nding [illegible] sed over

another Syngenta GM maize, Bt 11, destined for human consumption in 2004, if approved by the European Council of Ministers, because it contains the same protein that according to Syngenta, was in the Bt 176 maize fed to the German cows.

Despite the UK Food Standards Agency's recommendations to the Standing Committee of the Foodchain and Animal Health in December 2003 that GM maize Bt 11 is safe for human consumption, the five-year old *de-facto* moratorium remains in place in Europe, thanks to other member-countries who voted against approving Bt 11.

However, the approval process for Bt 11 as food is being processed under the Novel Food Regulation, which is not as strict as the new GM Food and Feed Regulation. The new legislation provides for approval under the old rules, if the application received a final scientific assessment before the new rules apply, as in the case of Bt 11. Nevertheless, Bt 11, if approved, will be subject to the new labelling and traceability legislation. Indeed Bt 11 sweet corn will fail to meet new EU food safety criteria, which clearly state that short term and long term effects of food safety on future generations must be taken into account, according to Article 14 (4) of EC Regulation 178/2002 (the general legislation on food law and food safety, not the Novel Food Regulation).

"Poison protein" in Bt maize?

A chief suspect for the death of the cows in Hesse is the Bt protein contained in Bt 176, which Syngenta says is Cry1Ab, the same as in Bt 11.

Studies conducted in Japan in 2003 clearly showed that undigested Bt toxin Cry1Ab is present in calf stomach, intestine and dung after being fed Bt 11

Figure 5 Cows ate Syngenta's GM maize and died; Syngenta criminally charged for covering up livestock deaths since 1996

Table 1 Hazards of GMOs

1. Uncontrollable, unpredictable impacts on safety due to the genetic modification process*
   - Scrambling the host genome*
   - Widespread mutations*
   - Inactivating genes*
   - Activating genes*
   - Creating new transcripts (RNAs) including those with regultory functions*
   - Creating new proteins*
   - Creating new metabolites or increasing metabolite to toxic levels*
   - Activating dormant viruses*
   - Creating new viruses by recombination of viral genes in GM insert with those in the host genome*
2. Toxicity of transgene protein(s) introduced (intentionally or otherwise)
   - Transgene protein toxic*
   - Transgene protein allergenic or immunogenic*
   - Trangenic protein becoming allergenic or immunogenic due to processing*
   - Unintended protein created by sequence inserted may be toxic or immunogenic
3. Effects due to the GM insert and its instability*
   - Genetic rearrangement with further unpredictable effects*
   - Horizontal gene transfer and recombination*
     - Spreading antibiotic and drug resistance*
     - Creating new viruses and bacteria that cause diseases
     - Creating mutations in genomes of cells to which the GM insert integrate including those associated with cancer*
4. Toxicity of herbicides used with herbicide tolerant GM crops*

*Documented in scientific literature

Transgenes tend to be integrated into gene-rich regions

after it has integrated into the genome. This has been documented in a study comparing herbicide tolerance in a transgene with the same trait from a mutation [42]. The transgene was up to 30 times more likely to escape and spread to neighbouring plants, and the most likely reason is via horizontal gene transfer.

The clearest evidence for the instability of GM constructs is transgene instability, the tendency for the transgenes not just to become silenced in transgenic lines, but to rearrange or become lost in successive generation. Transgene instability is an open secret buried under the permissive regulatory carpet.

In 2003, independent scientists characterized the GM insert in all the commercially approved transgenic lines in Europe, and found every one of them had undergone rearrangement [43] (see [44] Transgenic Lines Proven Unstable, *SiS* 20). According to European Directive 2001/18/EC, that would make them illegal, as they were not the 'event-specific' lines originally characterised and approved for commercial release [45] (Unstable Transgenic Lines Illegal, *SiS* 21). Event-specific characterisation and risk assessment is important because the transgenic process is utterly uncontrollable and unpredictable. The properties of the transgenic line depend entirely on where and in what form the transgenic DNA has landed and the collateral damage done to the genome in the event. Consequently, even-specific characterization is essential as well *as data confirming that the transgenic line derived from the event is genetically stable.*

Transgene instability makes a mockery of the risk assessment process, because any change in transgene expression, or worse, rearrangement or movement of the transgenic DNA insert(s) would create another transgenic plant different from the one that was

characterized and risk assessed. And it matters little how thoroughly the original characterization and risk assessment may have been done. I raised the issue with the European authority, to no avail. Later analyses of one of the lines indicated further rearrangements have taken place [46, 47] (see [48, 49] MON810 Genome Rearranged Again, Transgenic Lines Unstable hence Illegal and Ineligible for Protection, *SiS* 38). MON810 was analysed again a few year later and confirmed to have a different insertion site as well as new mRNA transcripts representing fusion proteins between cry1A transgene and host genome sequences, adding 2 or 18 amino acids to the Cry1A protein [50].

The legislature should take note: unstable transgenic lines are illegal. Not only should they not be still growing commercially, they are also strictly ineligible for patent protection. According to a review published in 2004, the loss of transgenes during reproduction occurs at a frequency of 10 to 50 % of transgenic plants, regardless of how they are produced [51]. Transgene instability appears to depend on the nature; of the transgene, the host genome, and the site of integration, and not on the transformation method. There may be

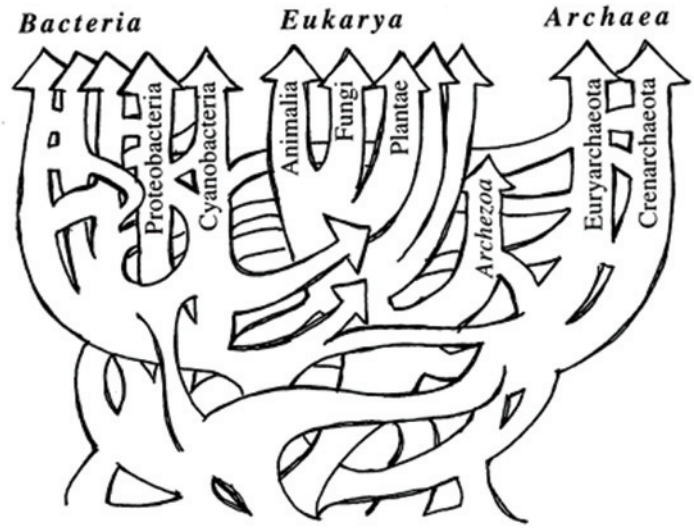

Figure 6 Horizontal gene transfer connects all kingdoms in evolution, Doolittle, 1999.

These ' hotspots' for integration may be sites that tend to be exposed and break more often, and hence also hotspots for disintegration.

integration hotspots in the genome that are inevitably also disintegration hotspots, as revealed by experiments in 'gene therapy' [52] (Gene Therapy Risks Exposed, *SiS* 19) in human cells, and confirmed in large scale analysis of transgenic integration in plants [53] and in the common carp [54].

In plants, transgene integration sites resulting from all transformation systems (except for homologous recombination) exhibit short sequence homologies between the integrated transgenic DNA and flanking genomic sequences of 1 to 8 bp, and between the rearranged transgene fragments [53]. Transgenes tend to be integrated into gene-rich regions, and reduced in the centromere regions of chromosomes. They also show propensity for AT-rich regions and at transitions between normal base composition to a poly-T or A-rich region. These ' hotspots' for integration may be sites that tend to be exposed and break more often, and hence also hotspots for disintegration. Another reason for transgenic instability is the genetic modification process itself, which may destabilise the genome by causing genome scrambling and chromosomal abnormalities.

Transgene instability is now widely reported in the scientific literature, and some examples are given below.

Apple cultivars were transformed using *Agrobacterium* vector to increase resistance to diseases like powdery mildew, apple scab and fire blight [55]. A total of 64 plants of 15 different transgenic apple lines were transferred to the greenhouse, half of them grown as own rooted trees, and half grafted in different non-transgenic scion-rootstock. When tested after an unspecified time, 22 of the plants (34 %) lost one or both genes. In the rest, four plants did not express the antibiotic marker gene, one had lost its promoter and in other three, the promoter was silenced (not functional). In a second experiment, 26 lines carrying the attacin E gene from *Hyalophora cecropia*, the β-glucuronidase (*gus*) gene and the *nptII* gene were propagated vegetatively *in vitro* without selective agents for 4 years (50 generations) and then analysed [56]. Neither expression nor integration remained stable in some lines, differences were found between plants of a single line and several plants were chimeras of expressing and non-expressing cell clones. For example, twenty-three lines kept all three genes (at least in some of the plants). One line lost *gusA* and two lines lost all genes. Low levels of *nptII* expression were found in 12 lines, increased expression in 10 lines and only two had the same level of protein expression. Stable expression of *gus* was found in eight lines, though some plants were mosaics of cells that expressed the gene and cells that did not, Two lines had no activity at all, even though one had the gene.

Researchers in Brazil identified a remarkable systematic elimination of transgenes [57]. A bean (*Phaseolus vulgaris* L.) line was obtained by particle bombardment with plasmid pMD4 containing the *gus* gene and the *rep-trap-ren* genes from bean golden mosaic geminiviru, both under the control of the CaMV (cauliflower mosaic virus) 35S promoter, to make it immune to the golden mosaic geminivirus. A soybean line was transformed with another plasmid pAG1 that contains a different combination of genes: the *gus* gene under the control of the *act2* promoter and the *ahas* (acetohydroxyacid synthase) gene under the control of its promoter from *Arabidopsis thaliana*. In both, the transgenes were stable during the vegetative phase, but were eliminated during meiosis, the cell division that makes germ cells.

The transgenic bean line contains at least 3 copies of the transgenes integrated at three separate loci (sites). None of the copies were transferred to the progeny by self-crossing or crosses to untransformed plants. Not a single progeny plant inherited any transgene. This phenomenon was systematically repeated for over two years in plants propagated by grafting (20 progenies of more than 300 plants from self-pollination, and 10 progenies of more than 100 plants from crosses to untransformed plants).

Analysis of the host genome flanking the transgene inserts revealed that one integrated plasmid disrupted a ribosomal RNA gene while another was integrated into a sequence with no significant homology to known sequences. The third integrated sequence could not be isolated because it lacked the necessary plasmid sequence.

The same phenomenon occurred in the soybean transgenic line.

The instability of the transgenic insert implies that it can jump out and insert at another place in the same genome, or it can transfer horizontally to the genome of cells in all organisms exposed to the GMO.

There is no doubt that transgenic DNA is far from natural for reasons already given, and all the indications are that it can spread more easily (see Box 2, adapted from [7]). I shall deal more substantially with horizontal gene transfer and specific elements of the GM constructs, the CaMV 35S promoter and *Agrobacterium* T-DNA vector in later sections.

6. Horizontal gene transfer from GMOs does happen

There is plenty of evidence that transgenic DNA can transfer from plant to bacteria in the laboratory, as noted by the European Food

Box 2
Transgenic DNA more likely to spread horizontally

1. Transgenic DNA is designed to jump into genomes, often through viral or bacterial plasmid vectors that can integrate into genomes.
2. Transgenic DNA tends to be structurally unstable and hence prone to break and join, giving rise to numerous deletions, duplications, and other rearrangements during the transformation process, which spread into the host genome; and this is in part responsible for the instability of transgenic varieties [46-57] (see main text).
3. The mechanisms that enable transgenic constructs to jump into the genome enable them to jump out again and reinsert at a different site or into another genome.
4. The borders of the most commonly used vector for transgenic plants, the T-DNA of *Agrobacterium*, are recombination hotspots (sites that tend to break and join). In addition, a recombination hotspot is also associated with the CaMV 35S promoter and many terminators, which mean that the whole or parts of the integrated transgenic DNA will have an increased propensity for secondary horizontal gene transfer and recombination (see later in main text).
5. The *Agrobacterium* vector and the bacteria remaining in transgenic plants is a vehicle for gene escape and can transfer genes into many bacteria as well as into human cells (see main text).
6. Transgenic constructs tend to integrate at recombination hotspots in the genome, which again, would increase the chances that they will *dis*integrate and transfer horizontally [52-54].
7. Transgenic DNA often has other genetic signals, such as the *origin of replication* left over from the plasmid vector. These are also recombination hotspots, and in addition, can enable the transgenic DNA to be replicated independently as a plasmid that is readily transferred horizontally among bacteria and other cells.
8. The metabolic stress on the host organism due to the continuous over-expression of transgenes linked to aggressive promoters such as the CaMV 35S promoter will increase the instability of the transgenic DNA, thereby facilitating horizontal gene transfer
9. Transgenic DNA is typically a mosaic of DNA sequences copied from many different species and their genetic parasites; these homologies mean that it will be more prone to recombine with, and successfully transfer to the genomes of many species and their genetic parasites. Homologous recombination typically occurs at one thousand to one million times the frequency of non-homologous recombination, and short homologous sequences could act as anchors for acquiring non-homologous sequences (see main text).

Safety Authority (EFSA) only relatively recently [58], just as it is well-known that transgenic DNA can persist in debris and residues in the soil long after the GM crops have been cultivated. But EFSA, like other regulatory agencies, has persistently denied that horizontal transfer of transgenic DNA can happen in the field or anywhere outside the laboratory. Even though phylogenetic studies have documented rampant cross-kingdom horizontal gene transfers in the course of evolution (Figure 6).

Geneticists at the University of Oldenburg in Germany demonstrated that while horizontal transfer of similar sequences (by homologous recombination) is the rule in bacteria, the horizontal transfer of *non*-homologous DNA also occurs at relatively high frequencies when a homologous DNA 'anchor sequence' is present, which can be as short as 99bp [59]. This certainly applies to transgenic DNA, which consists of viral, bacterial and other sequences cobbled together. In a review published in 2004, at least 87 species of naturally transformable bacteria was listed [60] – these are bacteria that can take up and integrate foreign DNA into their genome - representing 2 % of all known species. The authors pointed out that transgenic DNA can spread not only via the roots and plant debris, but also via pollen drift into fields that had never cultivated GM crops. They even developed a bio-monitoring technique for detecting transgenic DNA based on transformation of a competent strain of bacteria that depends on double cross-over (breaking and joining) event between the transgenic DNA and the bacterial chromosome, a theoretically much rarer event than a single cross-over. Nevertheless, the bio-monitoring technique is at least as sensitive as a routine polymerase chain reaction (PCR) for detecting minute amounts of specific DNA, *indicating that horizontal transfer of transgenic DNA is not a rare event*. This conflicts with the irrational conclusion in the same review that [60], "each of the many steps involved from the release of intact DNA from a plant cell to integration into a prokaryotic genome has such a low probability that a successful transfer event [is] extremely rare."

Researchers at Cardiff University in the UK have confirmed that horizontal transfer of transgenic DNA occurs at readily detectable levels using a similar system [61]. In sterile soil microcosms, transformation was detected using pure plant DNA at 3.6×10^{-8} and in ground up leaves at 2.5×10^{-11} transformant per recipient; for non-sterile soil using pure plant DNA, the frequency was 5.5×10^{-11} transformant per recipient.

However, it is very likely that transformation frequencies are routinely underestimated, as the overwhelming majority of natural bacteria cannot be cultured in the lab. Using methods that restore function to a green fluorescent protein transgene so the transformed bacteria can be seen without the need to culture and select for them, researchers were able to detect transfer of plant DNA to bacteria directly on the surface of intact leaves as well as on rotting, damaged leaves [62, 63]. Rotting and damaged leaves release nutrients that promote bacterial growth, and bacteria that can take up foreign DNA are at their most receptive (competent) state for horizontal gene transfer during exponential growth, thus 'opportunistic' hotspots for transfer of plant DNA to bacteria are present in plant material infected with pathogens. The experiment amply confirms that horizontal gene transfer in the field happens at much higher frequencies than previously supposed.

Antibiotic marker genes are routinely used to make GMOs as they offer a convenient way of selecting for cells that have taken up the GM DNA. A major concern is the spread of these antibiotic resistance genes by horizontal gene transfer to bacterial pathogens, making infections untreatable. However, there has been few if any proper studies that monitor the spread of antibiotic resistance genes from GM crops released into the environment until recently. A study in China found the antibiotic resistance marker gene, *blá*, for ampicillin resistance in all 6 of China's major rivers [64] (see [65] GM Antibiotic Resistance in China's Rivers. *SiS* 57); sequencing confirmed that the gene is a synthetic version derived from a lab and different from the wild type. It is the same as the version present in numerous GM crops released in China commercially or in field trials. The *blá* gene confers resistance to the most common class of antibiotics called β-lactams, which includes besides ampicillin (a □-lactam), the penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems. The researchers suggested that horizontal gene transfer of genetically engineered plasmids to microbes in the soil or from lactic acid bacteria to human and animal gut microbes is a likely consequence of the pollution of river water, and may underlie the rise in antibiotic resistance in animals as well as humans. This study again provides clear evidence that horizontal gene transfer from GMOs does happen, and very readily so.

Can GM DNA transfer to cells of animals feeding on the GMO? Several studies have documented the survival of DNA in food/feed throughout the intestinal tract in mice and pigs [66, 67 and references therein], in the rumen of sheep [68], and in the rumen and duodenum of cattle [69]. There is also evidence dating from the early 1990s that ingested DNA in food and feed can pass through the intestinal wall and enter the bloodstream (reviewed in [70] (DNA in

GM Food & Feed, *SiS* 23).

In the only feeding trial on human volunteers [71], a single meal was given in a milk shake containing GM soya flour with about 3 x 10^{12} copies of the soya genome. The complete 2 266 bp of the *epsps* transgene was recovered from the colostomy bag in six out of seven ileostomy subjects, at levels ranging from a high of 10^{11} copies (3.7 %) in one subject to 10^{5} copies in another. This is evidence that DNA is not rapidly broken down in the gastrointestinal tract, confirming earlier results from the same research group. Further, in three of the seven ileostomy subjects, about 1 to 3 per million bacteria cultured from the contents of the colostomy bag were positive for the GM soya transgene, showing that *horizontal transfer of transgenic DNA had occurred*, either before the single meal was taken, as claimed, or else as the result of the single GM soya meal, a possibility that cannot be ruled out [70]. Interestingly, no bacteria were found to have taken up non-transgenic soya DNA, despite the fact that non-transgenic soya DNA is vastly more abundant than the transgenic DNA, and humans have been exposed to non-transgenic soya DNA for millennia. *This is the clearest indication that transgenic DNA is much more readily transferred* for reasons given in Box 2.

The transfer of transgenic DNA demonstrated in the single human trial is just the tip of the iceberg, it shows how readily transgenic DNA, including antibiotic resistance genes, can transfer to bacteria, especially in the gastrointestinal tract. The gastrointestinal tract is a hotspot for horizontal gene transfer as successive reviews have made clear [72, 73].

Gene transfer from a GM probiotic in the bird gut is much higher than the rates observed by culturing the bacteria on a petri-dish [72], basically because the latter method depends on the diversity of bacteria being able to grow in culture at the same time. Besides, anaerobic bacteria make up 99 % of human gut flora, and these will not grow at all in ordinary culture. Organisms residing in the gastrointestinal tract are thought to be reservoirs of antibiotic resistance and virulence genes. Studies using simulated ileum of the pig gut provided clear evidence that antibiotic resistance could be transmitted between resident and pathogenic members of the Enterobacteriaceae passing through the gut [73].

