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Status and risk assessment of the use of transgenic arthropods in plant protection

Proceedings of a technical meeting organized by the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture and the Secretariat of the International Plant Protection Convention, held in Rome, 8 to 12 April 2002

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FOREWORD

New developments in the modern biotechnology have opened up the possibility of introducing genes into the germline of many insect species, including those of agricultural importance. This technology offers the potential to improve current pest control strategies that incorporate the Sterile Insect Technique (SIT). Potential improvements include the development of strains that (1) produce only male insects for sterilization and release and (2) carry a marker that distinguishes them from wild insects.

There are many institutions involved in the development of transgenic insect technology both for studies on basic gene regulation and for the creation of transgenic strains for use in a wide range of insect control programmes. It has been realized that the release into the environment of transgenic insects will not be an easy process considering the current public sensitivities in this area. The fact that insects are mobile and that once released cannot be recalled creates much concern. If fertile transgenic insects were to be released in any type of control programme, then the transgene would enter the wild population through mating. This strategy is fraught with, as yet, unknown risks and it is inconceivable that regulatory approval will be given for such a release in the near future. However, when transgenic strains are integrated into a sterile insect release then the concerns about transmission of the transgene to the wild population disappear as the matings between the released and the wild insects are sterile. This scenario is likely to be the first type of transgenic release.

Insects that are currently released in SIT programmes experience no significant regulatory problems, but this will not be the case if the insects that are released are transgenic, even if they are sterile. The meeting Status and Risk Assessment of the Use of Transgenic Arthropods in Plant Protection held in FAO Headquarters, Rome, in April 2002 was the first effort to bring together scientists and regulators in order to discuss risk assessment and regulation of transgenic insect release.

The event was organized by the Secretariat of the International Plant Protection Convention (IPPC), the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA). The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture is involved with the development of the transgenic technology and the IPPC would be involved with the regulation of any release of transgenic insects.

The objective of the meeting was to: (1) review the current state of the art of transgenic technology; (2) review the current regulatory framework in different countries; and (3) develop a set of guidelines for risk assessment of transgenic insects.

The Scientific Secretaries of the meeting were A. Robinson (IAEA) and C. Devorshak (IPPC). The IAEA officer responsible for this publication was A. Robinson of the FAO/IAEA Agriculture and Biotechnology Laboratory, Agency's Laboratories, Seibersdorf.

EDITORIAL NOTE

CONTENTS

1. SUMMARY

Genetic transformation technology is now routine for many arthropod pest species for which the Sterile Insect Technique (SIT) is being implemented and improvements in efficiency and application will continue to be made. Transformation vectors are functional in many arthropod orders and this technology can probably now be applied to any insect pest or biocontrol agent. However, major technical problems concern the stability of the transgenic strains, both genotypic and phenotypic, and their ability to express the transgene in a reliable and predictable way. A second area of concern is the biological fitness of transgenic strains when they have to compete with individuals in the field. However, of much broader significance in the whole area of trangenesis is the regulatory framework. The release of genetically modified insects will require a thorough risk assessment protocol, moving from the laboratory through field cages to open field release. The report deals not so much with the technical issues related to the development of tarnsgenic strains but focuses more on the development of a regulatory environment in which an appropriate risk assessment framework could be developed. This emphasis was reflected in the background and expertise of the consultants.

A joint meeting sponsored by the Secretariat of the International Plant Protection Convention (IPPC), the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA) brought together both scientists involved in transgenic technology and experts in the field of risk assessment and regulatory procedures. Three Working Groups (WG) were formed to discuss and report on the following topics:

- **WG1: Current Status of Transgenesis in Pest Arthropods**
- **WG2: Identification of Risks Associated with a Transgenic Release**
- **WG3: Development of Risk Assessment Protocols**

The problem of introducing genes into the germline of most arthropods has been essentially solved through the isolation of improved transformation vectors and better markers for the selection of transformed individuals. A major new thrust will be to develop transformation vectors that become immobilized following integration, which will go some way to satisfying both the needs of the end-user and the concerns of the environmental groups. Various ways to achieve this were discussed. The working group dealing with the transgenic technology identified this as a high priority.

A second scientific concern relating to any application of transgenic arthropods is the biological fitness of transgenic strains. Very little is known about this in arthropods despite the fact that transgenic strains have been available for many years. Initial data reported during the meeting suggested that transgenic strains can show considerably reduced fitness. Several more recent reports have confirmed this and it will be necessary to develop a substantial set of quality control (QC) protocols for any transgenic strain.

The regulatory aspects for any eventual release of transgenic arthropods are problematical to say the least, considering the current public perception of transgenic technology in general. Concerns related to horizontal transmission and impact on biodiversity will be at the forefront of interactions between scientists, regulatory authorities and various interest groups.

Two scenarios were used by the working groups to provide a framework for discussions and to try to create some commonality of understanding with the two scenarios having quite different risk associated factors. The first scenario was the release of sterile Mediterranean

fruit flies carrying a fluorescent marker gene and the second was the release of fertile mosquitoes carrying a gene conferring refractoriness to malaria with the aim of spreading that gene in the population. The strategy enabled the key risk elements of any future transgenic arthropod release to be identified although it was recognized that there will be specific constraints related to the biology and ecology of each particular species and regulatory approval is likely to be on a case-by-case basis. The vehement opposition to any use of transgenic organisms by many NGOs was noted and it was concluded that the lack of any common ground makes it currently difficult to enter into a dialogue with some of these groups.

Using the scenarios described above, each working group developed a working paper that is included in this publication. Rather surprisingly, the majority of risks identified for the two scenarios were common and there were very few concerns that were specific for the release of the fertile transgenic arthropods. The need/desirability of forming an IPPC expert working group to draft standards related to the use of transgenic arthropods in agriculture was also discussed.

It was very important that experts in both the technology of transformation and regulatory and risk assessment were present at the meeting. This publication of the outputs of the meeting will provide the basis for the rational development of the use of transgenic arthropods in plant protection.

2. INTRODUCTION

Insect pest control in agriculture and human health still relies very heavily on the use of chemical toxicants whether provided as a conventional application or delivered through transgenic plants. In fact world pesticide consumption is growing at ca 5% a year bringing with it the well-documented problems associated with environmental pollution, food safety, resistance and destruction of natural enemies. The intensification of cropping, reduced crop rotations and increased use of monocultures will create further biological imbalances of the agricultural environment. Future pest control will have to be conducted without impairing biological diversity and degrading the environment and with much less reliance on pesticides. Increased trade and movement of people and agricultural products will also facilitate the introduction of exotic pests into agricultural ecosystems. Current bio-rational approaches to insect control include use of predators and parasitoids, microbial and botanical insecticides, pheromones, insect growth regulators and the SIT.

The use of transgenic technology in insect control is currently restricted to the use of transgenic plants producing insecticidal proteins. In total there are about 44 million hectares planted with transgenic crops of which 25% are insect pest resistant crops. In the US there has been an overall reduction in insecticide use of about 1.2 million kg/year due to the use of these crops. Plant transformation technology began in the early 1980s and was rapidly adopted by industry as a very valuable new tool in plant breeding as the transformation system was of general applicability in many plant species.

The first insect to be transformed was *Drosophila melanogaster* in 1982 and this was followed by many attempts to use the same transformation system in different arthropod species. All these attempts failed and it has been necessary to identify new systems that now have been shown to have a much wider range of application than the original system developed in Drosophila. The first pest arthropod, the Mediterranean fruit fly, *Ceratitis*

capitata, was transformed in 1995 but over the past 3–4 years there has been rapid progress in the development of transformation technology for many pest arthropods.

To date, more than 12 different pest arthropod species have been successfully transformed. Considering the nature of the transformation vectors that are being used, there is little doubt that most pest species will be amenable to this technology, if required. The technology is being developed in the context of expanding the range of pest intervention strategies, some of which have already been validated in Drosophila as proof of principle and are now being tested in pest species. It is therefore, an opportune moment to review the field and to consider, how this technology needs to be regulated to facilitate its transfer to application in plant protection.

The proposed use of transgenic arthropods in pest control strategies has both scientific and regulatory concerns and both these issues are addressed in this document. There are two immediate scientific concerns related to the process of transformation, namely genomic stability of the transgene and its consistent expression in time and space. Current information is insufficient to provide an adequate understanding of this process in pest arthropods. Failure of transformed strains to maintain the stability and expression of the transgene will severely compromise this new approach to pest control. However, a stable and predictable transformation system is only the first step in the use of this technology; the most challenging component is the identification of transgenic constructs that in themselves can contribute to a viable pest control strategy.

Probably the most suitable pest control strategy for the integration of transgenic technology is the SIT, where the presence of the transgene in the environment is limited to the sterile flies that are released. It is now possible to introduce a genetic marker into pest arthropods that would greatly facilitate the evaluation of field SIT programmes. In the SIT there is no vertical transmission of the transgene to the population in the field.

Any uses of this technology will require the release of transformed arthropods into the environment and this demands the development of a strict regulatory framework. Some progress has been made on this at the national level in some countries. Ideally this framework would be developed by an international regulatory body and will involve aspects of risk identification, risk assessment and risk management.

