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ISIS Report 6 December 2000

Best Practice in the Design of GM Crops

Comment on Consultation Document from Advisory Committee on Releases to the Environment (ACRE) of the United Kingdom

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- Brief Biographies
- Executive Summary
- Introduction
- Detailed comments
- Conclusion and recommendations
- References
- Appendix 1

Brief Biographies

Dr. Mae-Wan Ho, Director of the Institute of Science in Society, ISIS, gained her B.Sc. in Biology (First Class) and Ph. D. in Biochemistry from Hong Kong University in 1967, and began postdoctoral research in human biochemical genetics in University of California at San Diego. Award of a competitive Fellowship of the National Genetics Foundation, USA, enabled her to further her research in London University, UK. She became Lecturer in Genetics, then Reader in Biology, and currently, Visiting Reader in Biology at the Open University, UK, where she has continued an outstanding career in research and teaching across many disciplines including molecular genetics. She is leading exponent of a new science of the organism, which has implications for holistic health and sustainable systems, and is currently visiting Professor of Biophysics in University of Catania, Sicily.

She became scientific advisor to the Third World Network in 1994, and co-founded the Institute of Science and Society in 1999 to promote social accountability of science and science for sustainability. Her written materials on genetic engineering and related issues (including a best-selling book) have been translated into many languages; and have been used by public interest organisations all over the world in submissions to their governments and posted on many websites. She has participated in numerous debates, lectures, and interviews for radio, TV, newspapers and magazines in close to 30 countries around the world. She has more than 200 publications including 10 books.

Dr. Joe Cummin, Professor Emeritus of Genetics, University of Western Ontario, London, Ontario, obtained his B.S. degree in Horticulture, Washington State University in 1955 and PhD degree in Cellular Biology from University of Wisconsin 1962. He did his postdoctoral work, successively at Edinburgh, Palermo, Stockholm (Karolinska) and the Macardle Laboratory for Cancer Research University of Wisconsin. He taught genetics at Rutgers University, the University of Washington and University of Seattle, USA before joining University of Western Ontario in 1972. His involvement in environmental issues dates from 1968 in a range of issues including mercury, asbestos and PCB and pesticide pollution, along with waste sites and incinerators.

His critiques of genetic modification began in 1988, when he encountered the power of multinational corporations over the federal government, and the refusal of corporations to undertake serious risk evaluations. Dr. Cummins has appeared in many public lecture and debates, and has been invited to make submissions to the Canadian and US Governments. He and has published over 200 scientific and popular articles. His most recent papers appeared in *Nature Biotechnology*, *The Ecologist*, and *Biotechnology and Development Review*.

Dr. Jeremy Bartlett obtained his B. Sc. in Genetics in 1985 from the University of Aberdeen. From 1985 to 1989, he was graduate student in the Genetics Department at the John Innes Institute in Norwich (now John Innes Centre), where he specialised in the genetic control of anthocyanin (flower pigment) synthesis in *Antirrhinum majus* (snapdragon). In 1990, he was awarded a PhD from the University of East Anglia for this research.

Since 1991, he has worked in computing, but has remained very interested in biology. The introduction of GM crops into the UK renewed his interest in Genetics in 1998, and he has taken part in many public debates since. His other current interests include sustainable

methods of agriculture and food production both in the UK and abroad.

Executive Summary

The *ACRE Subgroup on Best Practice in GM crop Design* has invited ISIS to comment on a draft "Guidance on Best Practice in the Design of Genetically Modified Crops" www.environment.detr.gov.uk/acre/bestprac . One of the main 'enabling technologies' considered in the document is the 'control of gene expression', dubbed 'terminator technology' by its critics, that genetic engineers seed or pollen to be sterile. A consultation exercise is simultaneously taking place in the United States by the US Department of Agriculture, on 'terminator' patents jointly owned by the USDA and Delta and Pine Land Company. The USDA is considering commercial development of the technology http://www.usda.gov/agencies/biotech/downloads/paper72000.html.

GM crops engineered with terminator technology for seed/pollen sterility are already undergoing UK government-funded 'farm-scale' field trials in the UK. Why has this ACRE consultation not taken place before the massive field trials were approved, especially in view of the serious new hazards introduced by the technology (see below)?