Gene transfer in the colon has been found in Bacteriodes species. Frequently, the environment of the gut is exposed to low levels of antibiotics used as therapeutic agents, growth promoters, or as contaminants in food. Antibiotics have been shown to stimulate the transfer of mobile genetic elements such as conjugative transposons (jumping genes involved in conjugation, a process whereby bacteria exchange genes via cell contact) and genomes of bacterial viruses which also reside in the bacterial genome. The mouse gut enabled a shiga toxin 1(Stx1)-encoding phage (bacteria virus) to be transmitted between two *E. coli* strains and produce infectious virions capable of infecting yet other *E. coli* strains in the gut.

The rumen is the first 'stomach' of cattle, sheep and goats, where high-fibre plant materials are digested by a microbes, both prokaryotes and eukaryotes, providing a great opportunity for horizontal gene transfer [73]. Transfer of antibiotic resistance in the rumen was first documented in sheep in the 1970s, and since then indirect evidence has mounted for rumen transfer events, with the protozoa in the rumen playing an important role in facilitating gene transfer between bacteria inhabiting the rumen [74].

We have reported [36] on how free DNA survives for a considerable period of time in saliva and was able to transfer to *Streptococcus gordonii*, a natural inhabitant of the mouth; so horizontal gene transfer is likely to start right away in the mouth [72].

All the more so, as foods such as ultra-heat treated milk, cacao drink and tomato juice were found to support horizontal gene transfer when external DNA was added along with bacteria [75]. The highest transformation frequencies of *E. coli* occurred in milk, soy drink, tomato and orange juice, and DNA was released and taken up by *E. coli* under food processing conditions.

Evidence is emerging that genomes of higher plants and animals may be softer targets for horizontal gene transfer than genomes of bacteria. We have been warning of this possibility at least since 2001, when experiments in 'gene therapy'- making transgenic human cells - were demonstrating how easy it is for transgenic constructs to be taken up by human and animal cells [76] (SLIPPING THROUGH THE REGULATORY NET: 'Naked' and 'free' nucleic acids, ISIS/TWN report). Similarly, information from transgenic *Arabidopsis* and rice, with sequenced genomes, and the huge amounts of relic viruses, transposons, retroelements, and chloroplast and mitochondrial DNAs found in these and other sequenced genomes are persuading geneticists that [51]: "nuclear genomes of plants, like those of other eukaryotes, are promiscuous in integrating nonhomologous DNA." We have spelt out what such consequences could be [76]: insertion mutations including cancer, activation of dormant viruses, and recombination with viral sequences in the genome to generate new viruses; all of which have been demonstrated in gene therapy experiments. Further evidence will be described at the end of this chapter.

In the only feeding trial on human volunteers, the complete 2 266 bp of the epsps transgene was recovered from the colostomy bag in six out of seven ileostomy subjects. Further, In three of the seven ileostomy subjects, about 1 to 3 per million bacteria cultured from the contents of the colostomy bag were positive for the GM soya transgene, showing that horizontal transfer of transgenic DNA had occurred

7. Hazards of the CaMV 35S promoter

A scientist from EFSA (European Food Safety Authority) belatedly discovered that major GM crops and products the regulatory agency has been approving for commercial release over the past 20 years contain a potentially dangerous virus gene. The gene – Gene VI - overlaps with the CaMV 35S promoter, the most widely used for driving gene expression in GM crops. This momentous discovery was published [77] in a little known journal. It would have passed unnoticed had it not caught the attention of Jonathan Latham and Alison Wilson of *Independent Science News*, who carried out a proper retrospective risk assessment on the Gene VI fragment, showing that the gene product is toxic to plants probably through, among other things, the inhibition of gene silencing, a necessary function universal to plants and animals (see later); hence it is also likely to be toxic to animals including humans (see [78, 79] Hazardous Virus Gene Discovered in GM Crops after 20 Years and Potentially Dangerous Virus Gene Hidden in Commercial GM Crops, *SiS* 57). They called for all GM crops containing CaMV 35S and similar viral promoters to be withdrawn.

This is not the first time that the safety of CaMV 35S promoter is being questioned.

ISIS first raised concerns over the CaMV 35S and similar promoters in a paper published in the journal *Microbial Ecology in Health and Disease* in 1999 [80] (Cauliflower Mosaic Viral Promoter - A Recipe for Disaster?) when the promoter was discovered to have a recombination (fragmentation) hotspot. We argued this would enhance unintended horizontal gene transfer and recombination, and in the process create new viruses or activate old ones, and trigger cancer in animal cells by 'insertion carcinogenesis'. The CaMV 35S promoter was known to be highly promiscuous in being able to function in most if not all species across the living world (including human cells, as it turned out). To make matters worse, many synthetic versions of the promoter have been constructed with additional enhancers for gene expression and sequences from other sources, all of which increase its instability (tendency to fragment) as well as its ability to drive inappropriate gene expression. (We also reported the overlap of the 35S promoter with Gene VI, so this knowledge must have been widely known, although its safety implications were not obvious, at least to us.)

As a precautionary measure, we strongly recommended that all transgenic crops containing CaMV 35S or similar promoters should be immediately withdrawn from commercial production or open field trials.

Our first paper brought a swift reaction. Within two days of its being published online, someone managed to solicit at least nine critiques, including one from Monsanto, which were posted on a website funded by the biotech industry and widely circulated on the internet. The critiques varied in tone from moderately polite to outright abusive. We wrote a detailed rebuttal, which was likewise circulated and posted to the same website, and have not received any replies from our critics since. But in January 2000, *Nature Biotechnology* published a distorted, one-sided and offensive account of our paper, concentrating on the criticisms and ignoring our rebuttal completely, which we published in the same journal that carried the first paper[81] (Hazards of Transgenic Plants Containing the Cauliflower Mosaic Viral Promoter).

It is significant but sad that regulators have denied any risk posed by gene VI in exactly the same way they argued against the dangers posed by the recombination hotspot in the CaMV 35S promoter. The first objection is that humans have been eating the CaMV for millennia without ill effects (if indeed people ate virus-infected vegetables); the second is that the CaMV 35S promoter is only active in plants and certainly not in animal or human cells.

Our rebuttal to the first objection is that the intact CaMV, consisting of the CaMV genome wrapped in its protein coat, even if eaten, is not infectious for human beings or for other non-susceptible animals and plants, as is well-known; for it is the coat that determines host susceptibility in the first instance. However, the naked or free viral genomes (and parts thereof) are known to be more infectious and have a wider host-range than the intact virus. Furthermore, the synthetic CaMV 35S promoters are very different from the natural promoters, often with extra sequences added, and are both much more aggressive as promoters driving inappropriate gene expression as well as more prone to fragment and recombine.

The second objection - that CaMV 35S is not active in animals and human cells - is simply false as we discovered in the scientific literature dating back to 1989, and pointed this out in a third report [82] (CaMV 35S promoter fragmentation hotspot confirmed, and it is active in animals). The CaMV 35S promoter supported high levels of reporter gene expression in mature *Xenopus* oocytes [83] and gave very efficient transcription in extracts of nuclei from HeLa cells (a human cell line) [84].

What of our original concern over the CaMV 35S promoter activating viruses in host genomes? There is new evidence suggesting that the CaMV 35S promoter may enhance the multiplication of disease-associated viruses including HIV and cytomegalovirus through the induction of proteins required for transcription of the viruses [85] (New Evidence Links CaMV 35S Promoter to HIV Transcription, ISIS scientific publication).

These known hazards of the CaMV 35S promoter are in addition to those due to gene VI; which fully justifies our original recommendation for a total recall of the affected GM crops.

8. Hazards of *Agrobacterium* vector

In 2010, scientists at Bristol University in the UK announced the [86] "discovery of a previously unknown route" whereby "GM genes may escape into the natural environment."

The "escape" referred to is horizontal gene transfer. The researchers showed that plant wounds that could be created by insect bites, abrasion and other mechanical damage, are hotspots for gene trafficking due to the wound hormones produced by the plant. Under such circumstances, the soil bacterium *Agrobacterium tumefaciens*, which causes crown gall disease in plants, could expand its host range to infect fungi, and insert foreign genes into the fungi's genome [87]. This has large implications on the safety of GMOs already widely released into the environment, according to the authors. It turns out that their discovery is nothing new.

A. tumefaciens causes crown-gall disease in plants, a tumour-like growth or gall on the infected plant, accompanied by the transfer of a DNA segment (T-DNA) from the tumour-inducing (Ti) plasmid of the bacterium. It is probably unique among natural plant pathogens in carrying out trans-kingdom horizontal gene transfer during an infection. And it is this ability that has been widely exploited for creating GM crops (Figure 7). But this was a big mistake, as I shall explain.

In the 1990s, it was shown that the range of organisms transformed by *Agrobacterium* could be extended if the wound hormone acetosyringone was used to induce the virulence (disease causing) system.

The researchers at Bristol University reasoned that as *A. tumefaciens* is a soil-dwelling pathogen that often infects plants through wounds, it is conceivable that the bacterium could encounter numerous species of microorganisms, including pathogenic fungi that use the same method to gain entry into the plant. The wound sites are likely to be exuding wound hormones such as acetosyringone, so the bacteria are primed for T-DNA transfer to the other species.

For their investigations, they used the wilt-causing fungus *Verticillium albo-atrum*, a strong candidate for encounters with *Agrobacterium* in the plant, as it has a similarly wide host range, infecting both root and crown. Previous lab experiments have shown that *V. albo-atrum* cannot be transformed by *Agrobacterium* in the absence of acetosyringone. So, if it is presented with *Agrobacterium* on plant tissue, and transformation does occur, it must be the plant that supplies the wound hormone.

Successful transformants of *V. albo-atrum* were obtained at high frequencies in every kind of plant tissue: 2 out of 17 potato slices, 1 out of 15 carrot slices; 14 out of 42 dishes each with 3-5 leaf pieces, and 10 out of 31 stem sections. The transformants were confirmed by molecular genetic analyses.

The researchers concluded [87]: "This work therefore raises interesting questions about whether the host range of *A. tumefaciens* in nature is greater than just plants. It is possible that evidence of such events could be looked for retrospectively in the increasing number of genome sequences becoming available....

"In addition, the result may well have implications for the risk assessment of GM plants generated via *Agrobacterium*-mediated transformation, as Agrobacterium can survive within plant tissue through transformation and tissue culture and can therefore be found within regenerated transgenic plants..."

This is an understatement of a serious risk that has been known almost since the first release of Agrobacterium-transformed GMOs into the environment. The risks are far greater than stated.

By the late 1990s, the Agrobacterium vector system became very widely used (see Figure 8), and many GM crops created were commercially released. Scientists at the Kinsealy Research and Development Centre in Dublin, Ireland, and the Scottish Crop Research Institute in Dundee, Scotland, were concerned that the inserted genes in plants would spread to wild populations by cross-pollination or by horizontal gene transfer to unrelated species, which was by then well-documented in the scientific literature.

The researchers considered it "imperative" to address the risk

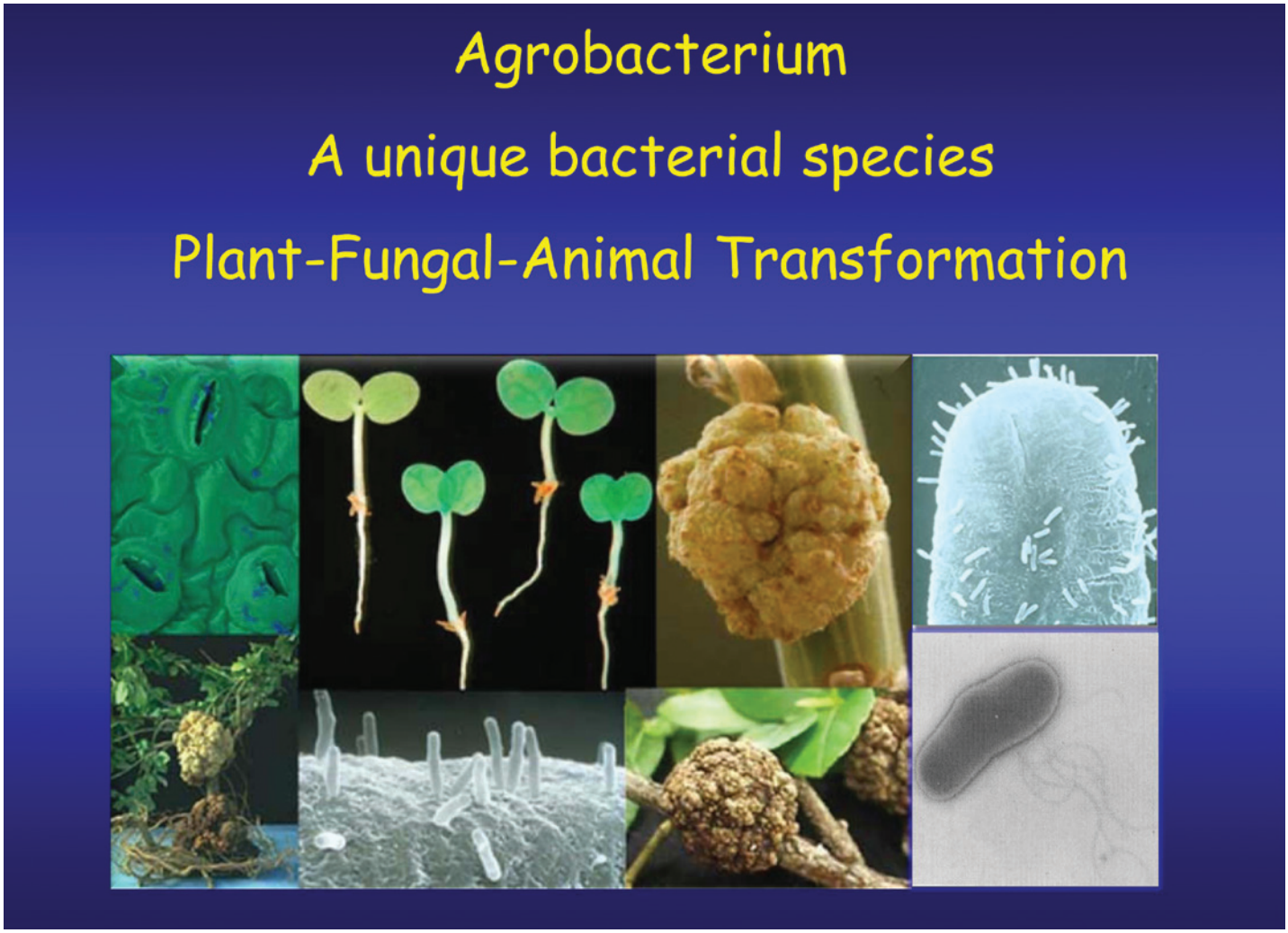

Figure 7 Agrobacterium is a promiscuous trans-kingdom gene transfer vector, from keyhani.ifas.ufl.edu

posed in using *Agrobacterium* as a tool in genetic engineering [88, 89], given its ability to transfer genes to other plants.

The transformation procedure involves inoculating the cells or tissue explants with the *Agrobacterium* vector system (consisting of the genetically modified Agrobacterium and its binary vector) and co-cultivation of the plant cells and bacteria for a short period, followed by the elimination of the bacteria with antibiotics.

However, if all the bacteria were not eliminated, then "release of these plants may also result in release of the *Agrobacterium* [with the foreign genes]", which will serve as a vehicle for further gene escape, at least to other *Agrobacterium* strains naturally present in the soil in the first instance.

Although various antibiotics have been used to eliminate *Agrobacterium* following transformation, the researchers stated that "very few authors actually test to ensure that the antibiotics succeed."

Moreover, the *Agrobacterium* can remain latent within the plant tissue. So putting transgenic plant material into culture medium without antibiotics and finding no *Agrobacterium* is no guarantee that the transgenic plant is free of it, as was often assumed.

In their study, they investigated the ability of antibiotics to eliminate *Agrobacterium tumefaciens* after transformation in three model systems: *Brassica* (mustard), *Solanum* (potato), and *Rubus* (raspberry). The antibiotics carbenicillin, cefataxime and ticaracillin were used respectively to eliminate the bacterium at four times the minimum bactericidal concentration, as recommended. They found that *none* of the antibiotic succeeded in eliminating *Agrobacterium*.

The contamination levels increased from 12 to 16 weeks to such an extent that transgenic *Solanum* cultures senesced and died. Contamination in shoot material decreased over 16 to 24 weeks possibly because only the apical node was used in further culture, but even that did not eliminate *Agrobacterium* from all the samples; 24 % remained contaminated at 24 weeks.