3. THE MEETING

A meeting was convened in FAO, Rome, from 8–12 April 2002 to address the issues described above. The goals of the meeting were: (1) to review the current status of transgenesis in pest arthropods, (2) to identify risks associated with the release of transgenic arthropods and (3) to draft provisional risk assessment protocols. The meeting was organised by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, IAEA, Vienna and IPPC Secretariat, Rome. It was attended by experts in the field of arthropod transgenesis, risk assessment and regulatory procedures. The working papers are summarized in this report. Three Working Groups were formed to discuss and report on the following topics:

- **WG1: Current status of transgenesis in pest arthropods**
- **WG2: Identification of risks associated with a transgenic release**
- **WG3: Development of risk assessment protocols**

Two scenarios were used by the working groups to provide a framework for discussions and to try to create some commonality of understanding with the two scenarios having different risk associated factors. The first scenario was the release of sterile Mediterranean fruit flies (*C. capitata)* carrying a fluorescent marker gene and the second was the release of fertile mosquitoes carrying a gene refractory to malaria with the aim of spreading that gene in the population.

4. SUMMARIES OF THE WORKING PAPERS

4.1. Overview

The problem of introducing genes into the germline of most arthropods has been essentially solved through the isolation of improved gene vectors and the use of better dominant markers for the selection of transformed individuals. A major new thrust will be to develop gene vectors that become immobilized following integration, which will go some way to satisfying both the needs of the end-user and the concerns of the environmental groups. A second scientific concern relates the biological fitness of transgenic strains. Very little is known about this in arthropods and it will be a major factor during evaluation of any strain. It will be necessary to develop a substantial set of quality control protocols for the evaluation of each transgenic strain. Currently every transgenic strain results from a specific integration event and hence is unique.

Current national regulatory processes, including the availability of suitable risk analysis protocols, may be insufficient to address any eventual release of transgenic arthropods. Likewise, the current negative public perception of transgenic technology in general will make the development and use of transgenic arthropods in pest control potentially difficult. Concerns related to horizontal transmission and any impact on biodiversity will have to be addressed during discussions between scientists, regulatory authorities and various other stakeholders. The participants concluded that regulatory approval of any transgenic arthropod release will be on this case-by-case basis. With transgenic technology, Intellectual Property Rights (IPR) of the strains will need to be addressed and the commercial deployment of a transgenic strain will require a complex set of negotiations related to licensing and royalty payments.

4.2. Arthropod transgenesis

The SIT could be the first insect control technique that can benefit from the integration of transgenic technology as there will be no transmission of any transgene to the field population as the released insects are sterile. Transgenic technology could be used to: (1) introduce of a marker gene into released insects, (2) produce male only strains and (3) induce molecular sterility in field populations. The use of these systems requires that the transgenic strain is able to be mass reared and produce insects that can successfully compete in the field. Experience with conventional Mediterranean fruit fly genetic sexing strains indicates this will not be a trivial undertaking. Factors related to stability, fitness and competitiveness will need to be thoroughly researched and understood before transgenic strains can be used in these programmes.

Many of the risks associated with the use of transgenic arthropods can probably be addressed using existing protocols, however there are unique risks, including the nature of the genetic change and the transgenic phenotype, which will require new experimental approaches. Risks associated with the use of this technology involve: (1) the host arthropod, (2) the vector,

(3) genes within the vector including markers and (4) the expected persistence of the transgenic strain in the environment. Approaches to assess the stability, mobility and risk of horizontal transfer of the genetic change will need to be identified. However, two issues remain intractable to direct analysis namely horizontal transfer and cross-mobility leading to instability. Both however can be approached indirectly leading to a quantitative measure of risk. A concern is intra-genomic movement that may influence the expected expression and activity of gene of interest within the transgene and thus affect programme effectiveness; plant molecular biologists are now attempting to stabilize gene expression by building in 'insulators'. This will probably also need to be considered for transgenic arthropods.

A limited field trial in the USA has been conducted with a transgenic insect using a strain of the pink bollworm, *Pectinophora gossypiella* marked with a gene expressing green fluorescent protein. Risk mitigation factors included removal of the wings from the released females, protection against vandalism and the use of pheromone traps to capture any escaped moths. Various biological parameters in transformed and non-transformed moths were measured in a large bio-secure field and very little effect of the transgene could be observed. The field trial generated much media and public comment during the preparatory phase and also in response to the Federal Register notice announcing availability of the Environmental Assessment for public comment.

Risk assessment will require methods for transformant identification and a full genetic analysis of the transformed genome. Genes of interest will need to be evaluated in terms of their affect on the host arthropod, and on the environment and other organisms. These factors must be considered individually, in combination and in the transgene persistence in the field. Risk assessments of fitness and host specificity of transgenic arthropods are relatively easy to assess in the laboratory, but evaluations of risk of horizontal gene transfer and unintended effects on ecosystem function are more difficult to analyse. If transgenic strains perform well in short term releases and risk assessments are completed satisfactorily, permanent releases into the environment may be allowed in the future. The use of active transposable elements or *Wolbachia* has been proposed to achieve population replacement but there has been little analysis of the potential risks of such drive mechanism.

4.3. International regulation

Currently, IPPC aims to protect plants and plant health from the introduction of pests of plants and to promote measures to control plant pests. It does this by the development of International Standards for Phytosanitary Measures (ISPM), two of which, ISPM 11 on Pest Risk Analysis and ISPM 3 on the Import and Release of Exotic Biological Agents are relevant to the release of transgenic arthropods. The IPPC also has a working group on pest risk analysis as it relates to transgenic plants. Although the IPPC could play a role in developing regulations for transgenic insects it is limited to plants and plant pests and although the risk analysis processes developed by the IPPC may be broadly applicable, arthropods that are medical or veterinary pests may not be subject to IPPC procedures.

The International Organization for Biological Control (IOBC) is another body involved with transgenic organisms. It has set up a global Working Group on "Transgenic Organisms in Integrated Pest Management and Biological Control" which will deal initially with transgenic plants. it has carried out a scientific analyses related to (a) needs and good agricultural practice, (b) characterization of the transgenic plant, (c) non-target effects and biodiversity, (d) plant resistance management and (e) gene flow.

4.4. National guidelines

In the USA, the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) is currently in the process of planning a rule change to clarify that all vectors, including those developed through biotechnology, are regulated and it is also developing comprehensive risk assessment guidance for the permit drafting process. The USA has also undertaken an Environmental Assessment under the National Environmental Policy Act and issued a USDA APHIS permit for a genetically modified pink bollworm that expresses green fluorescent protein. Guidance for risk assessment and the permit process for genetically modified vectors of animal diseases are also currently under development in the USA by APHIS in cooperation with other Federal agencies.

In New Zealand the Environmental Risk Management Authority, ERMA, is the organization responsible for the implementation of the Hazardous Substances and New Organisms Act (HSNO Act) and it regulates the development, importation, field-testing, and release of transgenic organisms. ERMA has been operating since 1998 and deals with risk identification, risk assessment and risk management. It has established risk assessment protocols that can be applied to the release of arthropods (transgenic or not) into the environment. In 2000/2001 a 'Royal Commission of Inquiry on Genetic Modification' was established and one of its conclusions was that the process that ERMA undertakes is fundamentally sound. The Commission also recommended to "preserve opportunities" for agricultural development with respect to gene technology but to proceed with caution. The New Zealand Government has adopted this position.

An extensive regulatory commission, The Inter-Ministerial Commission on Biosecurity and Genetically Modified Organisms (CIBIOGEM) has been set up in Mexico to coordinate federal policies for the production, import, export, movement, propagation, release, consumption and use of GMOs. The commission participated very actively in the development of the Cartagena Protocol and Mexico signed the protocol in 2000. The signing of this international instrument brings with it many obligations and the CIBIOGEM is charged with ensuring that all the relevant laws reflect its requirements. Current priorities include developing guidelines for the use of transgenic corn, labeling, and genetic transformation of potatoe, squash, papaya and fish species.

Zimbabwe has developed 'Biosafety Regulations and Guidelines' aimed at regulating the preparation and implementation of programmes in relation to research, production, importation and release of genetically modified organisms including arthropods. There are several areas where genetically modified arthropods are likely to make an impact in agriculture. Genetically modified arthropods in Zimbabwe's context are likely to stimulate new approaches to research and development in the protection of stored products against arthropod pests. Zimbabwe has established a Biosafety Board already with a mandate to advise the Government through the Research Council of Zimbabwe (RCZ) on the development, production, importation, application and release of GMOs.

Argentina established the National Advisory Commission of Agricultural Biotechnology (CONABIA) in 1991 that consists of both public and private sector stakeholders. The commission deals with all aspects related to GMOs and has detailed protocols in place for the implementation of field trials of various transgenic plant varieties. CONABIA is essentially focused on the environmental impact of GMOs and is not specifically concerned with food security or international trade. Argentina has considerable experience in the large scale use of transgenic plants in agriculture.

Biological control in the Caribbean region is largely focused on the control of the pink mealybug since infestations of this pest in any region can result in restrictions being placed on the export of fresh produce into non-infected countries. Control of economically important pests is of great importance for crops such as sugarcane, coffee and citrus fruit as they are major earners of foreign exchange. Permit applications for the importation, movement and release of these bio-control organisms are the task of the Pesticide Control Board of the Ministry of Agriculture, which follows the FAO code of conduct for the import and release of bio-control agents (ISPM 3). Several countries in the Caribbean region are currently examining their regulatory mechanisms to address the trans-boundary movement and environmental release of GMOs specifically for agricultural purposes. Although most attention has been focused on crop and food regulations, some attention will be placed on developing regulations for the use of transgenic arthropods to control common economically important pests.

5. THE WORKING GROUP REPORTS

5.1. Working Group 1: Status of transgenesis in pest arthropods

5.1.1. Overview

The field of arthropod transgenesis has advanced rapidly over the last few years, so that it is now possible to routinely produce transgenic arthropods. Therefore, it is timely to consider the current status of knowledge of the potential risks and hazards associated with transgenic release programmes. These may extend from the short term release of sterile male Mediterranean fruit flies to the long term release of mosquitoes that have been engineered to be refractory to the transmission of selected pathogens. The working group focused specifically on issues relating to the production of transgenic arthropods and how this impacts on assessing the risks and hazards of any potential release of these organisms into the environment.