The explicit aim of the UK ACRE Subgroup is to improve the safety of GM crops. The Draft Guidance admits many areas of ignorance and recommends rigorous testing of all new genes and technologies to ensure that they are safe and effective.

However, the Draft Guidance does not consider how the potential needs and benefits offered by the GM crops can be met by developing non-GM crops, or by means of alternative, sustainable agricultural practices with hundreds, if not thousands, of years of safety record behind them. Nor does the Draft Guidance address the socio-economic impacts of corporate control of agriculture through patents on seeds.

On the contrary, ACRE recommends using 'genetic protection systems' that engineer seed sterility to enforce corporate patents as a means of preventing gene transfer from GM crops. ACRE is either attempting to re-introduce a technology that even Monsanto corporation has abandoned as the result of universal rejection and condemnation, or else it is admitting that the transgenes and marker genes are unsafe, and have to be prevented from dispersal. The latter is surely a strong case for stopping GM crop development altogether, particularly, as we have argued, and as admitted by ACRE, the 'biological containment' offered by the technology is ineffective, and introduces serious new hazards.

The 'genetic protection systems' are ineffective on account of the 'leakiness' of genetic control, which is far short of 100%. Furthermore, the technology does nothing to prevent horizontal transfer of the genes. On the contrary, the increased complication of the constructs and consequent structural instability will tend to enhance horizontal gene transfer and recombination. In addition, the technology introduces significant hazards over and above those shared by all GM crops created to-date. First, the barnase enzyme encoded by the gene that makes pollen or ovules sterile is a non-specific RNAse, lethal to all cells, animals and humans included. Second, the recombinase enzyme required to control gene expression has the potential to scramble genes and genomes in unpredictable, harmful ways. Third, the

spread of sterility genes (or anther/ovule-lethal genes) will directly threaten food security and biodiversity.

We recommend the following as 'best practice' on GM crop design that ensures safety to health and biodiversity and minimises socioeconomic impacts on farmers.

1. A detailed case for the need and benefit of any GM crop should be presented before it is made.

2. No seed/pollen sterility techniques should be used, and no GM crops engineered with these techniques should be released into the environment.

3. All genes, gene products and gene constructs should be thoroughly assessed for safety before they are introduced.

4. Genes with harmful products, genes and constructs that may enhance horizontal transfer, or have other untoward consequences on genomes and organisms should not be used.

5. All antibiotic resistance marker genes should be eliminated.

6. No crop should be genetically modified to produce pharmaceuticals or industrial chemicals. The best practice is to use plant cell culture under strictly contained conditions.

7. No superfluous sequences, or uncharacterised sequences, should be included in any GMO destined for release into the environment.

8. No GM crop should be released into the environment unless it can be thoroughly identified and characterised, using the state-of-the-art molecular methods, with respect to unintended effects, as well as genetic uniformity and stability of the insert(s) for at least 5 successive generations.

9. Transformations should be precisely targeted as well as stable.

10. All patents on GM seeds should be revoked and banned.

11. Research on the safe design and construction of GM crops should be carried out by independent scientists, not subjected to any pressure to commercialise prematurely.

Introduction

The ACRE Subgroup on Best Practice in GM crop Design has invited ISIS to comment on a draft "Guidance on Best Practice in the Design of Genetically Modified Crops" www.environment.detr.gov.uk/acre/bestprac/. One of the main 'enabling technologies' considered in the document is the 'control of gene expression', dubbed 'terminator technology' by its critics,

that genetic engineers seeds or pollen to be sterile. A consultation exercise is simultaneously taking place in the United States by the US Department of Agriculture, on 'terminator' patents jointly owned by the USDA and Delta and Pine Land Company. The USDA is considering commercial development of the technology
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GM crops engineered with terminator technology for seed/pollen sterility are already undergoing government-funded 'farmscale' field trials in the UK (Aventis' spring and winter GM oil seed rape). We question why this ACRE consultation has not taken place before the field trials were approved, especially in view of the serious new hazards introduced by the technology, as we shall describe in detail.