The antibiotics carbenicillin, cefataxime and ticaracillin were used respectively to eliminate the bacterium at four times the minimum bactericidal concentration, as recommended. They found that none of the antibiotic succeeded in eliminating Agrobacterium

The binary vector was also present under non-selective conditions up to 6 months after transformation, where approximately 50 % of contaminated material still harboured bacterial cells with the binary vector at high levels of about 10^7 colony forming units per gram. The researchers pointed out: "Here is where the possibility of gene escape arises. The presence of the disarmed *Agrobacterium* in the tissue would not be a problem if the binary vector had been lost, but now its survival and spread are real possibilities." The binary vector contains the foreign genes as well as antibiotic resistance marker

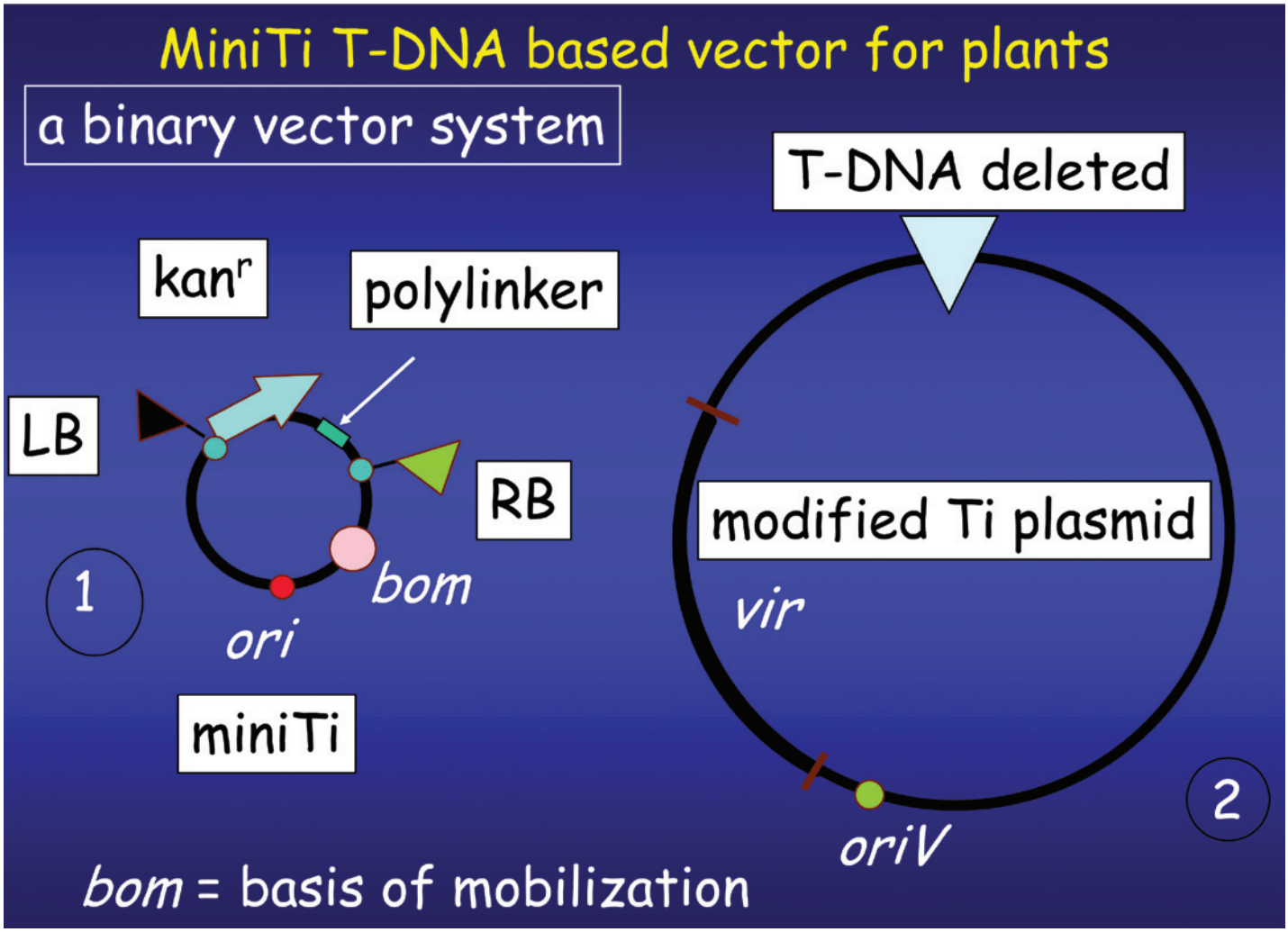

Figure 8 The Agrobacterium binary vector system consisting of the modified Ti *plasmid with the* T-DNA *deleted, but with virulence genes* vir *required for infection and* oriV *for origin of replication), and a mini* Ti *consisting of the right and left borders (*RB *and* LB*) of T-DNA, between which the transgenes are inserted, a kanamycin resistance marker gene* kan^r*, and genetic signals* ori *(origin of replication) for replicating the* mini Ti*, and* bom *(basis of mobilization) required for transfer of the* miniTi *into plant cells, from keyhani.ifas.ufl.edu*

gene(s).

There is no limit to the foreign genes that can be inserted into the binary vector. A few years earlier, a research group in Israel had inserted a viroid that causes disease in citrus fruits into the disarmed Ti plasmid of *Agrobacterium* and used that to infect and transform several plant species including tomato (*Lycopersicon esculentum*) *Gynura aurantiaca*, avocado (*Persea americana*), and grapefruit (*Citrus paradisi*) grafted on Troyer citrange (*Pancirus trifoliate* x *C. sinensis*) [90]. Extracts prepared from tissues of the infected plants 38-90 days after inoculation were plated on selective media and found to contain large amounts of the engineered bacteria.

The researchers warned of "newly formed combinations of persistently transmitted viruses" coupled with "the opportunistic and systemically moving *Agrobacterium* vector infectious to a wide host range might eventually cause infection and damage to crop plants or natural vegetation" that are "not presently visited by the traditional vectors of the virus disease."

In other words, *Agrobacterium* persisting in transgenic plants released into the environment has the potential to spread newly created viral diseases, and to plants that normally would not be infected by the disease agents. At the time, the researchers did not know that *Agrobacterium* would also infect animals and humans, and could spread new diseases to them as well (see below).

Have these warnings been heeded by other researchers? There is no evidence they have been taken on board. *Agrobacterium* has since been shown to transform at least 80 different non-plant species including yeasts and other fungi, algae, mammalian and human cells, also the gram positive bacterium *Streptomyces lividans*. A review published in 2008 stated [91]: "Future research has to show whether *Agrobacterium*-mediated transformation contributed to horizontal gene transfer between microorganisms in the rhizosphere."

We have repeatedly drawn attention to this possibility, most recently in 2011 [40] and before that, in 2010 [39] and in 2008 [92] (Horizontal Gene Transfer from GMOs Does Happen, *SiS* 38); the danger is even greater than envisaged by the early warnings in the 1990s.

In the gene transfer system based on *A. tumefaciens*, foreign genes are spliced into the mini-T-DNA binary that ends up integrated into the genome of the plant cell (see Fig. 8). But further investigations revealed that the process whereby *Agrobacterium* injects T-DNA into plant cells strongly resembles *conjugation*, the natural mating process between bacterial cells; and the crucial genetic signals are interchangeable [93].

Conjugation, mediated by certain bacterial plasmids requires a sequence called the origin of transfer (*oriT*) on the DNA transferred. All the other functions can be supplied from unlinked sources, referred to as 'trans-acting functions' (or *tra*).

It transpired that the left and right borders of the T-DNA are similar to *oriT*, and can be replaced by it. Further, the disarmed T-DNA, lacking the trans-acting functions (*virulence* genes that contribute to disease), can be helped by similar genes belonging to many other pathogenic bacteria.

That means transgenic plants created by the *Agrobacterium* binary vector system have a ready route for horizontal gene escape, via *Agrobacterium*, helped by the ordinary conjugative mechanisms of many other bacteria that cause diseases, which are present in the environment. I first pointed this out [94] in reviewing a book on

horizontal gene transfer which contained all the key information on the similarity between *Agrobacterium*'s gene transfer system and bacterial conjugation, *but still failed to sound the warning*.

Agrobacterium not only transfers genes into plant cells; there is possibility for *retro*transfer of DNA *from* the plant cell *to Agrobacterium* [95]. High rates of gene transfer are associated with the plant root system and the germinating seed, where conjugation is most likely [96].

Finally, *Agrobacterium* attaches to and genetically transforms several human cell lines [97, 98] (Common plant vector injects genes into human cells *ISIS News* 11/12). In stably transformed HeLa cells (a human cell line derived originally from a cancer patient), the integration of *T-DNA* occurred at the right border, exactly as would happen when it is transferred into a plant cell genome. This suggests that *Agrobacterium* transforms human cells by a mechanism similar to that which it uses for transforming plants cells.

It is worth citing Joe Cummin's comment on the scientific paper [98]: "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."

Cummins could have warned of exposure to *Agrobacterium* via ordinary soil, especially those contaminated with genetically modified plant debris and *Agrobacterium*.

The possibility that *Agrobacterium* is a vehicle for horizontal spread of transgenic DNA and the dangers of creating new pathogens remains unresolved to this day.

In 2008, *Agrobacterium* was linked to the outbreak of a strange disease.

8.1 *Agrobacterium* & Morgellons disease

The Centers for Disease Control (CDC) in the United States launched an investigation on 'Morgellons disease' in January 2008 [99, 100] (see [101] Agrobacterium & Morgellons Disease, A GM Connection?, *SiS* 38) after receiving thousands of complaints from people with this bewildering condition described as an unexplained skin condition with a reported range of symptoms including crawling, biting and stinging sensations; granules, threads, fibres, or black speck-like materials on or beneath the skin, and/or skin lesions; in some cases also fatigue, mental confusion, short term memory loss, joint pain, and changes in visions.

Morgellons disease first became known in 2001, when Mary Leitao created a web site describing the illness in her young son, which she named after a 17th century medical study in France describing similar symptoms. Until then, people with Morgellons disease have been diagnosed as cases of "delusional parasitosis", in which the symptoms are deemed entirely imaginary, and lesions allegedly due to self-inflicted wounds.

In a paper [102] published in 2006, researchers from the Morgellons Research Foundation (which no longer exists) identified the states of California, Texas and Florida as having the highest number of cases of Morgellons disease in the United States, but all 50 US states and 15 other nations, including Canada, the UK, Australia, and the Netherlands, have reported cases of the disease. The two main occupational groups reporting symptoms are nurses and teachers, with nurses outnumbering teachers three to one. The risk factor common to both groups was suspected to be a transmitted infectious agent. Contact with soil or waste products appears to be associated with the disease. Cases have been reported in cats and dogs, as well as horses. The list of people registered with Morgellons disease totalled 12 106 worldwide, as recorded by Morgellons Research Foundation on 12 April 2008. CDC's investigation was to be carried out in conjunction with Kaiser Permanente's Northern California Division of Research and the US Armed Forces Institute of Pathology.

What finally prompted CDC to investigate the disease was probably the discovery in January 2007 of *Agrobacterium* DNA in fibres of skin biopsies taken from Morgellons patients. The work was carried out by a team that included Vitaly Citovsky, a professor of molecular and cell biology at Stony Brook University in New York (SUNY), the very scientist who discovered *Agrobacterium* can transfer genes to human cells [97]. The team took scanning electron microscope pictures of the fibres in or extruding from the skin of patients suffering from Morgellons disease, confirming that they are unlike any ordinary natural or synthetic fibres (Figure 9).

The team also analysed patients for *Agrobacterium* DNA. Skin biopsy samples from Morgellons patients were subjected to high-stringency polymerase chain reaction (PCR) tests for genes encoded by the *Agrobacterium* chromosome and for *Agrobacterium* virulence (*vir*) genes and T-DNA on its Ti plasmid. They found that "all Morgellons patients screened to date have tested positive for the presence of *Agrobacterium*, whereas this microorganism has not been detected in any of the samples derived from the control, healthy individuals." Their preliminary conclusion is that "*Agrobacterium* may be involved in the etiology and/or progression" of Morgellons disease.

The unpublished findings, including electron micrographs, were posted on a website in January 2007 that no longer exists. A brief publication in *Journal of Investigative Medicine* reported the finding of *Agrobacterium* genes in two Morgellons patients and the authors including Citovsky explained why they looked for *Agrobacterium* [103]: "Morgellons skin fibers appear to contain cellulose. This observation indicates possible involvement of pathogenic *Agrobacterium*, which is known to produce cellulose fibers at infection sites within host tissues."

We wrote a report on the possible connection between the use of *Agrobacterium* in genetic modification and the widespread release of GM crops contaminated with genetically modified *Agrobacterium* and Morgellons disease [101], which was sent to the CDC. We urged the CDC to "clarify the role of *Agrobacterium* in the aetiology of Morgellons Disease as a matter of urgency."

In 2012, after a long delay, the CDC published its verdict [104]: "No common underlying medical condition or infectious source was identified, similar to more commonly recognized conditions such as delusional infestation." They had done no investigations on the *Agrobacterium* connection; and the list of authors did not include Citovsky or his associates. The case is far from closed.

9. RNA interference and double-stranded RNA

Most commercially grown GM crops are engineered to produce foreign proteins, but new ones are increasingly engineered to produce RNA of a special kind - double-stranded RNA (dsRNA) - that aims to interfere with the expression of a specific gene, usually to silence the gene [105]. The ability of dsRNA to interfere with gene expression was known since the 1980s; and the biochemistry of the phenomenon - referred to as RNA interference (RNAi) – was worked out in the roundworm *Caenorhabditis elegans* in the late 1990s [106]. Since then, the same RNAi pathway has been identified in practically all plant and animal kingdoms, and is part of the organism's defence against foreign nucleic acids including viruses.

DsRNA includes siRNA (short-inhibitory RNA), miRNA (microRNA), shRNA (short hairpin RNA) etc., all intermediates leading to RNA interference of protein synthesis. This can happen at transcription, or at translation. Typically, dsRNA originates from a long RNA molecule with stretches of complementary base sequences that base pair to form a stem ending in a non-base-paired loop. This stem-loop structure is then processed into a shorter dsRNA, and one strand, the guide strand does the job of interfering. It binds to a mRNA (messenger RNA) molecule in the cytoplasm by complementary base-pairing to prevent the mRNA from being translated into protein. Alternatively, the guide strand targets and chemically modifies DNA sequences in the nucleus by adding methyl groups to the DNA, and cause modification of histone proteins associated with the DNA. The nuclear pathway is known to inhibit transcription and to seed the formation of heterochromatin, an inactive, non-transcribed region of chromosomes.

Interestingly, the gene silencing effect of dsRNA can become inherited (either indefinitely, or through two or more generations) in cells and organisms that are not genetically modified, but simply exposed to the dsRNA for a period of time. It can happen via methyl

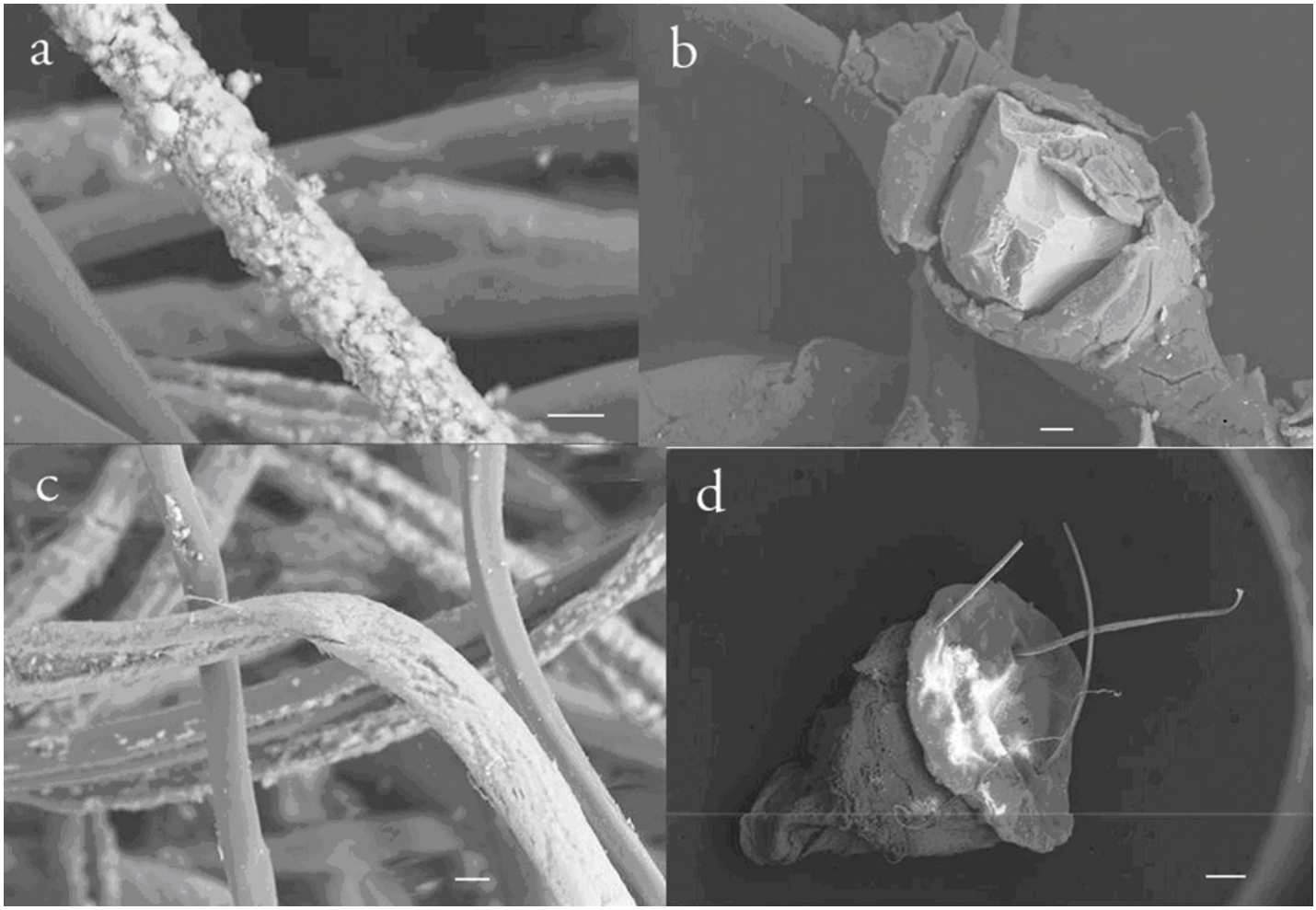

*Figure 9 Scanning electron microscope images of fibres from skin biopsies of patients with Morgellons Disease - **a**, white fibre with calcite, scale bar 10μm; **b**, green fibre with alumina 'rock' protruding, scale bar 20μm; **c**, various ribbon-like, cylindrical and faceted fibres all coated with minerals, scale bar 10 μm; **d**, skin lesion with fibres stabbing through the epidermis, scale bar 300 μm, from [101]*

groups added to the DNA, or the modification of histones [107, 108] or perhaps through RNA-dependent RNA polymerase that can amplify and perpetuate the dsRNA [109] without changing the base sequence of the DNA in the genome. This is another example of the inheritance of acquired characters now known to occur through many different mechanisms (see [110] Epigenetic Inheritance - What Genes Remember and other articles in the series, *SiS* 41) that makes genetic modification all the more hazardous.

DsRNA genetic modification has large implications on safety based on what is already known [111] (New GM Nightmares with RNA, *SiS* 58): dsRNA is stable, it resists digestion and can enter the bloodstream; its role in modifying gene expression is universal and acts across kingdoms; toxicity to animals have been amply demonstrated and exploited in targeting pests; although the intended target is a specific gene, many off-target effects have been identified; finally, plant dsRNA has been found circulating in the human bloodstream where it can be taken up into cells and tissues to interfere with the expression of genes. Consequently, animals including human beings eating the GM food containing dsRNA could well be harmed.

The dangers are real. Researchers in China showed that miRNA from food can circulate in the human blood stream and turn genes off in the human body [112] (see [113] How Food Affects Genes, *SiS* 53). They demonstrated that dsRNAs can survive digestion and be taken up in the circulatory system via the gastrointestinal tract. These plant-derived miRNA silenced a gene in human tissue culture cells, and in mouse liver, small intestine and lung. A survey of existing data of small RNA molecules (conducted by scientists working for Monsanto) from human blood and tissues sources, farm animals and insects confirmed that regulatory RNAs from plants can be found in animals including humans [114]. Thus, new dsRNA species in GM foods may be taken up by the animal cells to silence genes inappropriately.

They found that "all Morgellons patients screened to date have tested positive for the presence of Agrobacterium, whereas this microorganism has not been detected in any of the samples derived from the control, healthy individuals."

The gene-silencing depends on complementary base pairing for short sequences – 21 bases at most, but could be as few as 7 for miRNA - and there could be similar sequences all over the same genome and in genomes of other species. In particular, many miRNA target regulatory sequences of genes that are likely to be common to sets of genes expressed together [113] in certain tissues and cells. Worse yet, the matching need not be precise. Off-target effects are already well-known in gene therapy applications [115]. Over the past decade, investigations have produced a set of 'canonical rules' gov-

erning the interaction between miRNA and their target mRNA, but many exceptions to the rules have also been uncovered.