5.1.2. Scope

The working group report is divided into two sections:

(a)   An overview of the current status of the technology and tools that are available to create transgenic arthropods. The section encompasses the following elements:
   - a general review of the field in relation to producing transgenic arthropods;
   - a review of the current state-of-the-art relating to transformation vectors, markers, promoters, and target genes. For each, a short commentary is provided covering the advantages, disadvantages, and efficiency vs. risk trade-off.
   - perceived future priorities and research needs, together with a short discussion on the breadth of applicability of the technology.
(b)   A consideration of two potential examples of the future release of transgenic arthropods. The two examples considered are:
   - the release of transgenically marked, sterile, male Mediterranean fruit flies as part of an SIT programme, and
   - the release of fertile mosquitoes that have had a gene coding for refractoriness to a human pathogen (e.g. malaria), introduced into their germline, uses an active transposable element that could also drive the desirable gene through the target population.

These two examples are considered in terms of the following issue/questions:

- can they be produced now? If so, what is the timeline?
- if they can be produced, then how?
- if not, what is needed to be able to produce the new strain?
- what other new information is needed in order to fully assess the risks involved?
- what is the difference between the two examples in terms of evaluating the risks and hazards associated with their future release?

5.1.3. An overview of the current status of technology and tools

5.1.3.1. Background

The purpose of this working group report is to focus on arthropod transformation, mediated specifically by transposon-based vector systems. Such systems for heritable germ-line transformation are in most common use and are most likely to be used for near-term release programmes. Thus, the general properties of Class II transposable element vectors, associated marker genes, regulatory systems and target genes that can be incorporated into transgene vector systems will be considered. The context of the discussion will focus on how these features relate to the issues of assessing the risks involved in transgenic arthropod release. A number of other systems are also available for introducing transgenes into insect vectors, such as viral vectors and symbionts; these will not be considered. The Class II transposon-based vector systems that are now available will be discussed in order to provide some insight into how the use of each system may lead either to 'movement', or 'movement and replication' of the inserted sequence in the arthropod genome.

5.1.3.2. Class II transposon-based vector systems

Risk assessment of transgenic arthropods requires, primarily, an understanding of the systems and processes used for gene transfer. The systems currently available include germ-line transformation that results in the stable heritable integration of a transgene, as well as systems that allow the extrachromosomal transient expression of a genetic system, usually mediated by a viral or bacterial system. For gene expression systems in released arthropods, germ-line transformation typically mediated by a transposable-element-based system is currently the method of choice.

Critical to the assessment of risk factors and strain stability is a thorough understanding of the behaviour and regulatory properties of the vector used for genomic integration. While all the transformation vectors currently used for non-drosophilid germ-line transformation are Class II transposable elements that share common elements in terms of structure and mechanism of movement, significant differences exist among them and thus they must be considered independently when assessing risk. A primary consideration is that these transposons, along with other mobile genetic elements, are mutagenic by virtue of their ability to integrate into coding and non-coding genomic DNA sequences. Thus, they have the potential to disrupt normal gene function resulting in costs to fitness. On the other hand, a large percentage of most genomes are comprised of such mobile elements, and various mechanisms either have pre-existed or have evolved to regulate transposon function. A major concern relates to how a genome interacts with a transposon-based vector that has been newly introduced. Each of the

currently available Class II transposon-based vector systems are reviewed below and some of their key characteristics are highlighted.

Mariner

Of the transposons used as vectors in non-drosophilids, the functional *mariner* element, Mos1, was the first to be discovered but its ability to transform Drosophila was limited. It was several years before it was successfully tested in the mosquito *Aedes aegypti*, using the kynurenine-hydroxylase-white marker. Thus far, the use of *mariner* for transformation has only been extended to *Musca domestica*. It is notable that *mariner* is part of a superfamily of transposons that is widespread among arthropods; it has minimal requirements for function, with some elements shown to be active in prokaryotes and vertebrates.

Characteristics:

- relatively low transformation frequency (in Drosophila);
- replicative transposition by template dependent gap repair (TDGR), useful to drive genes;
- widespread function for use in most species;
- cross mobilisation and horizontal transmission potential due to widespread function and existence of related mariner/Tc elements.

Minos

The *Minos* element was discovered in *D. hydei* and was determined to be a member of the *Tc* family, originally described in nematodes. It was the first transposon to be used for germ-line transformation of a non-drosophilid, *C. capitata,* and it has been subsequently used to transform the mosquito *Anopheles stephensi* using a green fluorescent protein (GFP) marker. Although reports on its further use in transformation have been limited, transposition has been demonstrated in a wide range of insect cell lines and embryos as well as in mice.

Characteristics:
- widespread efficient function including vertebrates;
- member of widespread mariner/Tc family and thus there is potential for horizontal transmission and cross mobilization.

Hermes

The existence of *Hermes*, and other members of the *hobo, Ac, Tam3 (hAT)* family, was inferred from the cross-mobilization of *hobo* in species where *hobo* did not exist. *Hermes* and other *hAT* elements were then discovered by PCR amplification of genomic elements using common amino acid sequences in *hobo* (from Drosophila) and *Ac* (from maize) as priming sites. A complete *Hermes* element was first tested for function in *D. melanogaster* by germ-line transformation, and its function in other species was tested by transient transposition tests. *Hermes* has been used to transform *Ae. aegypti, Stomoxys calcitrans, Tribolium castaneum, C. capitata* and *Culex quinquefasciatus*. While most of these transformations resulted from *Hermes*-mediated cut-and-paste transpositions, the integrations in *Ae. aegypti* and *C. quinquefasciatus* were not precise transposon-mediated events, but included rearranged or abnormal integrations of the entire vector plasmid by some type of recombination event. These integrations were dependent upon the presence of *Hermes* transposase, and it has been theorized that replicative recombination resulted from an interaction between the injected *Hermes* and endogenous *hAT* elements. *Hermes* has also been demonstrated to have functional interactions with *hobo* as shown by cross-mobilization in plasmid and chromosomal excision

assays. Thus, the possibility exists that *Hermes* integrations in species harbouring functional *hAT* elements will not remain stable.

Characteristics:
- widespread efficient function;
- recombination (only in mosquitoes thus far) may reduce horizontal transmission potential, but this needs to be assessed for other species;
- cross mobilization with hobo and other hATs?
- potential for horizontal transmission;
- replicative recombination in mosquitoes that may involve entire vector including antibiotic resistance genes;
- necessary to determine basis of recombination and potential hAT interactions.

PiggyBac

The *piggyBac* element was discovered by its transposition from its host genome within a cabbage looper moth cell line, into an infecting baculovirus. Functional characterization by transient mobility assays indicated that it could potentially be used as a vector for germ-line transformation in several orders of arthropods. The first *piggyBac* transformations in *C. capitata* and *D. melanogaster* used an unmodified transposase gene indicating that the *piggyBac* vector has autonomous functions in different insect orders. Germ-line transformation with *piggyBac* was then achieved in other dipterans including *Bactrocera dorsalis, Anastrepha suspensa, M. domestica, Ae. aegypti, An. gambiae* and *An. albimanus*; two lepidopteran species, *Bombyx mori* and *P. gossypiella*; and a coleopteran, *T. castaneum*. There is currently no evidence to indicate that *piggyBac* is a member of a widespread family of related elements, though other elements share its specificity for integration into the tetranucleotide site, TTAA. *piggyBac* has been discovered in species other than the cabbage looper moth, with nearly identical elements in the Oriental fruit fly, *B. dorsalis*, and what are probably truncated elements in the lepidopteran, Spodoptera frugiperda. Thus, the existence of *piggyBac* in both closely and distantly related species indicates that it has recently traversed orders by horizontal transmission and probably exists in other species as well. This movement had to be facilitated by functional *piggyBac* elements or cross-mobilizing systems, and that raises concern for vector stability.

Characteristics:
- broad host range;
- widespread efficient use in three insect orders (Diptera, Lepidoptera, Coleoptera) by transformation and transient assays;
- stable integrants in medfly and other insects for six years (~70 generations) by phenotype, but only small scale rearing undertaken, not mass rearing;
- nearly identical elements in relevant target insects, including Diptera (B. dorsalis) and Lepidoptera (S. frugiperda); functionality is undetermined but the possibility remains for instability and horizontal transmission.

5.1.3.3. Markers for selection of transgenic arthropods

Eye colour markers

Advances in *Drosophila* transformation occurred rapidly due to the availability eye color mutant strains and cloned wild type alleles that could be used as markers in mutant-rescue

selections. The first non-drosophilid transformations also took advantage of available eye colour markers that included the medfly *white* gene, and the *Aedes aegypti kynurenine hydroxylase-white* mutation that is complemented by *D. melanogaster cinnabar+*. Another eye colour gene from *D. melanogaster, vermilion,* encodes tryptophan oxygensase and was used as a marker in early transformation experiments. It also complements the *green eye* color mutation in *M. domestica,* while the tryptophan oxygenase gene from *An. gambiae* complements *vermilion*. While the discovery and elucidation of these various eye colour genes is encouraging for their use as markers, in the absence of mutant host strains, such visible selection of transformed arthropods would be impossible for most species.

Characteristics:
- can use native wild type gene or Drosophila ortholog as marker;
- requires mutant host strain;
- subject to position effects that can eliminate a detectable phenotype;
- useful in the laboratory;
- it is not clear how these eye colour mutations affect the fitness of the arthropod.