The letter accompanying the ACRE consultation states,

> "The aim of the subgroup is to consider how the design and construction of GM plants might be used to further improve their safety and/or to simplify the risk assessment. For example, by preventing or minimising cross-pollination, avoiding antibiotic resistance marker genes or switching on inserted genes only when and where they are needed in the plant."
>
> "This guidance is particularly aimed at practitioners developing GM plants for commercial use. It is intended to be proactive. It establishes some general principles of best practice and reviews technologies that might enable these principles to be applied in the construction of the next generations of GM crops. The advice is based on experience gained from past applications to market GM crops in Europe, knowledge of emerging technologies and direct consultation."

The document offers no guidance on the socio-economic impacts, especially those resulting from GM patents and GM sterile seeds, both of which prevent farmers from replanting harvested seeds. Socio-economic impacts are part of risk assessment in accordance with the Cartegena Biosafety Protocol negotiated in Jan. 2000. As the summary of the Draft Guidance admits, "Where novel technologies have been developed, intellectual property rights may restrict access and have a large impact on how widely they are employed."

Our comments are mainly directed at the points raised in the Draft Guidance. For a more thorough representation of our views on agricultural biotechnology, please see World Scientists Statement, and Open Letter from World Scientists to All Governments posted on ISIS' website <www.i-sis.org.uk>.

Detailed comments

1. ACRE's Draft Guidance comes in four sections. Section 1 Aims and Scope of the Guidance, Section 2 Philosophy of Best Practice, Section 3 Best Practice, and Section 4 Enabling Technologies.

2. The intention of the Draft Guidance is to improve on safety to health and biodiversity. Significantly, it states in item 1.2, "Consent [on releases to the environment] will be issued only if ACRE considers that a proposed release will be *safe*." (italics ours)

3. Section 2 begins appropriately with uncertainty and the precautionary principle. However, we question whether safety assessments made on the basis of "the best

scientific evidence available at the time" (item2.1) is in accordance with the precautionary principle. As stated in paragraph 1.2 of the Draft Guidance, the scientific evidence required must indicate that the proposed GMO is *safe*. But to this day, "the best scientific evidence available" on the safety of GMOs turns out to be no evidence at all, at least, none that would stand up in a court of law or to scientific scrutiny. The industry must be vigorously challenged to provide such evidence, and make it widely available for public as well as scientific review.

4. In order to ensure that industry must provide evidence that the proposed GMO is safe, the first sentence of item 2.1 should state, "Safety assessments of GMOs are made on the basis of *all necessary scientific evidence indicating that the proposed releases are safe beyond reasonable doubt*."

5. Item 2.2 states "..there is no scientific evidence that demonstrates transfer of functional genes from plant material to bacteria in the environment." The qualifiers, 'functional' and 'in the environment' are typical of the misleading statements that allow regulatory bodies to ignore relevant scientific evidence (reviewed in reference [1]). First, transfer of *functional* genes from GM plant material to bacteria has been demonstrated in the laboratory suggesting at the very least that the same can occur in the environment. Second, experimental findings show that transfer of GM DNA, if not functional genes, may have occurred from GM plant residue to soil bacteria in the field, and from GM pollen to bacteria and yeast in the gut of bee larvae.

6. ACRE was right to reject GM plants that contained specific antibiotic resistance genes and to commission further research. We recommend that ACRE should reject *all* antibiotic resistance marker genes. The fact some antibiotic resistance is already widespread is not a reason to exacerbate the problem by large-scale release of the genes into the environment. More importantly, ACRE should interpret and accept scientific evidence itself in accordance with the precautionary principle. The absence of evidence is not evidence of absence, and the failure to show something is harmful is not evidence that it is safe, as argued in detail in an article by Dr. Peter Saunders, Professor of Mathematics, King's College, London [2].

7. We most definitely support the strict requirement for detection methods and unique identifiers of GMOs (item 2.3). ACRE should insist on detailed molecular genetic data documenting the structural as well as functional stability of transgenic insert(s) over at least five successive generations as argued elsewhere [3, 4]. We are aware of no such data on any GM line that has been released to the environment to-date. On the contrary, there are sufficiently numerous reports on the instability of transgenes and transgenic lines to make us suspect the worse [5-7].

8. The most glaring omission in environmental risk assessment (item 2.4) is the potential hazards of horizontal gene transfer (reviewed in ref. 1). This is inexcusable, particularly in view of ACRE's admission that antibiotic resistance genes may spread to bacteria in the environment.