Recently, researchers used a new technique to capture all the miRNAs bound to their targets by cross-linking them and sequencing the base-paired miRNA-target RNA duplexes. They found that the exceptions far outnumber the rule-based interactions [116] (see [117] RNA Interference "Complex and Flexible", *SiS* 59). Moreover, the results have been obtained in one cell type, human embryonic kidney cells, grown in culture. The report closed with the comment [116]: "More generally, the spectrum of miRNA-mRNA interactions is expected to rapidly change during differentiation and viral infection and following metabolic shifts or environmental insults." In other words, it is well-nigh impossible to predict or control off-target effects, as they vary according to the cells and tissues involved and their precise states.

A worst case scenario of toxic dsRNA came from a gene 'therapy' experiment in mice reported in 2006, which killed more than 150 animals [118] Gene Therapy Nightmare for Mice (*SiS* 31). The technique – hailed as 2002's 'breakthrough of the year' in 'precision' gene therapy - was found to have many off-target effects only a year later [119] Controversy over gene therapy 'breakthrough', *SiS* 26). Researchers were already finding dozens of genes affected by a single siRNA.

Jack Heinemann at the University of Canterbury, Christchurch, in New Zealand conducted a comparison between the DNA sequence of the human genome and a DNA sequence from the wheat SBE1 gene provided to the database Genbank by CSIRO. He found 4 perfect matches of 21 nucleotides and another 13 nucleotide stretch match, within a wheat gene sequence of just 536 nucleotides [120]. And this does not include comparisons of secondary unintended dsRNAs that may be induced in the GM plant, as indeed, in any GMO, including those not explicitly engineered to create dsRNA, nor the many mismatches that can give rise to a plethora of off-target effects under different environmental conditions in different cells and tissues.

How can anyone still think GM food in any form is safe? But this is not the end of the story.

10. The nucleic acids intercom

The conventional view of DNA is that it sits within the nucleus of the cell, somewhat static and metabolically inert, except during replication in dividing cells, or in repair when damaged, for example, by ultraviolet light. RNA, the other nucleic acid, has the job of transmitting the genetic message written in the DNA to the rest of the cell. RNA consists of faithful complementary copies of stretches of DNA that, after extensive processing, are exported outside the nucleus to the cytoplasm, where they form different parts of the necessary machinery to translate the messenger RNAs (mRNAs) into proteins (see Figure 1). And proteins are responsible for all the downstream biological functions. Consequently, knowing the sequence of DNA in the genome will decode the secret of life, will tell you how the organism, the human being, is constructed. That was how the human genome project as well as genetic modification was sold to the public [5].

Well, nothing could be further from the truth even before the human genome was conceived. The most important contribution of the human genome project to the advancement of science is to put the last nail on the coffin of the genetic determinist ideology that made the project seem so compelling ([121] Human Genome Map Spells Death of Genetic Determinism , *isisnews* 7/9). It has conspicuously failed to deliver even promises to identify the genes that predispose us to common diseases and other attributes, let alone how to construct a human being ([122] Ten years of the Human Genome, *SiS* 48; [123, 124] Mystery of Missing Heritability Solved?, No Genes for Intelligence, *SiS* 53; [125]. Instead, environmental, epigenetic effects that mark and change genes across generations have come onto centre stage [110].

Some of us knew that genetic determinism had died at least since the early 1980s when recombinant DNA (genetic engineering) technology enabled geneticists to scrutinize the genome in fine molecular detail and discovered to their astonishment what they called 'the fluid genome' [5, 7] (see earlier). Nevertheless, it is still amazing how dynamic and responsive the genetic material is, and how utterly entangled with everyday 'downstream' biological functions; no wonder it is impossible to pin down the genes predisposing us to diseases and other human attributes [120-123].

Darwin suggested in 1868 that all cells of an organism shed minute particles, gemmules, which circulate throughout the body and are passed on to the next generation through the germ cells, thereby transmitting the characteristics of the parents to their offspring. And if the cells of the parents undergo changes during their life time, those changes would also be transmitted to the offspring. Within the past decade, geneticists have discovered substantial amounts of nucleic acids circulating in the bloodstream which are taken up by cells and transported to the nucleus, where they could be integrated into the cells' genome. These nucleic acids appear very much like Darwin's gemmules

RNA not only acts as messenger RNAs, ribosomal RNAs and transfer RNAs for making proteins, it also acts as enzymes – ribozymes – that cut and join RNAs to make new ones that are not encoded in the genome, target specific mRNA for cleavage, and is the active enzyme of the ribosome that joins amino acids together to make proteins [126]. RNA is now known to regulate gene expression through thousands of miRNAs in RNA interference universal to plants and animals (see previous section). MiRNAs are part of the nucleic acid intercommunication system of the body ([127] Intercommunication via Circulating Nucleic Acids, *SiS* 42). This nucleic acid intercom has been rediscovered several times since Darwin proposed his theory of pangenesis to account for heredity including the inheritance of acquired characters [128, 129] (Darwin's Pangenesis, the Hidden History of Genetics, & the Dangers of GMOs, *SiS* 42). Darwin suggested in 1868 that all cells of an organism shed minute particles, *gemmules*, which circulate throughout the body and are passed on to the next generation through the germ cells, thereby transmitting the characteristics of the parents to their offspring. *And if the cells of the parents undergo changes during their life time, those changes would also be transmitted to the offspring.*

Darwin's cousin Francis Galton designed a series of blood transfusion experiments on rabbits with different pigments to test the theory of pangenesis, or at any rate, to test if gemmules existed; but found no evidence for them (probably because the volume of blood transfused was too small), and the theory was largely abandoned.

Within the past decade, geneticists have discovered substantial amounts of nucleic acids circulating in the bloodstream which are taken up by cells and transported to the nucleus, where they could be integrated into the cells' genome [127,128]. These nucleic acids appear very much like Darwin's gemmules. Many experiments subsequent to those carried out by Galton, on grafting in plants and transfusion in animals, showed that heritable characteristics could be transferred between organisms in the form of nucleic acids.

Furthermore, germ cells too can take up circulating nucleic

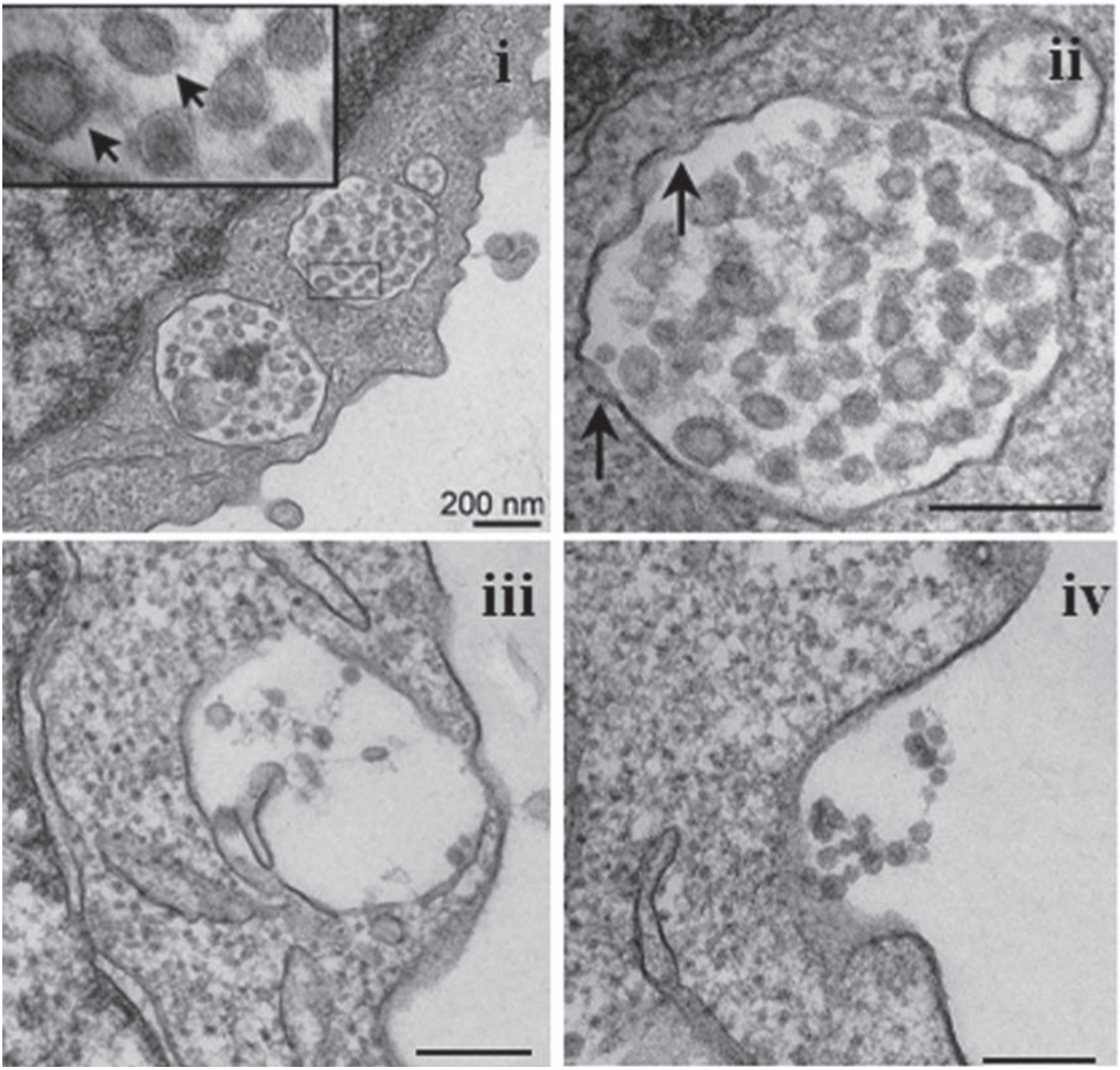

Figure 10 Formation of exosomes in multivesicular bodies and their extracellular release in cultured human mesenchymal stem cells, from [134]

acids, because there is really no 'Weismann's barrier' separating somatic from germ cells [5]. Cytoplasmic/maternal inheritance is well-known; but it turns out that sperms are very adept at taking up nucleic acids and transferring them into egg cells at fertilization. This process is so well-established that it is referred to as sperm-mediated gene transfer [130, 131] (Epigenetic Inheritance through Sperm Cells, the Lamarckian Dimension in Evolution, *SiS* 42); and applies not only to DNA, but also RNA. RNA sequences are reverse transcribed into complementary DNA (cDNA) sequences. The nucleic acids are delivered to the egg at fertilization, and inherited by the developing embryo in mosaic fashion; the foreign DNA being maintained stably as episomes (extrachromosomal DNA), but also occasionally integrated into the cell genome. Sperm mediated gene transfer can be made to happen easily by exposing sperm cells to nucleic acids, but it has also been observed to happen *in vivo* [131, 132], where either parent can transmit a mutant trait to an offspring that has two wild type copies of the gene, and hence not supposed to exhibit the mutant trait.

Pangenesis was expurgated from the mainstream neo-Darwinian account of evolution, as was any suggestion that circulating nucleic acids served as intercommunication between cells and tissues in the same organism and between different organisms, or that it could be transmitted to the next generation.

Both RNA and DNA are trafficked between cells, sent out from cells in overlapping but distinct vehicles to inform and transform other cells. Distinct populations of nucleic acids are exported from different cells in various conditions of health and disease, so much so that they are offering new opportunities for diagnosis. There is also evidence that nucleic acids exported by cancer cells both condition the body to accept cancer cells and spread cancer cells. Genetically modified nucleic acids, therefore, can take advantage of the system to enter and transform cells or do harm in other ways. There can be no doubt that this can happen; the ease with which nucleic acids are taken up by cells has been widely exploited in experiments in 'gene therapy' since the 1990s [76].

The best characterized vehicles for intercellular nucleic acid trafficking are exosomes [133]. Exosomes are membrane-bound vesicles (40-100 nm diameter) assembled in a membrane bound multivesicular body (MVB) inside the cell (see Figure 10i and ii) [134]. The membrane of the MVB invaginates to form the vesicles that are packed with enzymes, cytokines (cell-cell communication molecules of the immune system), nucleic acids and other signalling com-

pounds. In response to stimuli, the MVB fuses with the plasma membrane (Fig. 10 iii and iv) and the vesicles are released as exosomes into the extracellular space where they can interact with neighbouring cells or other more distant cells and induce changes in their state through the transfer of new receptor molecules or genetic material.

Exosomes are released *in vitro* by a wide range of cells in the blood and bone marrow as well as cancer cells. *In vivo*, exosomes have been isolated and characterized in practically all body fluids plasma, urine, saliva, cerebrospinal fluid, amniotic and synovial fluids.

Exosomes from different sources have a common set of proteins that regulate membrane cytoskeleton dynamics and membrane fusion; they also have a specific molecular repertoire that varies according to the cell type and conditions from which they originate. They are enriched in specific nucleic acids, in particular miRNAs and RNAs generally complexed with proteins. Exosomes are now considered an integral part of the intercellular communication system for immune modulation, as for example during pregnancy, to enable the mother's immune system to tolerate antigens from the foetus, or during oxidative stress, to increase the ability of other cells to withstand oxidative stress. However, cancer cells also make use of the same communication system to spread around the body.

DNA is known to be released in apoptotic bodies (membrane-bound vesicles containing fragmented DNA resulting from programmed cell death), which can be phagocytosed (engulfed) and transported into the nucleus of recipient cells for expression and integration into the genome (see [127]).

Apoptotic bodies derived from tumour cells induce foci (centres of malignancy) in p53-deficient fibroblast cultures *in vitro* and tumours in animals. Whole chromosomes or fragments are transferred by the phagocytosis pathway and integrated into the genome [127]. Horizontal gene transfer between cells may be important during tumour progression.

A further mechanism of horizontal DNA transfer has been suggested by studies in autoimmune disease [135]. The antimicrobial peptide LL-37, widely expressed in epithelia, bone marrow, and the genitourinary tract of humans, forms stable complexes with DNA and translocates extracellular DNA to the nucleus. LL-37-mediated delivery of self DNA may be an early event in autoimmune disease. The ability of LL-37 to transfer DNA across the plasma membrane is a shared property within the growing family of 'cell-penetrating peptides'.

Circulating DNA in cancer patients has many characteristics in common with the DNA of their tumours, and is suspected of being derived from apoptotic bodies of cancer cells. Furthermore, elevated concentration *per se* appears indicative of disease states, whether it is cancer, systemic lupus erthyematosus, rheumatoid arthritis, glomerulonephritis, pancreatic, hepatitis, inflammatory bowel disease, etc [136].

There is current debate as to whether circulating DNA is solely derived from dead cells or whether they are actively secreted by living cells (see [137]). It has been pointed out that the DNA circulating in healthy individuals simply do not have the characteristics of DNA from apoptotic or other dead cells, and any apoptotic DNA released from dead cells are immediately cleared and broken down by phagocytic cells nearby before it can reach the blood stream. Only in disease states when cells die in great numbers exceeding the capacity of phagocytic cells to clear the DNA do apoptotic bodies reach the circulation. Thus, in cell cultures with no dead cells, DNA is nevertheless actively secreted into the medium until a certain external concentration is reached. Replacing the medium leads to further secretion until the external equilibrium concentration is restored.

In fact, the active release of DNA from living cells has been known for at least 50 years, leading to the hypothesis that such DNA could be acting as a messenger (see [138, 139] and references therein). The main part of the DNA circulating in plasma and serum comes from active release of newly synthesized DNA by living cells. The released DNA is associated with RNA and a glycolipoprotein complex that in bacteria contains a DNA-dependent RNA polymerase, and in higher organisms, also a DNA-dependent DNA polymerase.

The most important contribution of the human genome project to the advancement of science is to put the last nail on the coffin of the genetic determinist ideology that made the project seem so compelling

Alu repeat sequences (transposons) are overrepresented compared to unique gene sequences. The spontaneously released DNA has a lower molecular weight than the typical genomic DNA. Both dividing and non-dividing cells release DNA, but not cells that are damaged or dying. Such release of DNA also takes place in bacteria, and cells of amphibians, birds, human, mammals, and plants. This DNA complex can be readily taken up by other cells where it can become integrated into the chromatin and expressed, both *in vitro* and *in vivo*.

High through-put parallel DNA sequencing of total circulating DNA from the serum of 51 healthy humans compared with 4 genomic DNA showed that the profile of circulating DNA resembled normal genomic DNA with the following exceptions [140]. Chromosome 19 sequences are under-represented; chromosome 19 contains most genes and has the highest amount of *Alu* elements. *Alu* sequences, are over-represented, accounting for 11.4 + 0.4 % in circulating DNA samples compared to 8.5 + 0.8 % in the genomic samples; while L1 and L2 long interspersed nuclear elements (LINEs) are under-represented, accounting for 19 % in serum DNA samples compared with 22.8 % in genomic samples. Also notable were the relatively large individual variations of circulating DNA for coding sequences, which ranged from 0.78 to 1.4 times genomic sequences; untranslated regulatory sequences, ranging from 0.58 to 1.3 times genomic sequences, and pseudogenes (relict genes previously believed to be no longer active) ranging from 0.85 to 1.15 times genomic sequences. The researchers conclude that non-specific release (due to cell death) is not the sole origin for circulating DNA.

It has been suggested that circulating DNA takes part in homologous recombination with genomic DNA, and that this process can correct mutations as well as induce genetic changes, with the external DNA fragments serving as reference molecules [141]. The team of researchers from Novosibirsk State University in Russia had used total genomic DNA preparations added to the culture medium to 'reprogram' cancer cells to normal cells. They injected fragmented wild type rat DNA into rats with diabetes caused by hereditary vasopressin deficiency, resulting in rapid improvement of the animals' physiological condition. Injection of fragmented wild-type mouse genomic DNA into mice lethally irradiated with ionising radiation saved the mice and accelerated the recovery of animals treated with a chemotherapeutic mutagen cyclophosphamide. They postulated that small genomic DNA fragments entered the cell nuclei and eliminated the mutations. In a later report, the team showed that short wild type human genomic DNA fragments added to the culture medium of proliferating human breast cancer cells entered the cell nuclei and repaired the extended 47 bp deletion in their CASP3 gene [142]. The fragments, ~ 200-3000 bp, were obtained by sonicating human placental DNA from a healthy consenting donor, followed by nuclease digestion. The fragments were labelled with radioactive ^{32}P isotope and added to the culture medium, and the time course of repair of the deletion followed by polymerase chain reaction (PCR) with primers flanking the deletion in the DNA isolated from the cells. The wild-type product is 125 bp while the mutant product is 78 bp. In the first experiment, cells treated for 6 and 12 days showed

wild-type: mutant product in a ratio of 1:1, indicating that some 50 % of the mutant genes had been repaired. In a second experiment monitored at 5 and 40 days, the team detected wild-type gene repair at 5 days, but at 40 days, the wild-type product greatly exceeded the mutant product.

The result obtained was remarkable considering that the correct CASP3 gene sequence was only one of millions of other genomic sequences in the random mixture of the genomic DNA fragments used. The researchers mentioned the possible risk [142] "of introducing mutations or causing a new disease if the DNA used for treatment contains such mutations." The same applies to the potential danger of extraneous transgenic DNA that may get integrated into the genome of host cells; considering that transgenic DNA may be especially invasive (see Box 2).