Chemical selection
The need for dominant-acting selections independent of pre-existing mutant strains initially focused on genes that would confer chemical or drug resistance. These included neomycin phosphotransferase II (NPT II) conferring resistance to neomycin analogues, organo-phosphorus dehydrogenase (*opd*) conferring resistance to paraoxon/ parathion, and the gene for dieldrin resistance (*Rdl*). Chemical selections can be very powerful, and if reliable, they would dramatically improve the efficiency of transformation screens for most arthropods by allowing selection *en masse*. However, most have been problematic due to protocols that vary with the treated species, arthropods that are selected as untransformed false-positives, and the need for highly toxic substrates. Since strain maintenance depends on resistance selection for each generation, the possibility of selecting for natural resistance mechanisms becomes greater with time. A possibility for utilizing the power of chemical resistance for mass screening would be the use of resistance markers in addition to a more reliable visible marker. Initial mass screens for G1 transformants were carried out by chemical resistance, with transformants verified and maintained as strains using the visible marker.

Characteristics
- widely applicable in all strains;
- can be used in mass selection;
- selection may be unreliable;
- natural resistance develops;
- substrates for selection are toxic;
- may be highly effective in use with a reliable visible marker for the creation of transgenic strains;
- arthropods containing such genes are unsuitable for release.

Fluorescent protein markers
Some of the most relevant dominant-acting marker systems are those using the green fluorescent protein (GFP) gene and its variants that include an enhanced GFP and spectral variants that emit blue, yellow, and cyan. The GFP gene from the jellyfish *Aequorea victoria* first showed heterologous function in a nematode, and subsequently it was used as a reporter gene in a variety of prokaryotic and eukaryotic organisms for both *in vivo* and *in vitro* studies.

Although widely used in Drosophila, GFP was rarely used as a primary transformant selection due to available eye colour markers and the need for fluorescent optical systems for detection. Some of the first uses of GFP in Drosophila were initial tests of the marker prior to use in non-drosophilids, and use of a dually marked *piggyBac* vector with *white+* and *polyubiquitin-*regulated. These experiments demonstrated that EGFP was considerably more efficient and reliable than *white* since less than half the G1 transformants detected by fluorescence exhibited visible eye pigmentation. This polyubiquitin-EGFP has since been used in three tephritid fruit flies, *An. albimanus,* and *Lucilia cuprina.* Other GFP marker constructs have been developed including the use of the Drosophila actin 5C promoter to select *Hermes* and *Minos* transformants in mosquitoes, and the actin A3 promoter to select *piggyBac* transformants in *Bombyx mori.* GFP regulated by promoters that are active in all cells throughout development provide the added benefit of allowing selection of transformants as late embryos or larvae, which is a major advantage for arthropods with long generation times. Another GFP marker construct with wide applicability uses an artificial Pax-6 promoter. It expresses most strongly from the brain, eyes, and ocelli in adults, but can also be observed from several structures in pupae and larvae. Notably, some insertions result in intense fluorescence that can be observed in pigmented eyes and it has allowed selection of transformants in *T. castaneum, M. domestica, A. stephensi* and in late embryos of *B. mori.*

Markers in addition to GFP will be critical for the use of multiple transgenes, or where reporter genes distinct from the transgenic marker are needed. The Pax-6 promoter has been linked to the yellow and cyan fluorescent proteins that should expand the number of independent marking systems available. In addition, a new red fluorescent protein (DsRed) has emission and excitation maxima that make it the fluorescent protein most easily distinguishable from GFP and the GFP variants. It has been shown to have high expression levels in Drosophila, *C. capitata, Anastrepha suspensa* and *A. stephensi.*

Fluorescent proteins will be useful as universal marking systems and testing should rapidly reveal the best regulatory systems for specific arthropod hosts, and specific applications. In addition to transformant selection, they will have initial use in released transgenic strains as genetic markers for detection of released arthropods. Drawbacks to the use of fluorescent proteins include quenching from hardened or melanized cuticles making detection difficult, and autofluorescence from a variety of tissues that vary in different arthropods.

Characteristics
- universally applicable;
- can track horizontal transmission to other organisms;
- highly stable as a marker system;
- can be quantified;
- automated embryo selection possible;
- requires an optical system;
- possible fitness costs;
- quenching may be a problem;
- DsRed protein has long maturation time.

The initial means of transformant identification depends on the marker system originally used to select or screen for the transformant line. Fully expressed dominant visible markers, such as wild type eye color genes, may be easily detected but difficult to distinguish from wild type non-transgenic arthropods. Incomplete expression of most transgenic markers due to chromosomal position effects actually helps in this regard since unique phenotypes, or

phenotypes distinguishable from wild type are not uncommon, and this allows identification of contaminating arthropods. Drug or chemical resistance is least reliable since natural resistance may be selected for in non-transgenic arthropods before fully homozygous lines can be created. If chemical resistance screens are used, it is most valuable to include an additional visible marker to verify transformation and simplify identification. The most reliable markers are dominant-acting visible neomorphs, such as fluorescent proteins, that are easily and clearly identified. False positives and mis-identification are only problematic when indistinguishable autofluoresence occurs. This can be minimized by using promoters that allow expression in unaffected tissues or times in development.

5.1.3.4. Promoters

Promoters are required to regulate the expression of genes necessary for transposon movement (transposase), selection of transformants (markers) and regulation of genes of interest. For widespread application, constitutive promoters are essential for transposase activity and marker function to be broadly effective. Highly specific promoters will be essential for driving the species-specific expression of genes of interest and in some cases, for specified transposase activity. The latter is a consideration for programmes where expression of a transgene or transposase could be limited by using highly species-specific promoters, thus reducing the risk of horizontal transmission. A wide range of promoters and regulatory systems have now been described and their function defined in a number of arthropod systems. Examples of promoter systems in use for transposase regulation, for selection markers and genes of interest are:

- polyubiquitin – constitutive;
- actin 5c (Drosophila) actin 3A (Bombyx) – constitutive;
- hsp70 – broadly active;
- Pax-6 (3xP3) – universal; tissue specific;
- yeast Gal4/UAS;
- bacterial tet operon (Tet-off/on system).

In addition, a range of tissue, sex or stage specific promoters have been identified and characterised to a greater or lesser extent, especially in Drosophila. However, there is still a need for more species and stage-specific promoters for certain targeted applications.

5.1.3.5. Potential target genes

A range of insect genes are of interest and are being investigated in terms of their suitability for introduction, modification, modulation of expression or other manipulation. Examples of the types of genes of interest might include: pathogen susceptibility/refractoriness, pathogen migration, replication and development/life cycle changes, immune system, insect sterility mechanisms, sex determination, olfaction, apoptosis and arthropod behaviour, including mate selection, host seeking, and feeding. This is a non-exhaustive list, but gives some indication of the genes that are being targeted for characterization, and may also give an impression of the types of manipulation of insect genes that may be possible using transgenic approaches.

5.1.3.6. Future research

Short term, high priorities

Of primary importance is the further study of mechanisms of transposition in general, and mechanisms specific to particular transposon systems. Greater understanding of transposon behaviour and regulation of normal function and repression is essential. This will include a detailed understanding of how transposons carrying specific genes behave in caged populations and hence how they may behave in wild populations. Risks associated with transgene instability and how horizontal transmission could be minimized by new vectors that can be immobilized subsequent to genomic integration need to be assessed. This is possible by the deletion or rearrangement of sequences required for mobility (terminal and sub-terminal) using recombination sites. Study of vector and host-genome interactions is needed, with a focus on epigenetic interactions that might result in unintended or unexpected transgene activity or repression. New gene transfer systems are needed that will also include recombination system target sites (*FRT/FLP; Cre-loxP*).

Medium term priorities

With the isolation and implementation of several effective vector systems, research efforts have reduced for the discovery and development of new vector systems. Of special need are systems that might be species-specific (for driven systems) and for higher risk applications. These may include synthetic systems whose mobility can be highly controlled.
Research on potential target genes, including:

- Refractory gene systems that specifically eliminate pathogen vector capacity
- Sex determination and dosage compensation genes for sex-specific gene expression
- Odorant receptor genes for manipulation of attraction and behaviour

Isolation and analysis of existing and new gene expression regulatory systems are needed that would include a definition of the range of phylogenetic activity. Genomics information is needed that will lead to new target gene, promoter and transposon discovery and characterization.

5.1.4. Two examples for future release of transgenic arthropods

5.1.4.1. Scenario 1

The SIT is currently an effective means of biological control for a variety of arthropod pests and it depends on the mass release of sterile insects. For medfly, a genetic sexing system is used that is based on *temperature sensitive lethal* (*tsl*) gene expression resulting in female-specific lethality. Additional needs for this programme to increase effectiveness are molecular sterility methods and the unambiguous marking of released males. The GFP and DsRed fluorescent proteins used for transformant selection may provide an efficient means of identifying released flies. Given the general use of GFP in numerous organisms and the short term release of sterile males in SIT, that would preclude persistence of the transgene in the environment, such a transgenic release programme is most likely to have the least amount of associated risks. The following is a suggested series of activities to achieve this goal

- Transgene vector construct would include GFP and/or DsRed fluorescent protein marker gene inserted within a *piggyBac* vector.

- Integration of the transgene would ideally be within the chromosome 5 inversion that carries the tsl-mutation and associated marker genes. Transgene vector/ marker constructs are available and have been integrated into wild type and *tsl* genetic-sexing strains. Chromosome 5 linkage is currently being tested and phenotypic and quality control studies are in progress. Transgene insertion will be characterized by Southern DNA hybridization, insertion site sequencing by inverse PCR, and possibly salivary gland chromosomal *in situ* hybridization. Transgene and genomic junction sequences would be used to create primers that would be able to rapidly assess transgene insertion.
- New transgenic lines may be required; this can be achieved by new injection experiments or re-mobilization (jumpstarter) of existing integrations.
- Appropriate strains should be available within 2 years and tested for stability and strain fitness within 3 to 4 years. Laboratory mass-rearing and controlled-release parameters will be possible once the strain is available.
- Information from concurrent studies on *piggyBac* and the marker system would allow ongoing improvements in the system and would be incorporated as appropriate.