9. Item 2.5 states, "Harm may result if hazards are realised. Risk assessment evaluates the likelihood of realisation and what the consequences will be; risk is therefore a product

of these two quantities." The statement is technically correct. Unfortunately, the likelihood of realisation -- the probability that the event will occur -- is impossible to evaluate in principle, due to the contingency of largely unknown, unpredictable natural conditions.

10. Nevertheless, the *hazards* can be identified unambiguously and supported by reasonable circumstantial and indirect evidence, and horizontal transfer of GM genes is a case in point (reviewed in ref. 1 and ref. 8). GM constructs contain new combination of genes, many from bacteria viruses, plasmids and transposons, including antibiotic resistance genes. GM constructs are designed to cross species barriers and to invade genomes, and they share homologies (similar base sequences) with a wide range of bacteria and viruses. All of these factors will facilitate horizontal gene transfer and recombination. There is already overwhelming evidence that horizontal gene transfer and recombination are responsible for creating deadly new viruses and bacteria and spreading drug and antibiotic resistance.

11. The excision of antibiotic resistance marker genes after they have served their function, recommended in item 2.10, is desirable, *provided it can be done precisely, and demonstrated to be done precisely*. We do not believe this is achieved in the current state of the technology, as our review (3.9) of relevant papers show.

12. The recommendations in item 2.11 should be made stronger, to *avoid* and not just minimise superfluous transgenes and sequences, *whether expressed or not*, as it is relatively easy for a gene to regain expression on being transferred horizontally and recombined. Special mobile units called *integrons* have sites that accept promoterless genes, so that the integrated gene is provided with a ready-made promoter to become expressed (see ref. 8). We must avoid dispersal of transgenes in the environment by horizontal gene transfer as well as by cross pollination. Practically, none of the means proposed by ACRE to avoid dispersal of transgenes, actually prevents horizontal gene transfer.

13. Item 2.13 states, "As we improve our understanding of plant molecular genetics, the technology for genetic modification of plants should become increasingly precise and more predictable in its outcome. This is in contrast to the complexity of predicting environmental impacts and their significance." These statements are an admission that current GM technology is imprecise and unpredictable. However, they should not be taken to mean that environmental impact assessment becomes unnecessary once GM becomes more precise and predictable. New genes and gene-combinations are still being introduced into crops, and new crops are still being released into the environment, all of which have to be subject to appropriate risk assessment.

14. The intention to adopt ‘bio-containment techniques’ is first raised in item 2.14. We note that bio-containment techniques have been used to ‘cripple’ bacterial strains in the laboratory, so that even if released into the environment, they would not be expected to survive. However, such bio-contained bacteria have now been shown to survive outside the laboratory, or to go dormant and come back with a vengeance, after acquiring genes to enable them to survive (see reference [8]). This should make us wary of the efficacy of bio-containment.

15. We agree with the good reasons offered to ‘minimise’ extraneous DNA (paragraph 3.2) although it would be better to eliminate extraneous DNA altogether. The reasons given are that,

    - it facilitates analysis (characterisation, including sequencing) of the insertion site;
    - it aids the monitoring of stability and inheritance of the transgene;
    - it reduces the chances of pleiotropic effects (ie, those due to gene interactions);
    - it simplifies the environmental risk assessment;
    - it removes one of the main criticisms of the technology regarding the propagation of plants containing antibiotic resistance genes and other marker traits e.g. herbicide tolerance.

16. Another important reason to minimise extraneous DNA left out in the Draft Guidance is that the extraneous sequences themselves may be unsafe, as for example, the origins of replication of plasmid vectors, which have often been included in GM crops. These will facilitate the maintenance and amplification of the transgenic DNA in bacteria to which the transgenic DNA is transferred. Extraneous unknown, uncharacterized sequences may also contain virulence genes that cause diseases.

17. Item 3.3 mentions ‘seed sterility traits’. These are part of the ‘terminator technology’ thoroughly rejected by all Third World Governments and non-Government organisations, on grounds that they are against the interests of farmers, so much so that the Monsanto corporation has announced it will not commercialise the technology. Terminator technology should not be resuscitated under the guise of preventing gene flow. It will not prevent gene flow; but will introduce new hazards other than those ACRE is attempting to address (see below).