8. To conclude

The rationale and impetus for genetic engineering and genetic modification was the 'central dogma' of molecular biology that assumed DNA carries all the instructions for making an organism. The mechanistic fallacy is inherent in the very term 'genetic *engineering*', for it goes against the grain of the fluid and responsive genome that already emerged since the early 1980s.

Instead of linear causal chains leading from DNA to RNA to protein and downstream biological functions, complex feed-forward and feed-back cycles interconnect organism and environment at all levels, marking and changing RNA and DNA down the generations.

In order to survive, the organism needs to engage in natural genetic modification in real time, an exquisitely precise molecular dance of life with RNA and DNA responding to and participating fully in 'downstream' biological functions. That is why organisms and ecosystems are particularly vulnerable to the crude, artificial genetically modified RNA and DNA created by human genetic engineers, as already indicated by abundant evidence presented in this and other chapters of this report.

It is the fluid and adaptable genome that defeats all mechanistic attempts at genetic modification, which is why genetic modification can almost never be safe. It is a clash of ideology with reality.

Stop Press

A new study published as this report is going to press finds significantly higher rates of severe stomach inflammation in pigs fed a diet of mixed GM corn and soybean for 22.7 weeks compared to an equivalent non-GM control diet: 32 % compared to 12 %. Female pigs fed the GM diet also had uterus heavier by 25 % on average [143]. The GM diet and duration of the feeding trial is representative of the commercial pig industry in the US. These results reaffirm the observations of independent scientists and farmers indicating that GM per se introduces health hazards.

References

Preface

1. ISAAA. Pocket K No. 16: Global status of commercialized biotech/GM crops in 2012. Internatioanl Service for the Acquisition of Agri-biotech Application, accessed 14 May 2013, http://www.isaaa.org/resources/publications/pocketk/16/
2. "The inconvenient truth about GM", Geoffrey Lean, Telegraph, 18 May 2013, http://www.telegraph.co.uk/earth/agriculture/geneticmodification/10064255/The-inconvenient-truth-about-GM.html
3. "Monsanto gives up fight for GM plants in Europe" Deutsche Wele, 31 May 2013, http://www.dw.de/monsanto-gives-up-fight-for-gm-plants-in-europe/a-16851701
4. "Monsanto to withdraw EU approval requests for new GMO crops", Charlie Dunmore, Reuters, 17 July 2013, http://www.reuters.com/article/2013/07/17/us-eu-monsanto-gmos-idUSBRE96G16R20130717
5. "Monsanto shares fall as South Korea joins pause in wheat imports", Steven Mufson, Wasington Post, 1 June 2013, http://www.washingtonpost.com/business/economy/monsanto-shares-fall-as-south-korea-joins-pause-in-wheat-imports/2013/05/31/5df79a3a-ca2c-11e2-8da7-d274bc611a47_story.html
6. "US Department of Agriculture probes Oregon Monsanto GM wheat mystery", Suzanne Goldenberg, *Guardian*, 22 June 2013, http://www.guardian.co.uk/environment/2013/jun/22/agriculture-oregon-monsanto-gm-wheat
7. "Eight European countries ban genetically modified crops, Poland the latest", 8 January 2013, http://www.examiner.com/article/eight-european-countries-ban-genetically-modified-crops-poland-the-latest
8. "Genetically modified crops and their significance for sustainable agriculture in Switzerland", *EuropaBio*, 22 March 2013, http://www.europabio.org/news/genetically-modified-crops-and-their-significance-sustainable-agriculture-switzerland
9. "This is the end of GM crops in Denmark", Niels C. Jensen, DR dk, 29 May 2013, http://www.dr.dk/Nyheder/Indland/2013/05/28/184747.htm (automatically translated by Google).
10. "Italy moves to ban growing of genetically modified maize type", Reuters, 12 July 2013, http://uk.reuters.com/article/2013/07/12/us-italy-gmo-idUKBRE96B0OS20130712
11. List of GMO-Free Regions, accessed 14 May 2013, http://www.gmo-free-regions.org/fileadmin/files/gmo-free-regions/full_list/List_GMO-free_regions_Europe_update_September_2010.pdf
12. Ho MW. GM crops facing meltdown in the USA. *Science in Society* 46, 24-27, 2010.
13. Sirinathsinghji E. US staple crop system failing from GM and monoculture. *Science in Society* 59 (to appear).
14. "New GMO labelling bill will be the ultimate test between the will of the people versus the greed and power of the biotech industry", Michelle Goldstein, *Natural News*, 29 April 2013, http://www.naturalnews.com/040118_Monsanto_GMO_labeling_Federal_Bill.html
15. "Green Party calls Monsanto a top risk to health and the environment, urges a moratorium on genetically modified food crops", Press Release, Green Party, 16 May 2013, http://www.gp.org/press/pr-national.php?ID=618
16. "Peru bans Monsanto and GMOs", Kristen M, Food Renegad, 3 December 2012, http://www.foodrenegade.com/
17. "Kenya bans the import of all GMOs with immediate effect", *Food Exposed*, 21 November 2012, http://www.foodexposed.co.za/kenya-bans-the-import-of-all-gmos-with-immediate-effect/
18. "Venezuela goes for law against transgenic seeds", *Prensa Latina*, 1 June 2013, http://www.plenglish.com/index.php?option=com_content&task=view&id=1470181&Itemid=1
19. "Indefinite moratorium on GM field trials recommended", Latha Jishnu, *Down to Earth*, 22 July 2013, http://www.downtoearth.org.in/content/indefinite-ban-gm-field-trials-recommended
20. "Mexico – Ground zero in the fight for the future of maize", Emilio Goday, Inter Press Service, Global issues, 8 May 2013, http://www.globalissues.org/news/2013/05/08/16502
21. "The GMO seed cartel", Ken Roseboro, The Organic & Non-GMO Report, 1 February 2013, http://www.non-gmoreport.com/articles/february2013/the-gmo-seed-cartel.php
22. Ho MW. Farmer suicides & Bt cotton nightmare unfolding in India. *Science in Society* 45, 32-39, 2010.
23. Ho MW and Lim LC. *The Case for a GM-Free Sustainable World*, Independent Science Panel Report, Institute of Science in Society and Third World Network, London and Penang, 2003; republished *GM-Free, Exposing the Hazards of Biotechnology to Ensure the Integrity of Our Food Supply*, Vitalhealth Publishing, Ridgefield, Ct., 2004 (both available from ISIS online bookstore http://www.i-sis.org.uk/onlinestore/books.php#1)
24. Mcintyre BD, Herren HR, Wakhungu J and Watson RT eds. Agriculture at a Crossroads, International Assessment of Agricultural Knowledge, Science and Technology for Development, Synthesis Report, Island Press, Washington D.C., 2009. http://www.unep.org/dewa/agassessment/reports/IAASTD/EN/Agriculture%20at%20a%20Crossroads_Synthesis%20Report%20(English).pdf
25. Ho MW, Burcher S, Lim LC, Cummins J. et al. Food Futures Now, Organic, Sustainable, Fossil Fuel Free, ISIS/TWN, London/Penang, 2008. http://www.i-sis.org.uk/foodFutures.php
26. Agriculture at a Crossroads, International Assessment of Agricultural Knowledge, Science and Technology for Development, Synthesis Report, 2009, Island Press, Washington DC, http://www.unep.org/dewa/agassessment/reports/IAASTD/EN/Agriculture%20at%20a%20Crossroads_Synthesis%20Report%20(English).pdf

1 Double Jeopardy of Glyphosate & Glyphosate Tolerant Crops

1. Séralini G-E, Clair E, Mesnage R, Gress S, Defarge N, Malatesta M, Hennequin D, de Vendômois J-S. Long term toxicity of a Roundup herbicide and a Roundup-tolera,nt genetically modified maize. *Food and Chemical Toxicology* published or the lonline September 2012. http://dx.doi.org/10.1016/j.fct.2012.08.005
2. Saunders PT. Excess Cancers and Deaths with GM Feed: the Stats Stand Up. *Science in Society* 56, 5-6, 2012.
3. "Study linking GM maize to cancer must be taken seriously by regulators", John Vidal, The Guardian, 28 September 2012, http://www.guardian.co.uk/environment/2012/sep/28/study-gm-maize-cancer
4. "Food herbicide residues set to rise as much as 150 times", Press Release, GM Freeze, 8 February 2012, http://www.gmfreeze.org/news-releases/180/
5. Environmental Protection Agency. 40 DRF Part 180 [EPA=HQ-P{{-2012-0132; FRL-9384-3] Glyphosate; Pesticide Tolerances. http://www.regulations.gov/#!submitComment;D=EPA-HQ-OPP-2012-0132-0009
6. Antoniou M, Habib M, Howard CV, Jennings RC, Leifert

C, Nodari RO, Robinson C, Fagan J. Roundup and birth defects: Is the public being kept in the dark? Earth Open Source, 2011.
7. Antoniou M, Habib MEM, Howard CV, Jennings RC, Leifert C, Nodari RO, Robinson CJ and Fagan J. Teratogenic Effects of Glyphosate-Based Herbicides: Divergence of Regulatory Decisions from Scientific Evidence. *J Environmental Analytical Toxicology* 2012, S4-006, doi: 10.4172/2161-0525.S4-006
8. Sirinathsinghji E and Ho MW. EU regulators Regulators and Monstanto Exposed for Hiding Glyphosate Toxicity. *Science in Society* 51, 46-48, 2011
9. Benbrook C. Impacts of genetically engineered crops on pesticide use in the US – the first sixteen years. *Environmental Sciences Europe* 2012, 24, 24 doi:10.1185.2190-4715-24-24.
10. Sirinathsinghji E. Study confirms GM crops lead to increased pesticide use. *Science in Society* 56, 8-9, 2012.
11. Benbrook C. GE crop risk assessment challenges: an overview. Food Safety News 6 May 2013, http://www.foodsafetynews.com/2013/05/ge-crop-risk-assessment-challenges-an-overview/
12. WHO (1994) Glyphosate. Environmental health criteria no.159.World Health Organization, Geneva
13. European Commission 2010. Commission Directive 2010/77/EU of 10 November 2010 in Annex I of certain active substances. Official Journal of the European Union, L 293, 11.11.2010.amending Council Directive 91/414/EEC as regards the expiry dates for inclusion Monsanto, Wikipedia, 16th February 2012 http://en.wikipedia.org/wiki/Monsanto
14. Monsanto guilty of chemical poisoning in France, Reuters, 16th February 2012 http://in.reuters.com/article/2012/02/13/france-pesticides-monsanto-idINDEE81C0FQ20120213
15. Steinrücken HC, Amrhein N The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimic acid-3-phosphate synthase. *Biochem Biophys Res Commun*, 1980, 94, 1207–1212
16. Ho MW and Cherry B. Glyphosate tolerant crops bring diseases and death. *Science in Society* 47.12-15, 2010.
17. Sirinathsinghji E. USDA Scientist Reveals All. Glyphosate Hazards to Crops, Soils, Animals and Consumers. *Science in Society* 53, 36-39, 2012
18. Perkins PJ, Boermans HJ & Stephenson G.R. Toxicity of glyphosate and triclopyr using the frog embryo teratogenesis assay – Xenopus. *Environmental Toxicology and Chemistry* 2000, 19, 940-945.
19. Howe CM, Berrill M, Pauli BD, Helbing CC, Werry K, Veldhoen N. Toxicity of glyphosate-based pesticides to four North American frog species. *Environmental Toxicology and Chemistry*. 2004, 23, 1928-38.
20. Soso AB, Barcellos LJ, Ranzani-Paiva MJ, Kreutz LC, Quevedo RM, Anziliero D, Lima M, Silva LB, Ritter F, Bedin AC, Finco JA. Chronic exposure to sub-lethal concentration of a glyphosate-based herbicide alters hormone profiles and affects reproduction of female Jundiá (Rhamdia quelen). *Environmental Toxicology and Pharmacology* 2007, 23, 308-13
21. Paganelli A, Gnazzo V, Acosta H, Lopez SL and Carrasco AD. Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signalling. *Chem Res Toxicol*, August 9 2010 http://pubs.acs.org/doi/abs/10.1021/tx1001749
22. Ho MW. Lab study establishes glyphosate link to birth defects. *Science in Society* 48, 32-33, 2010.
23. Benítez-Leite S, Macchi MA, and Acosta M. Malformaciones Congenitas asociadas a agrotoxicos. *Arch. Pediatr. Urug*. 2009, 80, 237 – 247.
24. WHO (World Health Organization). 1994. Glyphosate. Environmental Health Criteria. 159. http://www.inchem.org/documents/ehc/ehc/ehc159.htm#SectionNumber:7.3
25. Dallegrave, E, DiGiorgio, F, Mantese, Soares Coelho, R, Drawans Pereira, J, Dalsenter, P, and Langeloh, A. The teratogenic potential of the herbicide glyphosate-Roundup in Wistar rats. *Toxicology Letters* 2003, 142, 45 – 52.77
26. Romano RM, Romano MA, Bernardi MM, Furtado PV, Oliveira CA. Prepubertal exposure to commercial formulation of the herbicide glyphosate alters testosterone levels and testicular morphology. *Archives of Toxicology* 2010, 84, 309-17
27. Romano MA, Romano RM, Santos LD, Wisniewski P, Campos DA, de Souza PB, Viau P, Bernardi MM, Nunes MT, de Oliveira CA. Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression. *Archives of Toxicology* 2011, Nov 26. [Epub ahead of print]
28. Ermakova IV. Genetically modified soy leads to the decrease of weight and high mortality of rat pups of the first generation. Preliminary studies. *EcosInform* 2006, 1, 4-9 (in Russian).
29. Ho MW. GM soya-fed rats: stunted, dead, or sterile. *Science in Society* 33, 4-6, 2007.
30. ISIS. Science and scientist abused. Letter to *Nature Biotechnology*. *Science in Society* 36, 8, 2007.
31. Walsh LP, McCormick C, Martin C, Stocco DM. Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. *Environmental Health Perspectives* 2000, 108, 769-76.
32. Clair E, Mesnage R, Travert C, Séralini GE. A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells *in vitro*, and testosterone decrease at lower levels. *Toxicology In Vitro* 2011 Dec 19. [Epub ahead of print]
33. Sirinathsinghji EC. Glyphosate Kills Rat Testes Cells. *Science in Society* 54, 34-36.
34. Sirinathsinghji E. Pesticide Illnesses and GM Soybeans. Ban on Aerial Spraying Demanded in Argentina, *Science in Society* 53, 2012
35. Richard S, Moslemi S, Sipahutar H, Benachour N, Seralini GE. Differential effects of glyphosate and roundup on human placental cells and aromatase. *Environmental Health Perspectives* 2005, 113, 716-20.
36. Gasnier C, Dumont C, Benachour N, Clair E, Chagnon MC, Séralini GE. Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. *Toxicology* 2009, 262, 184-91. Epub 2009 Jun 17.
37. Hokanson R, Fudge R, Chowdhary R, Busbee D. Alteration of estrogen-regulated gene expression in human cells induced by the agricultural and horticultural herbicide glyphosate. *Hum Exp Toxicol* 2007, 26, 747-52.
38. Hardell L, Eriksson M, Nordstrom M. Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: pooled analysis of two Swedish case-control studies. *Leuk Lymphoma* 2002, 43, 1043-9
39. De Roos AJ, Zahm SH, Cantor KP, Weisenburger DD, Holmes FF, Burmeister LF, Blair A. Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. *Occup Environ Med* 2003, 60, E11.
40. Eriksson M, Hardell L, Carlberg M, Akerman M. Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis. *Int J Cancer* 2008, 123, 1657-63.
41. De Roos AJ, Blair A, Rusiecki JA, Hoppin JA, Svec M, Dosemeci M, Sandler DP, Alavanja MC. Cancer incidence among glyphosate-exposed pesticide applicators in the Agricultural Health Study. *Environ Health Perspect* 2005, 113, 49-54.
42. George J, Prasad S, Mahmood Z, Shukla Y. Studies on glyphosate-induced carcinogenicity in mouse skin: a proteomic approach. *J Proteomics* 2010, 73, 951-64. Epub 2010 Jan 4
43. Paz-y-Miño C, Sánchez ME, Arévalo M, Muñoz MJ, Witte T, De-la- Carrera GO, Leone PE. Evaluation of DNA damage in an Ecuadorian population exposed to glyphosate. *Genetics*