5.1.4.2. Scenario 2

Much research has been directed towards developing transgenic insect vector strains that are refractory to a range of pathogens, including malaria and dengue. In this context, refractory genes would render the insect vector and their progeny refractory or unable to transmit a specific defined pathogen to their host. In order for this approach to be effective, it would be necessary to release fertile transgenics incorporating a fully functional refractory gene integrated into a transposon vector. The transgene would be maintained and possibly driven into a large proportion of the target population. The vector construct would be autonomous and would be one that replicates as part of its transposition process, such as *mariner*. In addition, the vector construct would also be marked (e.g. with GFP) so that it could be tracked and monitored in the field. The following is a list representing the current status of this technology:

- Refractory genes are being investigated, but as yet, none are fully defined or characterized.
- Once identified, these genes would require full definition, which would include an assessment of the fitness costs to the arthropod and how this would be altered, for example, in relation to the number of transgenes.
- It may also be desirable to consider transforming near wild type arthropods to ensure hardiness in the field.
- In addition, it would be highly desirable to include some internal genetic safeguards to eliminate the transgene after a certain period following release.
- There is no genetic drive system available

5.1.5. General conclusions

- Scenario 1, as part of ongoing SIT programmes, would be most likely to be implemented first and would be possible in the near term. Such a release would be a precedent; hence it would be critical that it is implemented with greatest attention to environmental genetic safeguards and public transparency. There is an immediate need for the development of appropriate guidance for a regulatory framework to rigorously assess the environmental impact and determine if there are any risks that need to be investigated.

- Scenario 2 is not yet possible, and involves much more potential risk. It would, therefore, require a much higher level of scrutiny before a release could be contemplated.

- Transgenic arthropods and their application to biological and disease control is a new and rapidly developing field. Therefore, continuing to understand the technical aspects of the technology is an ongoing process that requires adequate and appropriate expertise at the regulatory level. This can only be achieved if regulators have the requisite academic training, experience and continuing education. Due to the high technical level and rapid developments in this field, regulatory assessments and decisions should be made in consultation with experts from academia, government and industry.

- By their very nature, released transgenic arthropods would not be limited by national borders and may migrate to neighbouring countries. Therefore, international agreements will be essential before the release of transgenic arthropods that may be capable of transcending national barriers.

- Due to the controversial nature of transgenic arthropod technology, it is essential that endeavours be made to communicate the science and its objectives to the public in a most understandable and responsible fashion.

5.2. Working Group 2: Identification of risks associated with a transgenic release

5.2.1. Overview

The purpose of the Working Group was to identify potential hazards that could arise from the release of transgenic arthropods. In considering these hazards, the working group agreed that the potential hazards could be presented by the transgenic organism (e.g. a transgenic medfly), the genetic construct used or the gene used in the transformation process. The working group considered the usage of terms related to risk analysis for transgenic arthropods. In keeping with generally accepted terminology for risk analysis, the terms hazard, likelihood, consequence, risk, risk assessment and risk analysis are used in this document.

5.2.2. Risk analysis

The working group report focuses on identifying potential hazards and does not address hazard assessment, exposure assessment or risk assessment. It is therefore important to note that the hazards identified here may occur with varying probabilities (e.g. improbable, frequent, highly probable), and the consequences of these hazards can vary in magnitude (e.g. negligible, low, catastrophic). Risk is a product of the probability of a hazard to occur times the magnitude of the consequences. Typically, risk analysis includes three stages: risk assessment, risk management and risk communication. The risk assessment stage is traditionally divided into four stages:

- hazard identification;
- hazard assessment (describing the hazards);
- exposure assessment (e.g. assessing the probability, likelihood, exposure or frequency of a hazard); and
- assessment of consequences (including assessing the magnitude of the economic, environmental, cultural and social consequences).

IPPC pest risk analysis (PRA) procedures follow this same general procedure for risk analysis using the following stages:

- initiation (pest or pathway initiated);
- pest risk assessment (including pest categorization, estimating introduction and spread potential, probability of introduction and spread and estimating consequences of introduction and spread); and
- pest risk management (including the determination of acceptability of risk and identification of risk management options).

Under IPPC PRA procedures, the initiation stage includes identifying all of the potential pests that may be associated with a commodity or product. Similarly, the working group considered that the hazard identification stage to be of particular relevance to transgenic arthropods. Hazard identification involves determining: what can happen, when it can happen and how it can happen. Identification of hazards does not involve the estimation of the likelihood or consequence of these events. A variety of methods can be used to identify hazards. These include, for example, brainstorming, review of similar historical cases, scenario or fault trees (e.g. diagramatic analyses of pathways and events) and literature reviews.

Stakeholder participation or public hearings may also be used in the process of identifying hazards. Stakeholders may include industry representatives, academia, consumer interest groups, environmental groups, and concerned citizens. Several factors must be identified in the process:

- sources of hazards — e.g. the inserted gene itself or the behaviour of the transgenic arthropod;
- areas of impact — e.g. agriculture or the environment (such as affecting natural enemies); human health, cultural or social aspects, the economy, ethics;
- exposure pathways — how is the intrinsic potential of the hazard realized, e.g. by unanticipated changes in behaviour, or by way of air, water, etc.

Hazards may be associated with the transgenic arthropod in general, with the genetic construct used, or with specific genes, promotors or sequence in the constructs or the gene product(s). Some hazards may be relevant while the transgenic arthropod is still in containment, while other hazards may need to be considered before the transgenic arthropod is released. Distinction should be made between hazards associated with the unmodified organism and those unique to the transgenic arthropod. In order to be as comprehensive as possible, the Working Group first listed potential hazards without considering specific transgenic arthropods. These are shown in Table 1, which is not necessarily a complete list. For example, reduced tolerance to environmental conditions may reduce the range of the pest. The working group also considered the relevance of these identified hazards in two specific cases as illustrative examples.

Table 2 compares two scenarios; transgenic sterile Mediterranean fruit flies containing a gene for a marker such as GFP and unrestricted release of transgenic mosquitoes transformed using transposable elements, representing two extremes of how transgenic arthropods may be released. Note that the scenario for the fruit fly is for one that has been transformed using a construct with very low or negligible mobility and in which fruit flies are sterile. Under these circumstances, the Working Group considered that the probability of most hazards occurring would be very low. Likewise the risk (i.e. hazard x probability x consequences) would be very low or negligible.

TABLE 1. Potential hazards associated with the release of transgenic arthropods

Area of Interest	Source of hazard	Hazard	Significance
Environment	Changes to biology of organism	change in host range	increased disease transmission
		change in environmental tolerances (temperature, humidity, etc.)	potential spread to new areas
		changes in other aspects of physiology (reproduction, pesticide resistance, susceptibility to post-harvest treatment)	compatibility with other pest management programmes
		change in reproductive behaviour	
		change in feeding behaviour on normal hosts	feeding more frequently may increase host exposure to pathogens
		change in pathogenicity	increase pathogenicity
		change in timing or length of development	disease transmission issues
	Effects on non-target organisms	change in suitability of LMO to parasites or predators	reduction in natural enemies,
		adverse effects on other beneficials (e.g. pollinators)	altered pollination
		effects on symbionts including gut symbionts, pathogens, etc.	altered survivorship, fitness, etc.
		adverse effects on soil species/community (or aquatic env.) (e.g. accumulation in soil of genetic material or gene product)	changes in soil productivity
	Stability of construct in organism	recombination potential	various effects on ecosystems
		mobility of the gene	
		transfer of transposable element by hybridization	
		horizontal transfer to a related species	
		horizontal transfer to other organisms including soil organisms or symbionts	
Human health	genetic modification	change in pathogenicity of parasites	could arise from horizontal transfer of gene from mosquito to parasite
		change in host range of mosquito	change in disease transmission potential
Cultural/ Social/ Economic	GMO	impact on ecosystem	cultural and religious concerns; effects on tourism or other industries; loss of trade opportunities

TABLE 2. Case specific examples of hazards. FF denotes sterile Mediterannean fruit fly containing the GFP marker gene. Mosquito denotes fertile mosquito containing a transposable element

Hazard	FF	Mosquito
Change in host range	0	+++
Effects on symbionts including gut symbionts, pathogens, etc.	0	+++
Adverse effects on soil species/community (or aquatic environment) (e.g. accumulation in soil of genetic material or gene product)	+	+
Change in suitability of GMO to parasites or predators	+ / 0	+
Horizontal transfer to other organisms including soil organisms or symbionts	0	+
Horizontal transfer to a related species	0	++

("0" denotes a neutral change or an effect that is not predicted to cause any significant risk; "+" denotes a change that could result in low or moderate risk; "++" denotes a change that could result in medium to high risk).

Note that Table 2 considers only a few of the hazards identified in Table 1. It is possible that in performing the complete risk assessment, certain hazards may be identified as more relevant or important than others (e.g. those with serious or irreparable consequences). The next stages of the risk assessment may, therefore, identify a few or some of the hazards that should undergo further analysis. Nonetheless, the initial list should be maintained in case that it is necessary to analyze additional hazards (i.e. when new information becomes available that changes the significance of the potential hazard). The working group also noted that each new application for the development and release of a transgenic arthropod should be considered on a case-by-case basis.