18. Item 3.4 suggests that GM constructs could be made to enable subsequent excision of extraneous sequences. One such method does indeed include terminator technology, in which site-specific recombination by a recombinase enzyme encoded by a transgene is used to splice out extraneous DNA. However, in a detailed review [9] of that paper, we show that significant, non-specific, non-target splicing has most likely taken place, resulting in genomic rearrangments and deletions. But the authors failed to investigate non-target effects. So-called site-specific recombinases are by no means completely specific or precise.

19. Similarly, biolistic methods of transformation are already known to introduce many rearrangments, repeats and deletions, even before integration takes place, leading to multiple insertions of repeated, rearranged sequences which cannot be properly characterised [10-13].

20. Item 3.5 advises screening to discard, at an early stage, those transformants with "unwanted vector sequences, such as those from outside the [*Agrobacterium*] T-DNA, especially plasmid replication origins and antibiotic resistance genes". Unfortunately, ACRE has already approved such crops for environmental releases and for the UK National Seed List, as in the case of Chardon LL [14]. ACRE should insist on much stricter molecular characterisation and criteria for approval consistent with their statement here.

21. Incorporation of transgenes into chloroplasts, as suggested in item 3.6, does not prevent gene transfer by pollen. That is because most pollen carries chloroplasts, as pointed out by one of us [15]. Homologous recombination is ideal, if it can be achieved, whether in the chloroplast or in the genome. But we know of no documented case of this being achieved in plants so far. 'Chimeroplasty' [16] using RNA-DNA hybrid 'hairpins' to base pair with specific gene sequences, claims to achieve site-specific mutations; but the actual results do not support the claims made, and no investigations on non-targeted mutations have been carried out.

22. Item 3.8 questions whether crops producing pharmaceutical products should be physically contained. *We believe all crops producing pharmaceuticals or industrial chemicals should be strictly contained*. Better still, plant cell cultures, rather than crops should be used for such purposes under strictly contained conditions.

23. Item 3.10 suggests various means of genetic isolation for GM plants, such as exploiting difference in flowering time, or using varieties naturally unattractive to insects. It should be pointed out that none of those means of isolation are completely effective, and *horizontal gene transfer to unrelated species is not eliminated by any means proposed in the Draft Guidance*.

24. Item 3.11 states, "Transgenic plants that cannot produce pollen already exist and have been developed to facilitate hybrid seed production. The production of transgenic plants that produce sterile seed is also feasible and this technology has been developed as a gene protection system to secure intellectual property rights. Both systems could also be used for risk management purposes. The benefit of linking a trait gene to a sterility gene to arrest pollen or seed development is that the frequency of both genes declines in subsequent populations as strong selection against them occurs. This happens because plants that inherit these genes do not produce pollen or seed." It is clear that ACRE is intending to use the universally condemned terminator technology as a means to prevent plants either from setting seeds or producing fertile pollen. And it is also clear from the next paragraph that ACRE is considering the widespread adoption of this technology.

25. Items 3.14-16 consider means of minimising unnecessary transgene expression, so that expression will only occur in specific tissues as required. The one concrete example cited is in item 3.16, "The use of promoters that are induced by chemicals, for example, offers the potential to regulate or control the fertility of a crop. Such systems could be manipulated so that crops that do not produce pollen (male sterility) are the norm and fertility is restored by treatment with a specific chemical. Thus, breeders and seed producers can carry out their work with the plant variety, but equally [sic], farmers can use the same variety in the sterile phase, minimising any potential risks to the environment." This is nothing other than the plant protection system that protects corporate intellectual property rights over the farmer's right to replant harvested seed. And, contrary to ACRE's claim, it does not minimise potential risks to the environment (see later).

26. The details of the pollen/seed sterility system and its specific hazards are described in Appendix 1, this Comment. We have presented it in the interest of clarity and

transparency, as the ACRE document has chosen not to give any relevant technical details. This is most unsatisfactory, as the public, are, in effect, being asked to comment without relevant knowledge and understanding.