and Molecular Biology 2007, 30, 456-460.
44. Peluso M, Munnia A, Bolognesi C, Parodi S. 32P-postlabeling detection of DNA adducts in mice treated with the herbicide Roundup. *Environ Mol Mutagen*. 1998, 31, 55-9.
45. Peluso M, Merlo F, Munnia A, Bolognesi C, Puntoni R, Parodi S. (32)P-postlabeling detection of DNA adducts in peripheral white blood cells of greenhouse floriculturists from western Liguria, Italy. *Cancer Epidemiol Biomarkers Prev*. 1996 5, 361-9.
46. Bolognesi C, Bonatti C, Degan P, Gallerani E, Peluso M, Rabboni R, Roggieri P, Abbondandolo A. Genotoxic Activity of Glyphosate and Its Technical Formulation Roundup. *Journal of Agricultural and Food Chemistry* 1997, 45, 1957–1962.
47. Koller VJ, Fürhacker M, Nersesyan A, Mišík M, Eisenbauer M, Knasmueller S. Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells. *Arch Toxicol* 2012 Feb 14. [Epub ahead of print]
48. Sirinathsinghji E. Glyphosate Toxic to Mouth Cells and Damages DNA, Roundup Much Worse, *Science in Society* 54, 38-39, 2012
49. Marc J, Mulner-Lorillon O, Bellé R. Glyphosate-based pesticides affect cell cycle regulation. *Biol Cell* 2004, 96, 245-9.
50. Bellé R, Le Bouffant R, Morales J, Cosson B, Cormier P, Mulner-Lorillon O. Sea urchin embryo, DNA-damaged cell cycle checkpoint and the mechanisms initiating cancer development. *J Soc Biol* 2007, 201, 317-27.
51. Cavas T, Konen S. Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (Carassius auratus) exposed to a glyphosate formulation using the micronucleus test and the comet assay. *Mutagenesis* 2007, 22, 263–268.
52. Guilherme S, Gaivão I, Santos MA, Pacheco M. European eel (Anguilla anguilla) genotoxic and pro-oxidant responses following short-term exposure to Roundup--a glyphosate-based herbicide. *Mutagenesis* 2010, 25, 523-30.
53. Jiraungkoorskul W, Upatham ES, Kruatrachue M, Sahaphong S, Vichasri-Grams S, Pokethitiyook P. Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia (Oreochromis niloticus). *Environmental Toxicology* 2003, 18, 260-7.
54. Kale PG, Petty BT Jr, Walker S, Ford JB, Dehkordi N, Tarasia S, Tasie BO, Kale R, Sohni YR. Mutagenicity testing of nine herbicides and pesticides currently used in agriculture. *Environmental and Molecular Mutagenesis* 1995, 25, 148-53.
55. Mañas F, Peralta L, Raviolo J, García Ovando H, Weyers A, Ugnia L, Gonzalez Cid M, Larripa I, Gorla N. Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests. *Ecotoxicology & Environmental Safety* 2009, 72, 834-7.
56. Benachour N and Séralini G-E. Glyphosate formulations Induce Apoptosis and Necrosis in Human Umbilical, Embryonic, and Placental Cells *Chem. Res. Toxicol.* , 2009, 22, 97–105.
57. Ho MW and Cherry B. Death by multiple poisoning, glyphosate and Roundup. *Science in Society* 42, 14, 2009.
58. Mesnage R, Bernay B and Séralini G-E. Ethoxylated adjuvants of glyphosate-based herbicids are active principles of human cell toxicity. *Toxicology* 2012, http://dx.doi.org/10.1016/j.tox.2012.09.006
59. Barbosa ER, Leiros da Costa MD, Bacheschi LA, Scaff M, Leite CC. Parkinsonism after glycine-derivate exposure. *Movement Disorders* 2001 16, 565-8.
60. Potrebić O, Jović-Stosić J, Vucinić S, Tadić J, Radulac M. [Acute glyphosate-surfactant poisoning with neurological sequels and fatal outcome]. *Vojnosanit Pregl* 2009, 66, 758-62.
61. Astiz M, de Alaniz MJ, Marra CA. Antioxidant defense system in rats simultaneously intoxicated with agrochemicals. *Environ Toxicol Pharmacol.* 2009, 28, 465-73.
62. Astiz M, de Alaniz MJ, Marra CA. Effect of pesticides on cell survival in liver and brain rat tissues. *Ecotoxicol Environ Saf* 2009, 72, 2025-32. Epub 2009 Jun 2.
63. Ho MW. Cancer a redox disease. *Science in Society* 54, 12-17, 2012.
64. Gui YX, Fan XN, Wang HM, Wang G, Chen SD. Glyphosate induced cell death through apoptotic and autophagic mechanisms. *Neurotoxicology and Teratology* 2012, Apr 4. [Epub ahead of print]
65. Garry VF, Harkins ME, Erickson LL, Long-Simpson LK, Holland SE, Burroughs BL. Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River Valley of Minnesota, USA. *Environ Health Perspect* 2002, 110 Suppl 3, 441-9.
66. Glusczak L, Miron Ddos S, Moraes BS, Simões RR, Schetinger MR, Morsch VM, Loro VL. Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (Rhamdia quelen). *Comp Biochem Physiol C Toxicol Pharmacol*. 2007, 146, 519-24.
67. Menéndez-Helman RJ, Ferreyroa GV, dos Santos Afonso M, Salibián A. Glyphosate as an acetylcholinesterase inhibitor in Cnesterodon decemmaculatus. *Bull Environ Contam Toxicol* 2012, 88, 6-9.
68. Jurewicz J, Hanke W. Prenatal and childhood exposure to pesticides and neurobehavioral development: review of epidemiological studies. *Int J Occup Med Environ Health* 2008, 21, 121-32.
69. Memorandum on Glyphosate Registration Standard Revision, United States Environment Protection Agency. 1986 http://www.epa.gov/pesticides/chem_search/cleared_reviews/csr_PC-103601_1-Mar-86_210.pdf
70. *The Case for a GM-Free Sustainable World*, Independent Science Panel Report, Executive Summary 2003, http://www.i-sis.org.uk/TheCaseforAGM-FreeSustainableWorld.php
71. Ho MW. Ban glyphosate herbicides *now*. *Science in Society* 43, 34-35, 2009.
72. Szarek J, Siwicki A, Andrzejewska A, Terech-Majewska E, Banaszkiewicz T. Effects of the herbicide Roundup on the ultrastructural pattern of hepatocytes in carp (Cyprinus carpio) *Mar. Environ. Res* 2000, 50, 263–266
73. Malatesta M, Perdoni F, Santin F, Battistelli S, Muller S, Biggiogera F. Hepatoma tissue culture (HTC) cells as a model for investigating the effects of low concentrations of herbicide on cell structure and function. *Toxicology In Vitro* 2008, 22, 1853–1860
74. Sirinathsinghji E. GM Feed Toxic, Meta-Analysis Reveals. *Science in Society* 52, 30-32, 2011.
75. Robinson C. Argentina's Roundup human tragedy *Science in Society* 48, 30, 2010
76. Samsel A and Seneff S. Glyphosate's suppression of cytochrome P450 enzymes and amino acid biowynthesis by gut microbiome: pathways to modern diseases. *Entropy* 2013, 15, 1-x manuscripts; doi: 19.3390/e140x000x.
77. Shinabarger DL and Braymer HD. Glyphosate catabolism by *Pseudomonas sp.* Strain PG2982. *J Bacteriol* 1986, 168, 702-7.
78. Nie CL, Wang XS, Liu Y, Perrett S and He RQ. Amyloid-like aggregates of neuronal tau induced by formaldehyde promote apoptosis of neuronal cells. *BMC Neurosci* 2007, 8, 9.
79. Rodloff AC and Krüger M. Chronic *Clostridium botulinum* infections in farmers. *Anaerobe* 2012, 18, 226-8.
80. Krüger M. Shehata AA, Schrödl W and Rodloff A. Glyphosate suppresses the antagonistic effect of *Enterococcus spp.* on *Clostridium botulinum*. *Anaerobe* 2013, 20, 74-78.
81. Cherry B. GM crops increase herbicide use in the United States. *Science in Society* 45 , 44-46, 2010
82. Ho MW. GM crops facing meltdown in the USA. *Science in Society* 46, 24-27, 2010.
83. WeedScience Database, International Survey of Herbicide Resistant Weeds. http://www.weedscience.org/In.asp, March 21st 2012.
84. Sirinathsinghji E. Monsanto Defeated By Roundup Re-

sistant Weeds. *Science in Society* 53, 40-41, 2011.
85. Purdue University, University News Service. "Waterhemp weed showing greater resistance to glyphosate", 20th March, 2012 http://www.purdue.edu/newsroom/general/2011/110929JohnsonWaterhemp.html
86. Bensch CN, Horak MN, Peterson D. Interference of redroot pigweed (Amaranthus retroflexus L.) in green bean (Phaseolus vulgaris L.). *Weed Science* 51, 37-43. 2003
87. "Glyphosate-resistant weed problem extends to more species, more farms", Farm Industry News, 29 January 2013, http://farmindustrynews.com/herbicides/glyphosate-resistant-weed-problem-extends-more-species-more-farms
88. Bernards ML, Crespo RJ, Kruger GR, Gaussoin R,. Tranel PJ. A Waterhemp (Amaranthus tuberculatus) Population Resistant to 2,4-D. *Weed Science* 2012, 60, 379
89. Report from the 1st National Meeting of Physicians in the Crop-sprayed Towns, Faculty of Medical Sciences, National University of Cordoba, 27th and 28th August 2010 http://www.reduas.fcm.unc.edu.ar/wp-content/plugins/download-monitor/download.php?id=34
90. Glyphosate-resistant weeds found in multiple Indiana counties. Purdue University News Service, 3rd September 2012 http://www.purdue.edu/newsroom/outreach/2012/120706JohnsonGlyphosate.html
91. Yamada T. Kremer RJ. De Carmargo e Castro and Wood BW. Glyphosate interactions with physiology, nutrition, and diseases of plants: threats to agricultural sustainability? *Europ J Agronomy* 2009 31, 111-3.
92. Kremer RJ and Means NE. Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. *European Journal of Agronomy* 2009, 31, 153-6.
93. Glyphosate, Wikipedia, 4 May 2010, http://en.wikipedia.org/wiki/Glyphosate#cite_note-EPAusage-2
94. Zobiole LHS, Oliveira RS Jr, Huber DM, Constantina J, Castro C, Oliveira FA, Oliveira A. Jr. Glyphosate reduces shoot concentrations of mineral nutrients in glyphosate-resistant soybeans. *Plant Soil* 2010, 328:57-69
95. Cakmak, I, Yazici, A, Tutus, Y, and Ozturk L. Glyphosate reduced seed and leaf concentrations of calcium, magnesium, manganese, and iron in non-glyphosate resistant soybean. *European Journal of Agronomy* 2009, 31, 114-119.
96. Zobiole LHS, Silvério de Oliveira RS Jr, Kremer RB, Constantina J, Bonatoc CM, Muniz AS. Water use efficiency and photosynthesis of glyphosate-resistant soybean as affected by glyphosate. *Pesticide Biochemistry and Physiology* 2010, 97, 182-193
97. Zobiole LHS, Bonini EA, Oliveira RS Jr, Kremer RJ, and Ferrarese-Filho O. Glyphosate affects lignin content and amino acid production in glyphosate-resistant soybean. *Acta Physiologiae Plantarium* 2010, 32, 831-837
98. Fernandes J, Falco WF, Oliveira SL, Caires AR. Changes in chlorophyll a fluorescence of glyphosate-tolerant soybean plants induced by glyphosate: *in vivo* analysis by laser-induced fluorescence spectroscopy. *Applied Optics* 2013, 52, 3004-11. doi: 10.1364/AO.52.003004
99. Johal CS & Huber DM. Glyphosate effects on diseases of plants. *European Journal of Agronomy* 2009 31, 144-152.
100. Neumann G. Risks of Glyphosate Pre-crop Applications inreduced-Tillage Winter Wheat Cropping Systems. Universitat Hohenheim, unpublished. 2012
101. Sirinathsinghji E. GM crops destroyed by US drought but non-GM varieties flourish. *Science in Society* 56, 6-7, 2012.
102. "Failure to Yield. Evaluating the Performance of Genetically Engineered Crops". Union of Concerned Scientists. 2009. http://www.ucsusa.org/assets/documents/food_and_agriculture/failure-to-yield.pdf
103. Shi G, Chavas JP, Lauer J. Commercialized transgenic traits, maize productivity and yield risk. *Nature Biotechnology* 2013, 31,111-4
104. Székács A & Darvas B. Forty Years with Glyphosate. Herbicides - Properties, Synthesis and Control of Weeds, Chapter 14. ISBN: 978-953-307-803-8
105. Tsui MT, Chu LM. Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. *Chemosphere* 2003, 52, 1189-97.
106. Ho MW. Scientists Reveal Glyphosate Poisons Crops and Soil. *Science in Society* 47, 2010, 12-15
107. Clair E, Linn L, Travert C, Amiel C, Séralini GE, Panoff JM. Effects of Roundup(®) and Glyphosate on Three Food Microorganisms: *Geotrichum candidum*, *Lactococcus lactis subsp. cremoris* and *Lactobacillus delbrueckii subsp. bulgaricus*. *Current Microbiology* 2012, 64, 486-91.
108. Helander M, Saloniem R and Saikkonen K. Glyphosate in northern ecosystems. *Trends in Plant Science* 2012, 17, 569-74.
109. Ho MW. Roundup Kills Frogs. *Science in Society* 26, 14, 2005.
110. Relyea R A. New effects of Roundup on amphibians: Predators reduce herbicide mortality; herbicides induce antipredator morphology. *Ecological Applications* 2012, 22, 634–647.
111. Glyphosate: lethal for amphibians in the Basque Country. 2nd February 2012 http://www.basqueresearch.com/berria_irakurri.asp?Berri_Kod=3735&hizk=I
112. Cuhra M, Traavik T, Bohn T. Clone- and age-dependent toxicity of a glyphosate commercial formulation and its active ingredient in *Daphnia magna*. *Ecotoxicology* 2012 Dec 6.[Epub ahead of print]
113. Vera MS, Lagomarsino L, Sylvester M, et al. New evidences of Roundup (glyphosate formulation) impact on the periphyton community and the water quality of freshwater ecosystems. *Ecotoxicology* 2010, 19, 710-21.
114. Vera MA, Di Fiori E, Lagomarsino L, et al. Direct and indirect effects of the glyphosate formulation Atanor ® on freshwater microbial communities. *Ecotoxicology* 2012, 21, 1805-16.
115. Ho MW. World water supply in jeopardy. *Science in Society* 56, 38-43, 2012.
116. Sirinathsinghji E. GM Crops and Water – A Recipe for Disaster. *Science in Society* 58, to appear.
117. Sirinathsinghji E. Glyphosate & Monarch Butterfly Decline. *Science in Society* 52, 32-33, 2011
118. Pleasants JM & Oberhauser KS. Milkweed loss in agricultural fields because of herbicide use: effect on the monarch butterfly population. *Insect Conservation and Diversity* 2012, doi: 10.1111/j.1752-4598.2012.00196.x
119. McLaren PJ, Cave JG, Parker EM, Slocombe RF. Chondrodysplastic calves in Northeast Victoria. *Veterinary Pathology* 2007, 44, 342-54
120. Sirinathsinghji E. GM Soy Linked to Illnesses in Farm Pigs. *Science in Society* 55, 8-9, 2012.
121. Sirinathsinghji E. GM crops and water – a recipe for disaster. *Science in Society* 58 (in press).
122. Sanchís J, Kantiani L, Llorca M, Rubio F, Ginebreda A, Fraile J, Garrido T, Farré M. Determination of glyphosate in ground water samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry. *Analytical and Bioanalytical Chemistry* 2012, 402, 2335-45.
123. Chang FC, Simcik MF, Capel PD. Occurrence and fate of the herbicide glyphosate and its degradate aminomethylphosphonic acid in the atmosphere. *Environmental Toxicology & Chemistry* 2011, 30, 548-55.
124. Herbicide in Urine. Ithaka Journal. http://www.ithaka-journal.net/herbizide-im-urin , 22nd April 2012.
125. Bill would ban pesticides and reassess the use of glyphosate. GMWatch, 20th September 2012, http://www.gmwatch.org/latest-listing/51-2012/14210-bill-would-ban-pesticides-and-reassess-the-use-of-glyphosate-in-brazil
126. Ho MW, Burcher S, Lim LC, et al. Food Futures Now

*Organic*Sustainable*Fossil Fuel Free, ISIS/TWN, London/ Penang, 2008. http://www.i-sis.org.uk/foodFutures.php

2 Bt Crops Failing & Harmful to Health and Environment

1. Benbrook C. Impacts of genetically engineered crops on pesticide use in the U.S. -the first sixteen years. *Environmental Sciences Europe* 2012, 24,24 doi:10.1186/2190-4715-24-24
2. Ho MW and Saunders PT. Transgenic cotton offers no advantage. *Science in Society* 38, 30, 2008.
3. Bt failure to hit cotton yield by 40%: Govt. Dnaindia.com, 26th November 2012 http://www.dnaindia.com/mumbai/report_bt-failure-to-hit-cotton-yield-by-40pct-govt_1769428
4. Maharashtra reports Bt cotton failure in 4 Million Hector – Thousands Cotton Farmers to Council Hall Nagpur on 11th December for compensation. http://vidarbhatimes.blogspot.co.uk/
5. Ministry blames Bt cotton for farmer suicides. hindustantimes.com, 26th March 2012 http://www.hindustantimes.com/News-Feed/Business/Ministry-blames-Bt-cotton-for-farmer-suicides/Article1-830798.aspx
6. Ho MW. Farmer suicides and Bt cotton nightmare unfolding in India. *Science in Society* 45, 32-39, 2010.
7. "Pesticides make a comeback", Ian Berry, The Wall Street Journal, 21 May 2013, http://online.wsj.com/article/SB10001424127887323463704578496923254944066.html
8. Cummins J. Bt toxins in Genetically Modified Crops : Regulation by Deceit. *Science in Society* 22, 32, 2004.
9. Gurian-Sherman D. Failure to yield: Evaluating the performance of genetically engineered crops. Union of Concerned Scientists. 2009. http://www.ucsusa.org/assets/documents/food_and_agriculture/failure-to-yield.pdf
10. Shi G, Chavas JP, Lauer J. Commercialized transgenic traits, maize productivity and yield risk. *Nature Biotechnology* 2013, 31,111-4
11. Benbrook C. GE crop risk assessment challenges: an overview. Food Safety News 6 May 2013, http://www.foodsafetynews.com/2013/05/ge-crop-risk-assessment-challenges-an-overview/
12. Séralini G-E, Mesnage R, Clair E, Gress S, Vendômois J, Cellier D. Genetically modified crops safety assessments: present limits and possible improvements. *Environmental Sciences Europe* 2011, 23, 10-20
13. Sirinathsinghji E. GM feed toxic, new meta-analysis confirms. *Science in Society* 52, 30-32, 2011
14. Mesnage R,Clair E, Gress S,Then C, Székács A,Séralini G-E. Cytotoxicity on human cells of Cry1Ab and Cry1Ac Bt insecticidal toxins alone or with a glyphosate-based herbicide. DOI 10.1002/jat.2712
15. Sirinathsinghji E. Bt Toxin Kills Human Kidney Cells. *Science in Society* 54, 36-38, 2012
16. Vázquez-Padrón RI, Gonzáles-Cabrera J, García-Tovar C, Neri-Bazan L, Lopéz-Revilla R, Hernández M, Moreno-Fierro L, de la Riva GA. Cry1Ac protoxin from *Bacillus thuringiensis* sp. kurstaki HD73 binds to surface proteins in the mouse small intestine. *Biochemical Biophysical Research Communications* 2010, 271, 54-8.
17. Finamore A, Roselli M, Britti S, Monastra G, Ambra R, Turrini A, Mengheri E. Intestinal and peripheral immune response to MON810 maize ingestion in weaning and old mice. *Journal of Agricultural and Food Chemistry* 2008, 56, 11533-9.
18. Mezzomo BP, Miranda-Vilela AL, Freire IdS, Barbosa LC, Portilho FA, Lacava ZG, Grisolia CK. Effects of oral administration of *Bacillus thuringiensis* as spore-crystal strains Cry1Aa, Cry1Ab, Cry1Ac or Cry2Aa on hematologic and genotoxic endpoints of Swiss albino mice. *Journal of Hematology Thromboembolic Diseases* 2013, 1,104. doi: 10.4172/jhtd.1000104
19. Ho MW. More illnesses linked to Bt crops. *Science in Society* 30, 8-10, 2006.
20. Aris A, Leblanc S. Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. *Reproductive Toxicolology*, 2011,31, 528-33
21. Ho MW. GM maize reduces fertility & deregulates genes in mice. *Science in Society* 41, 40-41, 2009.
22. Kranthi KR, Naidu S, Dhawad CS, Tatwawadi A, Mate K, Patil E, Bharose AA,. Behere GT, Wadaskar RM and Kranthi S. Temporal and intra-plant variability of Cry1Ac expression in Bt-cotton and its influence on the survival of the cotton bollworm, Helicoverpa armigera (Hübner) (Noctuidae: Lepidoptera). *Current Science* 2005, 89, 291-7.
23. Wan P, Zhang Y, Wu K, Huang M. Seasonal expression profiles of insecticidal protein and control efficacy against Helicoverpa armigera for Bt cotton in the Yangtze River valley of China. *Journal of Economic Entomology* 2005, 98, 195-201.
24. "The GMO Emperor has no Clothes" Navdanya International report, 2011 http://www.navdanyainternational.it/images/doc/Full_Report_Rapporto_completo.pdf
25. Gala R. Organic cotton beats Bt cotton. *Science in Society* 27, 49-50, 2005.
26. Lu Y, Wu K, Jiang Y, Xia B, Li P, Feng H, Wyckhuys KA, Guo Y. Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of Bt cotton in China. *Science* 2010, 328, 1151-1154
27. Saunders PT and Ho MW. From the Editors: GM spin meltdown in China. *Science in Society* 47, 2-3, 2010.
28. Ho MW. Mealy bug plagues Bt cotton in India and Pakistan. *Science in Society* 45, 40-43, 2010.
29. Hagenbucher S, Wäckers FL, Wettstein FE, Olson DM, Ruberson JR, Romeis J. Pest trade-offs in technology: reduced damage by caterpillars in Bt cotton benefits aphids. *Proceedings Biological Sciences* 2013, 280, 2013004
30. Nagrare VS, Kranthi S, Kumar R, Dhara Jothi B, Amutha M, Deshmukh AJ, Bisane KD and Kranthi KR.. Compendium of Cotton Mealybugs. Central Institute for Cotton Research, 2011. http://www.cicr.org.in/pdf/compendium_of_cotton_mealybugs.pdf
31. Growers Face Huge Losses in Brazil as Bt Cotton Eaten by Caterpillars. GMWatch.com, 13th May 2013. http://gmwatch.org/index.php?option=com_content&view=article&id=14683:growers-face-huge-losses-in-brazil-as-bt-cotton-eaten-by-caterpillars
32. Zeilinger AR, Olson DM, Andow DA. Competition between stink bug and heliothine caterpillar pests on cotton at within-plant spatial scales. *Entomologia Experimentalis et Applicata* 2011, 141, 59-70.
33. "Familiar insect pests haven't gone away", Tommy Horton, CottonFarming.com, June 2011, http://www.cottonfarming.com/home/issues/2011-06/2011_JuneCF-Lead.html
34. Bagwell RD, Burris E, Catchot AL, Cook DR, Gore J, Green JK, Lorenz JK, Musser FR, Robbins JT, Stewart SD and Studebaker G. Multistate Evalulation of Tarnished Plant Bug Sampling Methods in Bloomington Cotton. MP471-PD-10-07H. www.uaex.edu
35. Herbert A, Blinka E, Bacheler J, Van Duyn J, Green J, Toews M, Roberts P and Smith RH. Managing Stick Bugs in Cotton: Research in the Southeast Region, Virginia Cooperative Extension, Publication 444-390, 2009.
36. Then C. New pest in crop caused by large scale cultivation of Bt corn. Breckling, B. & Verhoeven, R. (2010) Implications of GM-Crop Cultivation at Large Spatial Scales, Theorie in der Ökologie, Frankfurt, Peter Lang. http://www.maps-erver.uni-vechta.de/generisk/gmls2010/beitraege/GMLS2_Then.pdf
37. EPA 'Memorandum to Open Docket Plant-Incorporated Protectant Insect Resistance Management (IRM)'. Briefing