The working group also considered that there could be other issues that may arise from the development and use of transgenic arthropods that may not necessarily be considered in the risk assessment. For instance, the Working Group discussed how the diet used to rear transgenic sterile fruit flies could be disposed of if it is found to be unacceptable as animal feed due to potential contamination with GMO products. Another potential problem is that of sabotage; radical groups may attempt to sabotage experiments or projects and thereby inadvertently release organisms from containment. The working group also recognized that issues such as the use of transgenic arthropods in bio-terrorism may need to be addressed, but that this was beyond the scope of this working group.

5.3. Working Group 3: Development of risk assessment protocols

5.3.1. Overview

Lack of relevant experience in releasing transgenic arthropods warrants a cautious, incremental approach to the development and implementation of the technology. Release of transgenic arthropods for pest management programmes should occur only after incremental and stepwise evaluations of identified hazards and potential risks in field trials. Table 1 includes questions relevant to contained field trials (see 5.3.3a) and release programs that will not result in establishment of the transgenic arthropod in the environment (see 5.3.3.b). Table 2 includes the questions from Table 1, as well as questions particular to release programs for the purpose of establishing organisms or alleles in the environment (see 5.3.3.b). Tables 1 and 2 are based on considerations by Tiedje et al. [1], but were expanded or revised to reflect issues relevant to arthropods. Possible consequences considered include effects on non-target species and biodiversity, disruption of ecosystem functions, threatened and

endangered species, and disruption of genomes of non-target species (potentially including humans). These tables reflect the Working Group's present knowledge, and we recognize that these issues may change as we gain more experience.

The amount of information on lateral gene transfer (or horizontal gene transfer) has "ballooned in recent years, in large measure due to the flood of data from the complete genome sequences. These genome sequences allow the full extent of DNA similarity between organisms to be appreciated for the first time." [2]. Bushman [2] reviewed our current state of understanding of this phenomenon. He noted that,

> *"It seems likely that lateral DNA transfer will be increasingly in the news. The effects of gene transfer on our daily lives are only starting to be appreciated. The transfer of sequence information among pathogens strongly influences the diseases they cause. Antibiotic-resistant infection, mobile pathogenic islands, and drug-resistant HIV are all examples of lateral transfer dictating the course of human disease."*

Bushman [2] concluded

> *"Given the frequency of lateral transfer generally, it seems likely that if large numbers of genetically modified organisms are introduced into the environment, at least a low rate of transfer will take place. However, this needs to be considered against the background of high rates of lateral transfer generally. If organisms are exchanging genes at a high rate, does it matter whether engineered genes are transferred occasionally? Obviously, the nature of the genes involved is a major determinant, and care must be taken in any setting where laboratory DNA constructions can be introduced into the environment. Still, in the seas, genes move between cells some 20 million billion times per second, leaving any human contribution vanishingly small by comparison. Looking forward, it will be crucial to carry out the debate on uses of genetically modified organisms against a background of solid information on natural rates of lateral gene transfer."*

Another review of mobile DNA was recently published [3], in which the authors also note in their preface,

> *"It would have been hard to anticipate the speed and scope of the advances that would take place in the study of mobile DNAs"*

since the book 'Mobile DNA' [4] was published in 1989. Craig et al. [3] also note that,

> *"To a degree unanticipated in Mobile DNA, work summarized here has revealed striking mechanistic similarities between transposition reactions involving elements that move exclusively by DNA intermediates and retrovirus-like elements for which the DNA substrate is generated via an RNA intermediate. The proteins that drive these related chemical reactions also share structural features"*

Most of the studies quoted above do not refer to higher eukaryotes but because considerable information is still lacking on the 'natural history', rate of transfer, mechanism of transfer and potential consequences of horizontal or lateral gene transfer. Caution is warranted in releases of transgenic arthropods, especially those containing active elements that could facilitate lateral movement. Important though the issue of horizontal gene transfer is, the debate is now

moving away from its likelihood to its consequences, i.e. whether the transferred material would provide a selective advantage to the recipient.

5.3.2. Definitions

(a) A transgenic arthropod, as referred to in this Working Group report, is an arthropod that possesses a novel combination of genetic material obtained through the use of modern biotechnology.
(b) A paratransgenic arthropod is an arthropod carrying a transgenic microorganism intended to affect the arthropod's phenotype with the microorganism in continual association with its host. This may include intra- or extracellular microorganisms in the gut or reproductive tract.
(c) The accessible environment consists of the region into which the organism will be released and the areas into which it could spread.
(d) A donor organism is one from which genetic material was obtained to create the transgenic organism.
(e) A recipient organism is the one into which the donor material has been introduced.
(f) A symbiont is an intra-or extracellular organism contained within a host (arthropod), which may have an obligate or facultative relationship with the arthropod host. This may include gut or reproductive tract inhabitants. The functional relationships of the organisms often are unknown. Such microorganisms also have been called 'biological associates'.

5.3.3. Scope

(a) Field trials are for the purpose of evaluating a transgenic or paratransgenic arthropod and are contained in some manner (i.e. time or/and space). Such experiments usually are conducted outdoors, are designed to be of limited duration, permit recovery, destruction, or decomposition (on site) of the experimental material, and can be terminated either as a normal conclusion of the trial or in the event of unforeseen consequences.

(b) Release programmes are for the purpose of:
   (i) short term presence in the environment with a low risk of establishment; for example, the autocidal control of a targeted pest population using the SIT. In that example, even if some small percentage of the released arthropods are not sterile or the released arthropods demonstrate F1 sterility, the persistence of these organisms or alleles is unlikely due to loss of intrinsic fitness relative to naturally occurring organisms or alleles. Other examples include inundative release of transgenic or paratransgenic arthropods unable to survive regular climatic fluctuations.
   (ii) release of fertile transgenic or paratransgenic arthropods carrying a 'suicide' trait or one of reduced fitness so that some intended purpose may be achieved by the released individuals or their progeny carrying self-limited suicide alleles, but not through their establishment in the environment.
   (iii) releases expressly intended for establishment of an allele or an organism in the environment for control of a pest or disease over time. Retrieval of the released organisms is difficult, if not impossible.

5.3.4. Report

5.3.4.1. Considerations
Elements of the questions in Tables 1 and 2 were based on ref [1]. The Working Group also considered language from the Cartagena Protocol on Biosafety to the Convention on Biological Diversity (CBD), the IPPC, its existing standards (in particular the glossary) and the specification for its Working Group that will draft guidance on risk assessment of living modified organisms (LMOs), and the Office International des Epizooties (OIE) and its Animal Health Code.

5.3.4.2. Low risk of establishment
This section addresses field trials of transgenic arthropods for evaluation, involving short term presence and low risk of establishment in the environment. The following is a list of questions that may need to be considered when developing a risk assessment protocol.

Attributes of the unmodified, recipient arthropod
(a) Is the arthropod subject to regulatory control?
(b) Taxonomy and distribution
- What is the origin and current distribution of the recipient species?
- What is its normal dispersal range?
- Is the recipient strain recognized as a specific biotype or strain? If so, what are the distinguishing characteristics?
    (1) What is the specific origin (point of collection) or acquisition location of the recipient strain?
    (2) Has the recipient and transgenic arthropod strain been identified by a qualified taxonomist and voucher specimens deposited in a permanent location to allow future morphological reconfirmation and isolation of DNA by conventional methods (e.g. after preservation at -80°C or in 95% ethanol)? Where were the voucher specimens deposited?

Ecological relationships and roles of the unmodified, recipient arthropod
(a) What is the recipient arthropod's trophic level (parasitoid, predator, parasite, plant feeder, or vector of animal or plant diseases) and host range?
(b) If the arthropod is a vector of plant, animal, or human disease(s), what are these?
(c) What other ecological relationships does the arthropod have?
(d) Is the arthropod involved in basic ecosystem functions and processes (e.g. decomposers, pollination)?
(e) What are the environmental limits to growth or reproduction (habitat, microhabitat)?
(f) How does the arthropod survive during periods of environmental stress?
(g) What is the potential for gene exchange with other populations of the same or related species? (before modification)
(h) What methods are available for detection of the arthropod, and what are their specificity, sensitivity, and reliability?

Attributes of the genetic alteration
(a) What is the intent of the genetic alteration (e.g., marker, altered function)?
(b) Have similar components of the present genetic material been evaluated in field tests, and if so, how do the present components differ?
(c) What is the nature and function of the genetic alteration?

- From what organism(s), are the transgene and molecules derived that were used to produce the transgenic organism? Describe any synthetic portions.
- What is the range of function of the components (e.g. effector expression or promoter function in other organisms)?
- Is the transgene donor (parental organism) pathogenic or subject to regulatory control?

(d) By what mechanism was the alteration made?

(e) What are the structures of the molecules used to alter the genome (primary sequences, maps and peptides)?

- Describe from what sources the above structural information was obtained and any additional confirmation.
- Are there undetermined sequences present in the inserted material or sequences not necessary for the intended effect?
- How many copies of the alteration(s) are present and what is known about each?
- Where is the alteration in the genome? (nuclear, mitochondrial, plasmid, symbiont, DNA sequence of the insertion site.)
- How stable is the genetic alteration?
- What is the mode of inheritance of the alteration(s), and how was this demonstrated.
  - (1) in the laboratory?
  - (2) in a contained environment similar to the release site?
- Is the copy number, sequence, and location of the insertion(s) stable, and how was this demonstrated?
  - (1) in the laboratory?
  - (2) in a contained environment similar to the release site?

Phenotype of the modified organisms compared to the unmodified

Organism in this section refers to the arthropod alone, or to the arthropod and symbiont in cases of paratransgenic arthropods. In the latter case, responses to the following questions should consider both organisms.