27. As made clear in Appendix 1 of our Comment here, the pollen/seed sterility system is ineffective on account of the 'leakiness' of genetic control, which is far from 100%. Furthermore, the technology does nothing to prevent horizontal transfer of the genes. On the contrary, the increased complication of the constructs and consequent structural instability will tend to enhance horizontal gene transfer and recombination. In addition, the technology introduces significant hazards over and above those shared by all GM crops. First, the barnase enzyme encoded by the gene that makes pollen or ovules sterile is a non-specific RNAse, lethal to all cells, animals and humans included [17]. Second, the recombinase enzyme required to control gene expression has the potential to scramble genes and genomes in unpredictable, harmful ways [9]. Third, the spread of sterility genes (or anther/ovule-lethal genes) will directly threaten food security and biodiversity.

28. Items 4.4 and 4.4a-d consider some alternatives to antibiotic resistance marker genes. 'Reporter' genes such as b-glucuronidase and the green fluorescent protein, which give visible signs of transformation, are already in use, but they do not offer the advantage of agents that kill all untransformed cells, leaving only the few that are transformed. Resistance to cytotoxic agents (cell poisons) other than antibiotics, does not always work in plants. Herbicide tolerance (HT) traits, not intended for agronomic use, might "tempt growers to use the HT trait inappropriately". All of the above should be vigorously assessed for safety, as ACRE points out. The safest approach appears to be auxotrophic (metabolic) markers. For example, the enzyme, phosphomannose isomerase (PMI) is not present in most plant cells. It converts mannose-6-phosphate to fructose-6-phosphate, thereby enabling plant cells to metabolise mannose. Nevertheless, potential toxic or allergenic changes in plant metabolism may result from this genetic modification, and should not be ignored, as ACRE makes clear.

29. Items 4.5 and 4.5a-c deal with technologies for removing extraneous DNA in GM plants. The first method is to make unlinked constructs of transgenes and antibiotic resistance marker genes, and transform plant cells simultaneously with them, so that the marker genes can be bred out of the co-transformed lines in later generations. The second method is to locate the marker gene on a transposon, which can be induced by the activity of an introduced transposase enzyme, to jump to another site and be selected out in later generations. The third method is to use site-specific recombination to excise the antibiotic resistance marker gene, by putting the latter between two sites recognised by the recombinase. The first two methods cannot be used in plants that have long generation times and depend largely on asexual propagation, such as trees.

30. We have already pointed out the hazards of site-specific recombination earlier. The second paragraph of item 4.5c states that risk assessment should include "potential unintended recombinase-mediated rearrangements". Transposons and transposases have similar effects in scrambling genomes, as stated in item 4.5 "There may be rearrangements at the site of transposition. Therefore rigorous molecular data will be required to define the site of insertion, confirm the absence of unwanted sequence and

that rearrangement have [sic] not occur."

31. Items 4.6 and 4/6a-e consider control of flowering and fertility in crop plants to minimise transgene dispersal. Methods include ‘apomixis’, the production of seeds without fertilisation, ‘cleistogamy’, the failure of flowers to open, ensuring self-fertilisation without pollen escape, strengthening hybridisation barriers between species, inhibiting flowering and finally, genetic engineering male sterility. Of these, only the last is "available now" though still "requiring further development". We have already explained in Appendix 1 why it should not be pursued. Item 4.6e points out, "the [male sterile] crop can still be fertilised by pollen to produce a hybrid" and hence gene escape *can* occur.

32. Seed sterility is considered in items 4.7 to 4.9, but no details on the technology involved are given. Item 4.9 states that "The benefit of linking a transgene to a sterility gene is that the frequencies of both decline in the population simply because of selection against the sterility gene due to the fact that plants that inherit these genes do not produce viable seed." However, it goes on to admit that there will be a "background frequency of legitimate pollination and seed set", ie, the system will be leaky, and where gene flow is high, this could "significantly affect population viability".