30th November 2011. (Docket No: EPA-HQ-OPP-2011-0922) http://www.regulations.gov/#!searchResults;rpp=10;po=0;s=EPA-HQ-OPP-2011-0922
38. Sirinathsinghji E. Bt resistant Rootworm Spreads. *Science in Society* 52, 34-35, 2011
39. "Monsanto's Superweeds & Superbugs", Pesticide Action Network, 12th September 2011 http://www.panna.org/blog/monsantos-superweeds-superbugs
40. Brévault T, Heuberger S, Zhang M, Ellers-Kirk C, Ni X, Masson L, Li X, Tabashnik BE, Carrière Y. Potential shortfall of pyramided transgenic cotton for insect resistance management. *Proc Natl Acad Sci U S A* 2013, 110, 5806-11.
41. Bøhn T, Primicerio R, Hessen DO, Traavik T. Reduced fitness of *Daphnia magna* fed a Bt-transgenic maize variety. *Archives of Environmental Contamination and Toxicology* 2008, 55, 584-92
42. Hilbeck A, McMillan JA, Meier M, Humbel A, Schläpfer-Miller J, Trtikova M. A controversy re-visited: Is the coccinellid Adalia bipunctata adversely affected by Bt toxins? *Environmental Sciences Europe* 2012, 24, 10-22
43. Sirinathsinghji E. Bt Toxicity Confirmed: Flawed Studies Exposed. *Science in Society* 55, 9-10, 2012
44. Losey JE, Rayor LS, Carter ME. Transgenic pollen harms monarch larvae. *Nature* 1999, 399, 214.
45. Brower LP, Taylor OR, Williams EH, Slayback DA, Zubieta RR, Ramírez MI. Decline of monarch butterflies overwintering in Mexico: is the migratory phenomenon at risk? Insect Conservation and Diversity 2011, doi: 10.1111/j.1752-4598.2011.00142.x
46. Sirinathsinghji E. Glyphosate and Monarch Butterfly Decline. *Science in Society* 52, 32-33, 2011
47. Holst N, Lang A, Lövei G, Otto M. Increased Mortality is Predicted of Inachis io larvae caused by Bt-Maize Pollen in European Farmland. *Ecological Modelling* 2013, 250, 126-133.
48. Ho MW, Cummins J. The Mystery of the Disappearing Honeybees *Science in Society* 34 2007
49. Ramirez-Romero R, Desneux N, Decourtye A, Chaffiol A, Pham-Delègue MH. Does Cry1Ab protein affect learning performances of the honey bee Apis mellifera L. (Hymenoptera, Apidae)? *Ecotoxicological Environmental Safety* 2008, 70, 327-33.
50. Ho MW and Cummins J. GM food & feed not fit for "man or beast". ISIS Report 7 May 2004, http://www.i-sis.org.uk/ManorBeast.php
51. Dutton A, Klein H, Romeis J and Bigler F. "Uptake of Bt-toxin by herbivores feeding on transgenic maize and consequences for the predator Chrysoperia carnea",*Ecological Entomology* 2002, 27, 441-7.
52. Romeis J, Dutton A and Bigler F. "*Bacillus thuringiensis* toxin (Cry1Ab) has no direct effect on larvae of the green lacewing Chrysoperla carnea (Stephens) (Neuroptera: Chrysopidae)". *Journal of Insect Physiology* 2004, 50, 175-83.
53. Navdanya. Monstanto's Bt Cotton Kills the Soil as Well as Farmers. ISIS report 23/02/09. http://www.i-sis.org.uk/BtCottonKillsSoilandFarmers.php
54. Quist D, Chapela IH.2001. Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature* 2001, 414, 541-3.
55. Rosi-Marxhall EJ, Tank JL, Royer TV, Whiles MR, Evans-White M, Chamgers C, Griffiths NA, Pokelsek J and Stephen ML.Toxins in transgenic crop byproducts may affect headwater stream ecosystems. *Proceedings of the National Academy of Sciences* 2007, 104, 16204-8.
56. Ho MW. Bt crops threaten aquatic ecosystems. *Science in Society* 36, 49, 2007
57. Tank JL, Rosi-Marshall EJ, Royer TV, Whiles MR, Griffiths NA, Frauendorf TC, Treering DJ. Occurrence of maize detritus and a transgenic insecticidal protein (Cry1Ab) within the stream network of an agricultural landscape. *Proc Natl Acad Sci U S A* 2010 107, 17645-50.

3 New Genetics & Hazards of GMOs

1. Ho MW. FAQs on genetic engineering. ISIS Tutorial. http://www.i-sis.org.uk/FAQ.php
2. Crick FHC. On protein synthesis. *Symp Soc Exp Biol* 1958, 12, 139-163.
3. Crick F. Central dogma of molecular biology. *Nature* 1970, 227, 561-3.
4. Watson JD and Crick F. A structure for deoxyribose nucleic acid. *Nature* 1953, 171, 737-8.
5. Ho MW. Genetic Engineering Dream of Nightmare? The Brave New World of Bad Science and Big Business, Third World Network, Gateway Books, MacMillan, Continuum, Penang, Malaysia, Bath, UK, Dublin, Ireland, New York, USA, 1998, 1999, 2007 (reprint with extended Introduction). http://www.i-sis.orucg.uk/genet.php
6. Ho MW. Death of the central dogma. *Science in Society* 24, 4, 2004.
7. Ho MW. Living with the Fluid Genome, ISIS/TWN, London/Penang 2003. http://www.i-sis.org.uk/fluidGenome.php
8. Ho MW. Development and evolution revisited. In Handbook of Developmental Science, Behavior and Genetics (Hood D, Halpern C, Greenberg G and Lerner R. eds.) pp. 61-108, Wiley-Blackwell, Chicheter, 2010.
9. Ho MW, Cummins J and Saunders PT. GM Food Nightmare Unfolding in the Regulatory Sham, *Microbial Ecology in Health and Disease* 2007, 19, 66-77. http://www.i-sis.org.uk/pdf/GM_Food_Nightmare_Unfolding.pdf
10. Séralini G-E, Clair E, Mesnage R, Gress S, Defarge N, Malatesta M, Hennequin D, de Vendômois J-S. Long term toxicity of a Roundup herbicide and a Roundup-tolera,nt genetically modified maize. *Food and Chemical Toxicology* published or the lonline September 2012. http://dx.doi.org/10.1016/j.fct.2012.08.005
11. Saunders PT and Ho MW. GM cancer warning can no longer be ignored. *Science in Society* 56, 2-4, 2012.
12. Saunders PT. Excess cancers and deaths with GM feed: the stats stand up. *Science in Society* 56, 4-5, 2012.
13. Sirinathsinghji E. GM soy linked to illnesses in farm pigs. *Science in Society* 55, 8-9, 2012.
14. Séralini G-E, Mesnage R, Clair E, Gress S, Spiroux de Vendôme J and Cellier D. Genetically modified crops safety assessment: present limits and possible improvements. *Environmental Sciences Europe* 2011, 23, 10, http://www.enveurope.com/content/23/1/10
15. Sirinathsinghji E. GM Feed Toxic, Meta-Analysis Reveals. *Science in Society* 52, 30-32, 2011.
16. Ho MW. Emergency! Pathogen new to science found in Roundup Ready GM crops. *Science in Society* 50, 10-11, 2011.
17. Ho MW. Scientist defends claim of new pathogen linked to GM crops. *Science in Society* 50, 12-13, 2011.
18. Ermakova IV. Genetically modified soy leads to the decrease of weight and high mortality of rat pups of the first generation. Preliminary studies. *EcosInform* 2006, 1, 4-9 (in Russian).
19. Ho MW. GM soya-fed rats: stunted, dead, or sterile. *Science in Society* 33, 4-6, 2007.
20. Ho MW. More illnesses linked to Bt crops. *Science in Society* 30, 8-10, 2006.
21. Ho MW. Mass deaths in sheep grazing on Bt cotton. *Science in Society* 30. 12-13, 2006.
22. Prescott VE, Campbell PM, Moore A, Mattes J, Rothenberg ME, Foster PS, Higgins TJV and Hogan SP. Transgenic expression of bean a-amylase inhibitor in peas results in altered structure and immunogenicity. *J Agricultural and Food Chemistry* 2005, 53, 9023-30.
23. Ho MW. Transgenic pea that make mice ill. *Science in Society* 29, 28-29, 2006.
24. Malatesta M, Caporaloni C, Rossi L, Battistelli S, Rocchi MBL, Tonucci F and Gazzanelli G. Ultrastructural analysis of

pancreatic acinar cells from mice fed on genetically modified soybean. *J Anat* 2002, 201, 409-415.L.
25. Malatesta M, Biggiogera M, Manuali E, Rochhi MBL, Baldelli B, Gazzanelli G. Fine structural analyses of pancreatic acinar cell nuclei from mice fed on genetically modified soybean. *European Journal of Histochemistry* 2003, 47, 385-8.
26. Malatesta M, Caporaloni C, Gavaudan S, Rocchi MBL, Serafini S, Tiberi C and Gazzanelli G. Ultrastructural morphometrical and immunocytochemical analysis of hepatocyte nuclei from mice fed on genetically modified soybean. *Cell Structure and Function* 2002, 27, 175-80.
27. Malatesta M, Tiberi C, Baldelli B, Battistelli S, Manuali E Biggiogera M. Reversibility of hepatocyte nuclear modifications in mice fed on genetically modified soybean. *European Journal of Histochemistry* 2005, 49, 237-42.
28. Vecchio L, Cisterna B, Malatesta M, Martin TE, Biggiogera M. Ultrastructural analysis of testes from mice fed on genetically modified soybean. E*uropean Journal of Histochemistry* 2004, 48, 449-54.
29. Ho MW. GM ban long overdue. Dozens ill & five deaths in the Philippines. *Science in Society* 29, 26-27, 2006.
30. "French experts very disturbed by health effects of Monsanto GM corn" GMWatch, 23 April 2004. www.gmwatch.org
31. Ho MW and Burcher S. Cows ate GM maize and died. *Science in Society* 21, 4-6, 2004.
32. Sirinathsinghji E. Syngenta charged for covering up livestock deaths from GM corn. *Science in Society* 55, 4-5, 2012.
33. Pusztai A, Bardocz S and Ewen SWB. Genetically modified foods: Potential human health effects. In Food Safety: Contaminants and Toxins, (J P F D'Mello ed.), Scottish Agricultural College, Edinburgh, CAB International, 2003.
34. 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.
35. Novotny E. Animals avoid GM food, for good reasons. *Science in Society* 21, 9-11, 2004.
36. Ho MW and Lim LC. *The Case for a GM-Free Sustainable World*, Independent Science Panel Report, Institute of Science in Society and Third World Network, London and Penang, 2003; republished *GM-Free, Exposing the Hazards of Biotechnology to Ensure the Integrity of Our Food Supply*, Vitalhealth Publishing, Ridgefield, Ct., 2004 (both available from ISIS online bookstore http://www.i-sis.org.uk/onlinestore/books.php#1)
37. Ho MW, Traavik T, Olsvik O, Tappeser B, Howard V, von Weizsacker C, and McGaein GC. Gene technology and gene ecology of infectious diseases. *Microbial Ecology in Health and Disease* 1998, 10, 33-39.
38. Ho MW. Horizontal gene transfer – the hidden hazards of genetic engineering, TWN, 2000.
39. Ho MW. GM DNA does jump species. *Science in Society* 47, 30-33, 2010.
40. Ho MW. Scientists discover new route for GM gene escape. *Science in Society* 50, 14-15, 2011.
41. "GMOs: Gene transfer is neither unnatural nor dangerous", Michael Eisen, Science 2.0, 19 June 2012, http://www.science20.com/profile/michael_eisen
42. Bergelson J, Purrington CB, Wichmann G. Promiscuity in transgenic plants. *Nature* 1998, 395, 25.
43. Collonier C, Berthier G, Boyer F, Duplan M-N, Fernandez S, Kebdani N, Kobilinsky A, Romanuk M, Bertheau Y. Characterization of commercial GMO inserts: a source of useful material to study genome fluidity. Poster courtesy of Pr. Gilles-Eric Seralini, Président du Conseil Scientifique du CRII-GEN, www.crii-gen.org
44. Ho MW. Transgenic lines proven unstable. *Science in Society* 20, 35-36, 2003.
45. Ho MW. Unstable transgenic lines illegal. *Science in Society* 21, 23, 2004.
46. Hernández M, Pla M, Esteve T, Prat S Puigdomènech P and Ferrando A. A specific real-time quantitative PCR detection system for event MON810 in maize YieldGard based on the 3'-transgene integration sequence. *Transgenic Research* 2003, 170-89.
47. Singh CK, Ojka A, Kamle S and Kachru DN. Assessment of cry1Ab transgene cassette in commercial Bt corn MON810: gene, event, construct & GMO specific concurrent characterization. Nature Protocols 2007, DOI: 10.1038/nprot.2007.440, http://www.natureprotocols.com/2007/10/23/assessment_of_cry1ab_transgene.php
48. Ho MW. MON 810 rearranged again. *Science in Society* 38, 27, 2008.
49. Ho MW. Transgenic lines unstable hence illegal and ineligible for protection. *Science in Society* 38, 28-29, 2008.
50. Rosati A, Bogani P, Santarlasci A and Buiatti M. Characterisation of 3' transgene insertion site and derived mRNAs in MON819 YieldGard maize. *Plant Mol Biol* 2008, 67, 271-81.
51. Yin Z, Plader W and Malepszy S. Transgene inheritance in plants. *J. Appl Genet* 2004, 45, 127-44.
52. Ho MW and Cummins J. Gene therapy risks exposed. *Science in Society* 19 48+50, 2003.
53. Somers DA and Makarevitch I. Transgene integration in plants: poking or patching holes in promiscuous genomes? *Current Opinion in Biotechnology* 2004, 15, 126-31.
54. Wu B, Sun YH, Wang YW, Wang YP and Zhu ZY. Characterization of transgene integration pattern in F4hGH-transgenic common carp (Cyprinus carpio L.) Cell Research 2005, 15, 447-54.
55. Reim S and Hanke V. Investigation on stability of transgenes and their expression in transgenic apple plants (Malus x domestica Borkh.) Xith Eucarpia Symp. On Fruit Breed. & Genetics (F Laurens and K Evans, eds), *Acta Hort* 2004, 663, 419-24.
56. Flachowsky J, Riedel M, Reim S and Hanke M-V. Evaluation of the uniformity and stability of T-DNA integration and gene expression in transgenic apple plants. *Electronic Journal of Biotechnology* 2008, 11, DOI: 10.2225/vol11-issue1-fulltext-10
57. Roman E, Soares A, Proite K, Neiva S, Grossi M, Faria JC, Rech EL and Aragão FJL. Transgene elimination in genetically modified dry bean and soybean lines. *Genetics and Molecular Research* 2005, 4, 177-84.
58. Statement of EFSA. EFSA-Q-2009-00589 and EFSA-Q-2009-00593. Consolidated presentation of the joint Scientific Opinions of the GMO and BIOHAZ panels on "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants" Prepared by GMO and BIOHAZ Units. *The EFSA Journal* 2009, 1108, 1-8.
59. de Vries J, Herzfeld T and Wackernagel W. Transfer of plastid DNA from tobacco to the soil bacterium Acinetobacter sp. By natural transformation. *Molecular Microbiology* 2004, 53, 323-34.
60. de Vries J, Meier P and Wackernagel W. Microbial horizontal gene transfer and the DNA release from transgenic crop plants. *Plant and Soil* 2004; 266: 91-104.
61. Simpson DJ, Fry JC, Rogers HJ and Day MJ. Transformation of Acinetobacyer baylyi in non-sterile soil using recombinant plant nuclear DNA. *Environ Biosafety Res* 2007 6, 101-12.
62. Rizzi A, Pontiroli A, Brusetti L, Borin S, Solini C, Abruzzese A, Sacchi GA, Vogel TM, Simonet P, Bazzicalupo M, Nielsen KM, Monier J-M and Daffoncho D. Strategy for in situ detection of natural transformation-based horizontal gene transfer events. *Applied and Environmental Microbiology* 2008, 74, 1250-4.
63. Pontiroli A, Rizzi A, Simonet P, Daffonchio D, Vogel TM and