(a) Have strains similar to the present material been evaluated in field tests, and if so, how does the present strain differ?

(b) What function has been deliberately enhanced, introduced, or diminished?

(c) Have any phenotypic traits been modified unexpectedly by the introduced alteration?

(d) What is the host/prey range relative to the unmodified organism?

(e) Are there detectable changes in behaviour (e.g. mating, dispersal)?

(f) Have changes in life table attributes occurred in the altered strain?

(g) What is the level and pattern (stage, tissue) of expression of the trait?

(h) Does the altered phenotype persist in any way in dead material?

(i) Has the alteration changed the organism's susceptibility to control by natural or artificial means?

(j) Have the environmental limits to growth or reproduction (habitat, microhabitat) been altered as a result of the modification?

(k) Has the alteration affected the expression of an existing gene(s)?

- Is the alteration in a gene?
- What is its effect on that (or other) genes' function?

(l) What quality control measures are available to detect changes in the desired function of the material during production or after release?

(m) What detection methods are available to distinguish the modified from unmodified material and what are their specificity, sensitivity, and reliability?

Attributes of the accessible environment
(a) Describe the accessible environment or dispersal range, given the field conditions. (Under the IPPC process, this is the "endangered area".)
(b) Are there artificial or natural agents that could move the transgenic or paratransgenic arthropod or genetic components from within the release environment? What are they?
(c) Are there alternative hosts or prey in the accessible environment?
(d) What relatives/related arthropods occur within the accessible environment or dispersal range?
(e) Are endangered or threatened species present that could be affected?
(f) How effective is the monitoring of the goals of the release? Monitoring for unintended consequences?
(g) How can unintended and undesired outcomes be reversed (bioremediation)?
  - Who would finance and implement the process and how much would it cost?
  - How has the feasibility of this bioremediation been verified?
  - What are the social, economic and environmental consequences if this remediation is required and conducted?
(h) What monitoring survey programs are in place to assess the characteristics of the modified arthropod population?

5.3.4.3. Fertile transgenic arthropod releases

The following points relate to releases of fertile transgenic or paratransgenic arthropods into a favourable environment that are not intended for establishment or releases expressly intended for establishment (see 5.3.3). Issues related to the release of driving factors, which include mobile DNAs (transposable elements [TEs], viral vectors) or other drive mechanisms (meiotic drive, symbionts, and *Wolbachia*), raise additional questions.
(a) Does the exposure time or period of persistence in the environment have a known impact on the hazards identified below?
(b) What is the host range of the driving factor?
(c) What is the potential host range of the target organism? Will the modification or driving factor affect transmission of parasites?
(d) What are the potential outcomes of movement of the driving factor(s), or components of the transgene, to a new host?
(e) Is selection pressure necessary to drive or maintain the driving factor in the population?
(f) What means of resistance to the drive factor exist or may arise?
(g) What is the likelihood that dissociation between the drive and effector mechanism(s) occurs?
(h) To what extent is the alteration likely to become established in the target population?
(i) What predictive models exist for predicting the behaviour of the material upon release?
(j) What monitoring survey programs are in place to assess the characteristics of the modified arthropod population? or the disease vectored by this arthropod?

5.3.5. Conclusions

Previous experience indicates sterile arthropod releases pose few risks provided that accepted quality control procedures are in place. Similarly, releases of transgenic sterile arthropods will be of lower risk than releases of transgenic arthropods for other control tactics. Release of transgenic or paratransgenic arthropods for permanent establishment in the environment will likely involve higher levels of risk.

It is important to note that different stakeholders will have different attitudes to risk (i.e. some will be more risk averse than others). It is important for the risk assessment process to make it clear what the attitude to risk is so that the process is transparent and others can understand how the conclusions were drawn. For example, if qualitative descriptions are used ('low', 'medium' or 'high') it should be clearly stated what these mean so that the process is transparent.

Uncertainties exist about the behaviour of mobile DNAs and other drive mechanisms in wild populations. Research needs include: issues related to the horizontal transfer of DNAs from the transgenic organism into naturally occurring species: mechanisms, frequency, consequences, and remediation methods in the event of harmful consequences of horizontal transfer. The assessment should be clear in identifying where uncertainties occur so that evaluations can be made on the basis of the quality and relevance of the information. Identifying areas of uncertainties can also help identify the types of information or research that is required to reduce the uncertainty.

During risk assessment of applications for field trials or release, broad consultation of all affected and interested parties should be sought and taken into account. Maximum transparency of the process should be assured. The outcomes and experience gained from field trials and release programmes should be made available to all affected and interested parties. It is recommended that the risk assessment and release programmes be conducted in consultation with governments of countries into which entry of the transgenic material or arthropods may occur. Consignment of similar material in transit is covered by agreements outside of this document.

It will be necessary to predict the geographic and biological scope of the release and, therefore, the scope of the risks/hazards. Knowledge of the population biology and ecology of many arthropod species may be inadequate for new situations. For example, research may be required to resolve the probability of intrapopulation, interpopulation, and interspecific movement of genetic material. Because unintended and undesired outcomes might occur, long term monitoring of transgenic or paratransgenic arthropod populations should be conducted after release programs, especially when the population becomes permanently established.

The level of knowledge about the relationships between microorganisms associated with arthropods (symbionts) and microorganisms in the environment is limited. Therefore, research is needed to understand the potential risks of horizontal gene transfer between transgenic symbionts and microorganisms in the environment should arthropods that carry a transgenic symbiont (paratransgenic arthropod) be released into the environment.

Because knowledge and experience with transgenic organisms and the biology of natural populations continually change, risk assessment protocols and estimations of relative risk will require periodic revision.

6. OVERALL CONCLUSIONS

(1) From the technical presentations it was clear that insect transgenesis is now an established technology in many insect species, i.e. it is now possible to introduce functional foreign genes into a variety of insect genomes. This has been made possible by the identification of new gene vectors and the use of improved markers to identify transgenic individuals.

(2) Current transgenic techniques involve random integration events into the genome and this can have two consequences, firstly that the expression of the transgene can be compromised due to position effects and secondly that the insertion itself can affect the fitness of the insect carrying the transgene. These observations have important consequences for eventual use of transgenic insects in SIT programmes.

(3) New vectors will need to be developed that can be immobilized following insertion so as to prevent further mobilization, i.e. suicide vectors. In addition, targeted insertion sites will need to be developed to reduce the problem of position effects. In non-insect systems these types of vectors have been developed.

(4) Although many transgenic strains have been produced, to date none have been evaluated in terms of their suitability for inclusion in an SIT programme e.g. mass rearing characteristics, transgene stability during mass rearing, mating competitiveness. Based on experience with other specialized strains for use in SIT, extrapolating from laboratory experimentation to the operational scale is not straightforward.

(5) Currently there are no regulatory frameworks addressing the release of transgenic insects but in many countries these are available for transgenic plants. However, the IPPC does have a regulatory procedure, ISPMs, which could form the basis for any future regulation. The IPPC already has a working group drafting specifications related to transgenic plants.

(6) National guidelines as they relate to transgenic organisms are extremely varied. Most of them contain guidelines for pest risk assessment including limited field trials. The contained release of a pink bollworm strain marked with a fluorescent protein has been carried out in the USA and the results showed no obvious effects of the transgene on some components of behaviour.

(7) A draft risk assessment protocol was developed that identifies the different ways in which transgenic insects could be deployed in a pest control programme and the different types of information that would be required by the regulators.

(8) Considering the public sensitivity of the subject of transgenic organisms in the public domain, field trials will need to be carried out with broad consultation of all affected and interested parties.

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WORKING PAPERS

THE INTERNATIONAL PLANT PROTECTION CONVENTION, LIVING MODIFIED ORGANISMS AND PEST RISK ANALYSIS

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Abstract

As technology improves, the types of organisms that are being transformed using the techniques of modern biotechnology are steadily increasing. Concurrently, the movement of these organisms across borders in trade or for other purposes has also increased. Many countries, in particular developing countries, require guidance on how to assess the potential risks that may be associated with transgenic organisms, and how these organisms can or should be regulated. The International Plant Protection Convention produces international standards for phytosanitary measures that can provide countries with guidance on how to conduct pest risk analysis, and how to implement measures to protect plant life or health within their territories. This paper will provide an overview of:

- the International Plant Protection Convention and other international instruments relevant to the transboundary movement of transgenic organisms;
- the legal basis for risk analysis for transgenic organisms in international instruments;
- procedures for pest risk analysis under the IPPC;
- the application of international standards for phytosanitary measures (including the pest risk analysis standards) for assessing and managing plant pest risks of LMOs; and
- future activities of the IPPC with regard to LMOs.

INTERNATIONAL INSTRUMENTS, RISK ANALYSIS AND LIVING MODIFIED ORGANISMS[1]

The International Plant Protection Convention (IPPC) is a multilateral treaty with the purpose of protecting plants and plant health from the introduction and spread of pests[2] of plants, and to promote measures for the control of plant pests. The scope of the IPPC covers plants, including wild flora and forests, and plant products and applies to both direct and indirect damage from pests (including weeds). Biological control agents used to control plant pests also fall under the scope of the IPPC. There are currently 117 contracting parties (nations) to the IPPC.

The IPPC is identified in the World Trade Organization's Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) as the international standard setting organization for plant health. Both the IPPC and the SPS Agreement state that measures to protect plant life or health should not be disguised barriers to trade, and that these measures should only be applied to the extent necessary to protect plant life or health. However, both agreements also affirm the sovereign right of all Members to take necessary measures to protect plant life or health from the introduction and spread of pests. Members of the WTO are legally obligated to base their phytosanitary measures on international standards for phytosanitary measures (ISPMs) developed under the auspices of the IPPC. Countries can put in place measures that go beyond what is called for in ISPMs (or for cases where a standard

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[1] The CBD and the IPPC use the term "living modified organism" or LMO instead of "genetically modified organism" or GMO when referring to those organisms that fall under the scope of the CP.