33. Plastid (chloroplast) transformation technology is considered in items 4.10 and 4.11. It offers two potential advantages, first, in being more precise, as complete nucleotide sequences of 16 chloroplasts genomes have already been determined, and second, that it may limit transgene dispersal through pollen, although it is admitted that some paternal (pollen) transfer does occur [15]. Chloroplast genes have the advantage of being able to create elevated gene dosage without the problem of ‘dosage compensation’ encountered in nuclear genes (which leads to inactivation of extra gene copies). However, pollen does contain chloroplasts and pollen is the primary transmitter of chloroplast genes in gymnosperms (to which pines and other confers belong) and some angiosperm species. Many angiosperm species transmit chloroplasts through both pollen and egg, while others are solely maternal in transmission but under environmental stress from, for example, near-ultraviolet radiation or herbicide exposure, chloroplast transmission becomes paternal or biparental.

34. Strategies to minimise transgene expression are considered in items 4.12 and 4.12a-c. The gene excision systems, mentioned in 4.12a, is site-specific recombination. Here, ACRE makes clear it is aware of the new hazards involved. The second paragraph states, " . . . the [excision] process would have to be 100% efficient, or specific acceptability levels of non-excision would have to be set. Another problem may result if the excised gene were to reintegrate at another site." And again in the next paragraph, "..the recombinase gene may remain in the plant line and result in recombination at other sites in the genome, a possibility that carries uncertainty about its subsequent effects. Therefore, it may be desirable to remove ..the introduced recombinase. The risk assessment will have to consider the possibility of less than 100% excision efficiency and possible rearrangements and their effects." We explain these effects in Appendix 1 of our Comments here.

35. Item 4.12b, significantly, considers introducing introns and chloroplast sequences as

biological containment to prevent these from being expressed in the "unlikely event of environmental gene transfer from GM-plants to bacteria". This is the only technology addressing horizontal gene transfer in the entire document. We re-iterate that in our view, the evidence for horizontal gene transfer is sufficiently compelling for it to be taken seriously into account in risk assessment, particularly in accordance with the precautionary principle.

36. Item 4.12c considers chemically inducible promoters, and cautions that "The reliability of these systems will need to be demonstrated so that for example, fertility is not restored by freak environmental conditions in the field." Such a possibility is likely in view of the 100 000 industrial and agricultural chemicals that currently pollute our environment, and our knowledge of naturally occurring phyto-chemicals is woefully inadequate.

Conclusion and recommendations

ACRE's attempt to improve the safety of GM crops through a consideration of design and construction technologies is to be welcomed. The Draft Guidance admits many areas of ignorance and recommends rigorous testing of all new genes and technologies to ensure that they are safe and effective.

However, ACRE does not consider how the potential needs and benefits offered by the GM crops can be met by developing non-GM crops, or by means of alternative, sustainable agricultural practices with hundreds, if not thousands of years of safety record behind them. Nor does the Draft Guidance address the issue of corporate control of agriculture through patents on seeds.

On the contrary, ACRE recommends using 'genetic protection systems' that engineer seed sterility to enforce corporate patents, dubbed 'terminator technology' by its critics, as a means of preventing gene transfer from GM crops. ACRE is either attempting to re-introduce a technology that even the Monsanto corporation has abandoned as the result of universal rejection and condemnation, or else it is admitting that the transgenes and marker genes are unsafe, and have to be prevented from dispersal. The latter is surely a strong case for stopping GM crop development altogether, particularly, as we have argued, and as admitted by ACRE, the 'biological containment' offered by offered by the technology is ineffective, and introduces serious new hazards.

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We recommend the following as 'best practice' on GM crop design that ensures safety to health and biodiversity and minimises socioeconomic impacts on farmers.

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9. Transformations should be precisely targeted as well as stable.

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*** References to some of our own papers are contained in the following supplements enclosed:**

S1. Horizontal gene transfer-hidden hazards of genetic engineering by Mae-Wan Ho (ref.1)

S2. *ISIS News#6*, refs. 2, 6, 17.

S3. *ISIS News#5*, ref. 7.

S4. *ISIS News#3*, ref. 9.

S5. Fatal flaws in food safety assessment, by Mae-Wan Ho and Ricarda Steinbrecher, ref. 4.

S6. Biosafey Alert by Mae-Wan Ho, ref. 4.

S7. Gene technology and gene ecology of infectious diseases, by Mae-Wan Ho, *et al*, ref. 8.

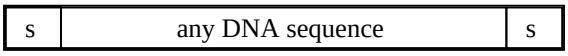
S8. The cauliflower mosaic viral promoter, a recipe for disaster? By Mae-Wan Ho, Angela Ryan and Joe Cummins, ref. 12.