Monier JM. Visual evidence of horizontal gene transfer between plants and bacteria in the phytosphere of transplastomic tobacco. *Appl Environ Microbio* 2009, 75, 3314-22.
64. Chen J, Jin M, Qiu ZG, Guo C, Chen ZL, Shen ZQ, Wang XW, Li JW. A Survey of Drug Resistance bla Genes Originating from Synthetic Plasmid Vectors in Six Chinese Rivers. Environmental Science & Technology 2012, 46, 13448-54.
65. Sirinathsinghji E. GM antibiotic resistance in China's rivers. *Science in Society* 57, 6-7, 2013.
66. Chowdhury EH, Mikami O, Nakajima Y. Hino A, Kuribara H, Suga K, Hanazumi M and Yomemochi C. Detection of genetically modified maize DNA fragments in the gastrointestinal contents of pigs fed StarLink CBH351. *Vet Hum Toxicol* 2003; 45: 95-6.
67. Reuter T and Aulrich K. Investigations on genetically modified maize (Bt-maize) in pig nutrition: fate of feed-ingested foreign DNA in pig bodies. *Eur Food Res Technol* 2003, 216, 185-92.
68. Duggan PS, Chambers PA, Heritage J and Forbes JM. Fate of genetically modified maize DNA in the oral cavity and rumen of sheep. *British Journal of Nutrition* 2003, 89, 159-66.
69. Phipps RH, Deaville ER, Maddison BC. Detection of transgenic and endogenous plant DNA in rumen fluid, duodenal digesta, milk, blood and feces of lactating dairy cows. *J. Dairy Sci.* 2003: 86: JDS 3275 Take H502.
70. Ho MW. DNA in GM food & feed. *Science in Society* 23, 34-36, 2004.
71. Netherwood T, Martin-Orue SM, O-Donnell AG, Gockling S, Graham J, Mathers JC and Gilbert JH. Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. *Nature biotechnology* 2004; 22: 204-209.
72. Netherwood, T., Bowden, R., Harrison, P., O'Donnell, A.G., Parker, D.S., Gilbert, H.J., 1999. Gene transfer in the gastrointestinal tract. *Applied and Environmental Microbiology* 65, 5139–5141.
73. Kelly BG, Vespermann A, Bolton DJ Gene transfer events and their occurrence in selected environments. *Food Chem Toxicol* 2009, 47, 978–83.
74. McCuddin, Z., Carlson, S.A., Rasmussen, M.A., Franklin, S.K., 2006. Klebsiella to Salmonella gene transfer within rumen protozoa: implications for antibiotic resistance and rumen defaunation. *Veterinary Microbiology* 2005, 114, 275–84.
75. Mercer DK, Scott KP, Bruce-Johnson WA, Glover LA and Flint HJ. Fate of free DNA and transformation of the oral bacterium Streptococcus gordonii DL by plasmid DNA in human saliva. *Applied and Environmental Microbiology* 1999, 65, 6-10.
76. Ho MW, Ryan A, Cummins J and Traavik T. Slipping Through the Regulatory Net, 'Naked' and 'Free' Nucleic Acids, TWN, Penang, 2001, http://www.i-sis.org.uk/onlinestore/books.php
77. Podevin N and du Jardin P. Possible consequences of the overlap between the CaMV 35S promoter regions in plant transformation vectors used and the viral gene VI in transgenic plants. *GM Crops and Food* 2012, 3, 1-5.
78. Ho MW. Hazardous virus gene discovered in GM crops after 20 years. Editorial. *Science in Society* 57, 2-3, 2013.
79. Latham J and Wilson A. Potentially dangerous virus gene hidden in commercial GM crops. *Science in Society* 57, 4-5, 2013.
80. Ho MW, Ryan A, Cummins J. Cauliflower mosaic viral promoter – a recipe for disaster? *Microb Ecol Health Dis* 1999, 11, 194–7.
81. Ho MW, Ryan A, Cummins J. Hazards of transgenic plants with the cauliflower mosaic viral promoter. *Microb Ecol Health Dis* 2000, 12, 6–11.
82. Ho MW, Ryan A, Cummins J. CaMV35S promoter fragmentation hotspot confi rmed and it is active in animals. *Microb Ecol Health Dis* 2000, 12, 189.
83. Ballas N, Broido S, Soreq H, Loyter A. Efficient functioning of plant promoters and poly(A) sites in *Xenopus* oocytes. *Nucl Acids Res* 1989, 17, 7891–903.
84. Burke C, Yu XB, Marchitelli L, Davis EA, Ackerman S. Transcription factor IIA of wheat and human function similarly with plant and animal viral promoters. *Nucleic Acids Res* 1990, 18, 3611–20.
85. Ho MW and Cummins J. New evidence links CaMV 35S promoter to HIV transcription. *Microb Ecol Health Dis* 2009, 21, 172-4.
86. "Hazards of GMOS: Agrobacterium mediated transformation" http://www.bristol.ac.uk/news/2010/7279.html
87. Knight CJ, Bailey AM, Foster GD. Investigating Agrobacterium-mediated transformation of Verticillium albo-atrum on plant surfaces. PLOS ONE 2010, 5(10): e13684. Doi:10.1371/journal.pone.0013684
88. Mc Nicol MJ, Lyon GD, Chen MY, Barrett C and Cobb E. Scottish Crop Research Institute. Contract No RG 0202. The Possibility of *Agrobacterium* as a Vehicle for Gene Escape. MAFF. R&D and Surveillance Report: 395.
89. Barrett C, Cobb E, MacNiol R and Lyon G. A risk assessment study of plant genetic transformation using *Agrobacterium* and implication for analysis of transgenic plants. *Plant Cell Tissue and Organ Culture* 1997, 19, 135-144.
90. Mogilner N, Zutra D Gafny R and Bar-Joseph M. the persistence of engineered *Agrobacterium tumefaciens* in agroinfected plants. *Molecular Plant – Microbe Interactions* 1993, 6(50), 673-5.
91. Soltani J, van Heusden PH and Hooykaas PJJ. Agrobacterium-mediated transformation of non-plant organisms. In *Agrobacterium: From Biology to Biotechnology* (Tzfira T and Citovsky V eds.), pp. 649-74, Springer, New York, 2008,
92. Ho MW. Horizontal gene transfer from GMOs does happen. *Science in Society* 38, 22-24, 2008.
93. Ferguson G and Heinemann J. Recent history of transkingdom conjugation . In *Horizontal Gene Transfer* 2nd ed., Syvanen M and Kado CI. (eds.) Academic Press, San Diego, 2002.
94. Ho MW. Horizontal gene transfer, book review. *Heredity* 2003, 90, 6-7.
95. Kado C. Horiontal transmission of genes by Agrobacterium species. In *Horizontal Gene Transfer* 2nd ed., Syvanen M and Kado CI. (eds.) Academic Press, San Diego, 2002.
96. Sengelov G, Kristensen KJ, Sorensen AH, Kroer N, and Sorensen SJ. 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* 2001, 42, 160-7.
97. 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.
98. Cummins J. "Common plant vector injects genes into human cells. *ISIS News* 2002, 11/12, p. 10.
99. "CDC to launch study on unexplained illness" 16 January 2008, Centers for Disease Control and Prevention, http://www.cdc.gov/od/oc/media/transcripts/2008/t080116.htm#id=45169
100. Unexplained Dermopathy (aka "Morgellons"), Centers for Disease Control and Prevention, 17 January 2008, http://www.cdc.gov/unexplaineddermopathy/general_info.html
101. Ho MW and Cummins J. *Agrobacterium* & Morgellons disease, a GM connection? *Science in Society* 38, 33-36, 2008.
102. Savely VR, Leitao MM and Stricker RB. The mystery of Morgellons Disease, infection or delusion? *Am J Clin Dermatol* 2006, 7(1), 1-5.
103. Stricker RB, Savely VR, Saltsman A and Citovsky V. Contribution of Agrobacterium to Morgellons disease. *Journal of Investigative Medicine* 9 2007, 55 (supplement), S123.
104. Pearson ML, Selby JV, Katz KA et al. Clinical, epidemiologic, histopathologic and molecular features of an unexplained dermopathy. PLoS One 2012, 7, e29908.

105. Heinemann JA. Agapito-Tenfen SZ and Carman JA. A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessment. Environment International 2013, 55, 43-55, http://bit.ly/14i7pyG
106. Fire A, Xu S, Montgomery MK, Kostas SA, Diver SE and Mello CC. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 1998, 391, 806-11.
107. Cogoni C and Macino G. Post-transcriptional gene silencing across kingdoms. *Curr Opinion Genet Dev* 2000, 10, 638-43.
108. Hawkins PG, Sharon Santoso S, Adams C, Anest V and Morris KV. Promoter targeted small RNAs induce long-term transcriptional gene silencing in human cells. Nucleic Acids Research 2009, 37, published online 20 March 2009, doi:10.1093/nar/gkp127.
109. Alder MN, Dames S, Gaudet J and Mango SE. Gene silencing in *Caernorhabditis elegans* by transitive NA interference. *RNA* 2003, 9, 25-32.
110. Ho MW. Epigenetic inheritance, what genes remember. *Science in Society* 41, 4-5, 2009.
111. Ho MW. New GM nightmares with RNA. *Science in Society* 58, 6-7, 2013.
112. Zhang L, Hou D, Chen X. et al. Exogenous plant MiR168a specifically targets mammalian LDIRAP1: evidence of cross-kingdom regulation by microRNA. Cell Res 2012a, 22, 107-26.
113. Ho MW. How food affects genes. *Science in Society* 53, 12-13, 2-12.
114. Zhang Y. Wiggins E. Lawrence C, Petrick J, Ivashuta S and Heck G. Analysis of plant-derived miRNAs in animal small RNA datasets. *BCM Genomics* 2012, 13.
115. Thermo Scientific Tech Support. Off-target effects: disturbing the silence of RNA interference (RNAi). Tech Review. 2010, accessed 17 April 2013, http://www.thermoscientificbio.com/uploadedFiles/Resources/off-target-tech-review.pdf
116. Helwak A, Kudla G, Dudnakova T and Tollervey D. Mapping the human miRNA Interactome by CLASH reveals frequent noncanonical binding. Cell 2013, 153, 654-65. MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh
117. Ho MW. RNA interference "complex and flexible". *Science in Society* 59 (to appear).
118. Ho MW. Gene therapy nightmare for mice could human be next? *Science in Society* 31, 25, 2006.
119. Ho MW. Controversy over gene therapy breakthrough. *Science in Society* 26, 38, 2005.
120. Heinemann JA. Update on "Evaluation of risks from creation of novel RNA molecules in genetically engineered wheat plants and recommendations for risk assessment". 21 March 2013 jack.heinemann@canterbury.ac.nz
121. Ho MW. Human genome map spells end of genetic determinism. i-sis news7/8, February 2001.
122. Ho MW. Ten years of the human genome. *Science in Society* 48, 22-25, 2010.
123. Ho MW. Mystery of missing heritability solved? *Science in Society* 53, 26-27+31, 2012.
124. Ho MW. No genes for intelligence. *Science in Society* 53, 28-31, 2012.
125. Ho MW. No genes for intelligence in the fluid genome. *Adv in Child Develop and Behav* 2013, 45, 67-92.
126. Ribozyme. Wikipedia, 11 March 2013, http://en.wikipedia.org/wiki/Ribozyme
127. Ho MW. Intercommunication via circulating nucleic acids. *Science in Society* 42, 46-48, 2009.
128. Liu Y. A new perspective on Darwin's Pangenesis. *Biol Rev* 2008, 83, 141-9.
129. Ho MW. Darwin's pangenesis, the hidden history of genetics & the dangers of GMOs. *Science in Society* 42, 42-45, 2009.
130. Spadafora C. Sperm-mediated 'reverse' gene transfer: a role of reverse transcriptase in the generation of new genetic information. *Human Reproduction* 2008, 23(4), 735-40.
131. Ho MW. Epigenetic inheritance through sperm cells, the Lamarckian dimension in evolution. *Science in Society* 42, 40-42, 2009.
132. Rasoulzadegan M, Grandjean V, Gounon P, Vicent S, Gillot I, Cuzin F. RNA-mediated non-Mendelian inheritance of an epigenetic change in the mouse. *Nature* 2006, 441, 469-74.
133. Corrado C, Raimondo S, Chiesi A, Ciccia F, De Leo G and Alessandro R. Exosomes as intercellular signaling organelles involved in health and disease: basic science and clinical applications. *International Journal Molecular Science* 2013, 14, 5338-66.
134. Sahoo S, Klychko E, Thorne T. et al. Exosomes from human CD34+ stem cells mediate the proangiogenic paracrine activity. *Circ Res* 2011, 109, 724-8.
135. Bergsmedh A, Szeles A, Henriksson M, Bratt A, Foldman MJ, Spetz A-L and Holmgren L. Horizontal transfer of oncogenes by uptake of apoptotic bodies. *PNAS* 2001, 98, 6407-11.
136. Beling M and Wittrup A. Nanotubes, exosomes, and nucleic acid-binding peptides provide novel mechanisms of intercellular communication in eukaryotic cells: implications in health and disease. *The Journal of Cell Biology* 2008, 183, 1187-91.
137. Van der Vaart M and Pretorius PJ. Circulating DNA, its origins and fluctuation. *Ann N Y Acad Sci* 2008, 1137, 18-26.
138. Gahan PB, Ander P and Stroun M. Metabolic DNA as the origin of spontaneously released DNA? *Ann N Y Acad Sci* 2008, 1137, 7-17.
139. Gahan PB and Stroun M. The biology of CNAPS. In: Rykova EY, Kikuchi Y (eds) Extracellular nucleic acids. In NAMB series "Nucleic Acids and Molecular Biology". Springer, Berlin. 2010.
140. Beck J, Urnovitz HB, Riggert J, Cierici M, and Schütz E. Profile of the circulating DNA in apparently healthy individuals. *Clin Chem* 2009, 55(4), 730-8.
141. Yakubov LA, Petrova NA, Popova NA, Semenov DV, Nikolin VP and Os'kina IN. The role of extracellular DNA in the stability and variability of cell genomes. *Doklady Biochemistry Biophysics and Molecular Biology* 2002, 382, 31-4.
142. Yakubov LA, Rogachev VA, Likhacheva AC, Bogachev SS, Seeleva TE, Shilov AG, Baiborodin SI, Petroa N, Mechelina LV, Shurdov MA and Wickstrom E. Natural human gene correction by small extracellular genomic DNA fragments. *Cell Cycle* 2007, 6(18), 2293-301. Epub 2007 Jul 12.
143. Carman JA, Vlieger HR, Ver Steeg LJ, Sneller VE, Robinson GW, Clinch-Jones CA, Hayes JI and Edwards JW. A long-term toxicology study on pigs fed a combined genetically modified (GM) soy and GM maize diet. *Journal of Organic Systems* 2013, 8, 39-54.

Authors' Biographies

Mae-Wan Ho, B Sc Hon Biology (1st Class) and Ph D Biochemistry, Hong Kong University, Director and co-founder of the Institute of Science in Society (ISIS, www.i-sis.org.uk), Editor in Chief and Art Director of its trend-setting art/science magazine *Science in Society*, is on the Roster of Experts of the Cartagena Protocol on Biosafety. She is best known for pioneering work on the physics of organisms and sustainable systems; also a staunch critic of neo-Darwinian theory and early proponent of the epigenetic theory of evolution. Much in demand as a public speaker and a prolific writer, Mae-Wan has more than 170 scientific publications, 18 books, and over 650 popular articles and essays across many disciplines.

Eva Sirinathsinghi, B Sc Neuroscience, Edinburgh University and Ph D Neurogenetics, King's College, London University, is researcher at ISIS and staff writer for Science in Society since 2011. She is passionate about going beyond reductionist science to science that serves society, focusing on the effects of corporate science on people's health and welfare.

About ISIS

The Institute of Science in Society (ISIS) was co-founded in 1999 by scientists Mae-Wan Ho and Peter Saunders to provide critical yet accessible and reliable information to the public and policy makers.

ISIS aims to reclaim science for the public good; to promote a contemporary, holistic science of the organism and sustainable systems; and influence social and policy changes towards a sustainable, equitable world. ISIS is a partner organisation of the Third World Network based in Penang, Malaysia, and works informally with many scientists who are members of ISIS or of the Independent Science Panel that ISIS initiated (see below).

ISIS works through lively reports posted on its popular website www.i-sis.org.uk, archived by the British Library since 2009 as part of UK's national documentary heritage. The reports are circulated to a large e-mail list that includes all sectors of civil society worldwide, from small farmers in India to policy-makers in the United Nations. We publish an art/science, trend-setting quarterly magazine *Science in Society*, and topical in-depth, influential, and timely reports (see below) as well as monographs including *Genetic Engineering Dream or Nightmare* (1998, 1999, 2000, 2007), *Living with the Fluid Genome* (2003), *Unravelling AIDS* (2005), *The Rainbow and the Worm, the Physics of Organisms*, 3rd edition (2008); *Living Rainbow* H_2O (2012).

ISIS also initiates major campaigns from time to time:

World Scientists Open Letter, February 1999, calling for a moratorium on genetically modified (GM) organisms, ban on patents on life, and support for sustainable agriculture; eventually signed by 828 scientists from 84 countries http://www.i-sis.org.uk/list.php

Independent Science Panel, constituted May 2003, consists of dozens of scientists from many disciplines. Its report, *The Case for a GM-Free Sustainable World*, calling for a ban on GM crops and a comprehensive shift to sustainable agriculture was presented in the UK Parliament and European Parliament, circulated worldwide, and translated into 5 or more languages.

Sustainable World Global Initiative, launched April 2005, http://www.i-sis.org.uk/SustainableWorldInitiativeF.php, held its first international conference 14/15 July 2005 in UK Parliament, followed by a weekend workshop 21 January 2006, out of which came a proposal for an innovative food and energy self-sufficient 'Dream Farm 2' for demonstration/education/research purposes. Its first report, *Which Energies?*, appeared in 2006, followed by a second definitive report, *Food Futures Now* (2008) showing how organic agriculture and localized food and energy systems can provide food and energy security and free us from fossil fuels. The third and final report, Green Energies - 100% Renewable by 2050 (2009) was also launched in UK Parliament November 2009, and struck a chord among politicians and opinion formers. It marks the turning point in the world's commitment to green renewable energies.

Reclaiming Beauty and Truth in Science and Art, was launched in a unique art/science event 26-27 March 2011, when a wholefoods factory was transformed overnight into an art gallery and music/lecture hall around the theme of 'quantum jazz', the sublime aesthetics of quantum coherence in living systems and the living universe http://www.i-sis.org.uk/Avant_Garde_ArtScience_Event.php. The event was marked by a commemorative volume of essays and artworks, *Celebrating ISIS, Quantum Jazz Biology *Medicine*Art*, a *Quantum Jazz Art* DVD of artworks with a special selection of music, plus four DVDs of performances and interviews at the actual event itself. Our second act was an extended art/science/music festival, Colours of Water, 12-28 March 2013, a resounding success featuring an amazing cast of scientists, artists, musician, and other social leaders from around the world, all inspired by water and aiming to raise awareness on sustainable water use and conservation (http://www.i-sis.org.uk/coloursofwater/).

Ban GMOs *Now*
Published by ISIS 2013
www.i-sis.org.uk
ISBN: 978-0-9544923-7-3
Price: £12.00