S9. Hazards of transgenic plants with the CaMV promoter , by Mae-Wan Ho, Angela Ryan and Joe Cummins, ref. 13.

Appendix 1

The seed/pollen sterility systems and specific hazards involved

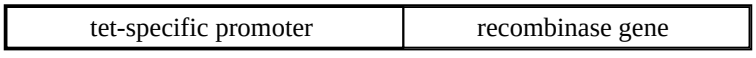
The seed/pollen sterility systems consist of two key elements. The first is 'site-specific recombination', carried out by a recombinase enzyme that recognises specific 'sites', or short DNA sequences, labelled 's' in the diagram below. Any stretch of DNA sequence flanked by two such sites will be spliced out by the recombinase.



The other key element is barnase, an enzyme breaking down RNA, *which is lethal to all cells in which it is expressed*, unless its specific inhibitor, barstar, is also present in the cell. The *barnase* gene is placed next to the transgene of interest, say, a gene coding for herbicide tolerance. One way to engineer pollen sterility is to place the barnase gene under the control of a promoter that works only during anther development. Theoretically, there will be no fertile pollen from this transgenic crop. In the case of crops that are normally self-fertilized, there will be no seeds set. Otherwise, the only fertile seeds set will be those fertilized by non-GM varieties nearby, which will not be herbicide tolerant; so farmers who want the herbicide tolerant trait, will have to buy fresh seeds from the company every season.

To propagate the line, the company may make use of site-specific recombination. For example, the promoter of the barnase could normally be blocked by a sequence flanked by sites recognised by a recombinase

The recombinase can be engineered into the same transgenic line, or it could be introduced by crossing the GM line containing barnase with another that contains the recombinase to generate a hybrid. The recombinase is placed under the control of a promoter that responds to an external chemical, say, the antibiotic tetracycline.



When tetracycline is applied, the recombinase is expressed, splicing out the blocking sequence in the barnase promoter, so barnase is expressed. By treating harvested seed with tetracycline before they are sold to the farmer, the company can ensure that the plants grown from the seeds will be pollen sterile.

If female-sterility is required, the barnase gene could be placed under the control of a promoter that works only during ovule development, and the rest is similar.

Alternatively, the recombinase is engineered into a GM line with the gene coding for barstar, which, when crossed with the GM line containing barnase, will produce a hybrid. The hybrid treated with tetracycline, will produce plants that will set seed, because the barstar inactivates the barnase. However, if the farmer tries to resow the harvested seeds, he or she will find that only about half (7/16) of the seeds will have the same characteristics as those he bought from the company, and about one fifth (3/16) of the seeds may be completely sterile.

This system is ineffective for preventing gene flow for the following reasons:

a. All gene control systems are known to be 'leaky' in the sense of not being 100% effective, and the proposed system is no exception, particularly as so many elements have to be engineered perfectly, which is beyond current capability. As a result, some fertile pollen/seeds are very likely to be produced.

b. Pollen sterile GM plants can still be fertilised by non-GM pollen, just as GM pollen from ovule-sterile plants can cross with non-GM plants, thus enabling gene escape.

c. Horizontal gene transfer is not at all prevented by this system, if anything it may be enhanced due to increased structural instability of the complicated constructs involved. Horizontal gene transfer to bacteria and viruses in all environments can be envisaged. Plant residues, dust and pollen may all contribute. Insect pollinators or feeders may also be significant vectors for horizontal gene transfer.

Significant hazards are introduced by this system, over and above those due to GM crops in general.

a. Barnase is a potent RNAse that breaks down RNA indiscriminately, and is known to be lethal to all cells, animals and humans included. *It should not be permitted in any GM crop, let alone GM crop intended for animal feed or human food*.

b. The 'site-specific' recombinases are known not to be 100% specific. There is already evidence suggesting that unintended rearrangements and deletions of genomic sequences have resulted from the use of such recombinases (9). In other words, the recombinases have the potential to scramble genomes in unpredictable, harmful ways.

c. The increased complication of the transgenic constructs will only increase structural instability and horizontal gene transfer.

d. Transfer of sterility genes will have drastic consequences on agriculture on biodiversity.

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