[2] The IPPC definition of a "pest" is: any species, strain or biotype of plant, animal or pathogenic agent, injurious to plants or plant products (Glossary of Phytosanitary Terms, 2001).

does not exist), so long as those measures are technically justified and based on an assessment of the risks involved. Countries may also put in place provisional measures, or emergency measures, without performing a risk assessment (e.g. as in the case of foot and mouth disease) so long as they undertake to perform the risk assessment within a reasonable period of time to determine whether the measures are justified. Although the IPPC is identified in a trade agreement, and makes provision for trade in plants and plant products, it is not limited to trade in its application.

Requirements for conducting pest risk analysis as the principal means for establishing phytosanitary measures appear in the Convention under Art. VII (Requirements in relation to imports). In particular, Art. VII.2a states that "Contracting parties shall not, under their phytosanitary legislation, take any of the measures specified in paragraph 1 of this Article unless such measures are made necessary by phytosanitary considerations and are technically justified." Art. VII.2c includes requirements for countries to furnish the rationale for their phytosanitary measures. As with the SPS Agreement, the IPPC requires that in addition to being technically justified, measures do not unnecessarily impede trade. Art. VII.2g states "Contracting parties shall institute only phytosanitary measures that are technically justified, consistent with the pest risk involved and represent the least restrictive measures available, and result in the minimum impediment to the international movement of people, commodities and conveyances." Like the SPS Agreement, the IPPC makes specific mention in Art. VII.6 that a country may put in place provisional measures, but that the country is obliged to seek information and evaluate the measures as soon as possible.

The Convention on Biological Diversity (CBD) is an international instrument with the purpose of promoting the conservation and sustainable use of biological diversity and ensuring an equitable distribution of the benefits derived from biological diversity. It is a wide ranging agreement that addresses many key areas including measures and incentives for the conservation and sustainable use of biological diversity, conservation of genetic resources (in both wild and cultivated environments), and technical and scientific cooperation. In terms of ecosystems, the agreement covers all types of habitats including agricultural and marine environments.

The CBD also addresses the benefits and risks associated with technology, including biotechnology. The Cartagena Protocol on Biosafety (CP), a subsidiary agreement of the CBD, is the international instrument that sets forth requirements regarding the transboundary movement of living modified organisms (LMOs). The CP was adopted by the Conference of the Parties (COP) of the CBD in January 2000, but is not yet in force[3]. The decisionmaking body of the CP is the Meeting of the Parties (MOP), while the Intergovernmental Committee for the Cartagena Protocol on Biosafety (ICCP) administers certain activities on behalf of the CP.

The CP's scope applies to the transboundary movement of any LMO that is intended for release into the environment (plants, animals, etc.), but does not apply to LMOs that are intended for feed, processing for food or for pharmaceutical uses. This is in contrast to the scope of the IPPC, which in principle could apply to LMOs intended for feed, processing for food or pharmaceuticals in the event that those organisms could pose a threat to plant health.

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[3] The CP will enter into force on the ninetieth day after the date of deposit of the fiftieth instrument of ratification, acceptance, approval or accession by States or regional economic integration organizations that are Parties to the Convention.

Like the SPS Agreement and the IPPC, the CP requires countries to base measures for LMOs on a risk assessment. However, neither the CBD nor the CP have the mandate nor the capacity to create internationally agreed guidance (e.g. in the form of standards); rather, guidance on the conduct of risk assessment for LMOs under the CP is restricted to the agreement itself and to Annex III (Risk Assessment) of the agreement. In this light, internationally agreed guidance developed by other bodies, including the IPPC, will be especially important for countries to understand their rights and obligations of the relevant agreements, and to develop technically sound risk analysis procedures to assess and manage potential risks associated with LMOs.

Over the past few years, the Secretariat of the IPPC has undertaken collaborative efforts with the Secretariat of the CBD as an initiative of the IPPC's decisionmaking body, the Interim Commission on Phytosanitary Measures (ICPM). The collaborative efforts are aimed at addressing areas of overlap between the two Conventions, specifically with regard to alien invasive species (AIS) and LMOs. An open-ended expert Working Group which met in June 2000 that included phytosanitary experts and representatives of the CBD, agreed that in cases where either AIS or LMOs have the potential to be plant pests, these organisms fall under the scope of the IPPC and procedures (e.g. pest risk analysis) developed under the IPPC clearly apply. It was agreed that organisms that do not pose a threat to plant health (e.g. transgenic mosquitos) do not fall within the scope of the IPPC.

It should be noted that countries which are signatory to the CBD are the same countries that are signatory to the WTO and to the IPPC. Efforts to enhance cooperation are especially important at the national level to reduce overlaps or possible conflicting regulations that address AIS and LMOs which result from poor coordination between national agencies. In practice, national plant protection organizations (NPPOs) are being increasingly called upon to address risks associated with AIS and LMOs, where those organisms can pose a threat to plant health. As such, the IPPC has been called upon, both by national governments, the CBD and its subsidiary bodies (e.g. Conference of Parties, the Intergovernmental Committee for the Cartagena Protocol on Biosafety) to provide guidance to NPPOs on how to assess and manage the potential plant pest risks associated with AIS and LMOs.

One key difference between the above agreements is with whom the responsibility lies in conducting the risk analyses. Under the SPS Agreement and the IPPC, the responsibility for conducting the risk assessment is shared between the importer and exporter. The importing country, in imposing conditions for import, is in the position to justify whatever measures it requires based either on an international standard or on a risk analysis. The exporting country is responsible for providing certain types of information to the importing country in order to enable to importing country to conduct the risk analysis. This may include information on the pest status of the exporting country, growing conditions, field certification procedures or other factors that affect the likelihood of quarantine pests moving in a commodity. Similarly, both importing and exporting countries should seek out mutually acceptable risk management options. On the other hand, procedures under the CBD generally require the exporting country to fulfill the requirements for the risk analysis. This key difference has, and will continue, to create difficulties in capitals as countries strive to implement the requirements of these agreements.

A common element of all of these agreements (SPS Agreement, IPPC, CBD and CP) is that they apply principally to the transboundary movement of products (e.g. international trade). Each agreement specifically states that every nation retains the sovereign right to regulate products or organisms within its respective territory as it sees fit. For example, at the national

level, a country is within its rights under these agreements if it chooses not to impose domestic restrictions on the production or movement of certain organisms, including LMOs (and so long as those domestic products are not treated more favorably than similar imported products).

PEST RISK ANALYSIS REQUIREMENTS AND PROCEDURES

As mentioned above, the IPPC requires that countries base their measures on international standards when possible. However, countries may deviate from standards if their measures are justified using pest risk analysis. Requirements for conducting PRA as the principal means for establishing phytosanitary measures appear in the Convention under Art. VII (Requirements in relation to imports). In particular, Art. VII.2a states that *"Contracting parties shall not, under their phytosanitary legislation, take any of the measures specified in paragraph 1 of this Article unless such measures are made necessary by phytosanitary considerations and are technically justified."* Art. VII.2c includes requirements for countries to furnish the rationale for their phytosanitary measures. As with the SPS Agreement, the IPPC requires that in addition to being technically justified, measures do not unnecessarily impede trade. Art. VII.2g states *"Contracting parties shall institute only phytosanitary measures that are technically justified, consistent with the pest risk involved and represent the least restrictive measures available, and result in the minimum impediment to the international movement of people, commodities and conveyances."* Like the SPS Agreement, the IPPC makes specific mention in Art. VII.6 that a country may put in place provisional measures, but that the country is obliged to seek information and evaluate the measures as soon as possible.

The current ISPMs for pest risk analysis (PRA) developed by the IPPC include ISPM Pub. No. 2 *Guidelines for pest risk analysis* and ISPM Pub. No. 11 *Pest risk analysis for quarantine pests* (see Annex I). It is envisaged that there will also be an ISPM for pest risk analysis for regulated non-quarantine pests. ISPM Pub. No. 2 is in need of revision, and as such, ISPM Pub. No. 11 is the most important of the pest risk analysis concept standards at this time. Other ISPMs, such as the Code of conduct for the import and release of exotic biological control agents rely heavily on many elements of pest risk analysis.

The IPPC defines **pest risk analysis** as
"The process of evaluating biological or other scientific and economic evidence to determine whether a pest should be regulated and the strength of any phytosanitary measures to be taken against it."

The related terms of **pest risk assessment** and pest **risk management** are defined as follows:
Pest risk assessment is defined as *"Determination of whether a pest is a quarantine pest and evaluation of its introduction potential."*

Pest risk management is defined as *"The decision-making process of reducing the risk of introduction of a quarantine pest."*

The IPPC makes distinctions between regulated pests, quarantine pests and regulated non-quarantine pests as follows:

A regulated pest is "a *quarantine pest or a regulated non-quarantine pest*".

A quarantine pest is "a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled."

A regulated non-quarantine pest is "a *non-quarantine pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party*".

The distinctions above are key concepts in the sense that a pest must meet the definition of a regulated pest before a country can put measures in place against it for products entering the country from another country.

Overview of the IPPC PRA Process
Although the terminology used in the IPPC differs slightly from risk analysis procedures developed in other fields (e.g. engineering, toxicology, etc.) the general concept of risk is the same. Risk is generally defined as a product of the probability of a hazard occurring (e.g. pest introduction) times the magnitude of the consequences. Namely, risk analysis includes the elements of risk assessment, risk management and risk communication. In general terms, risk assessment includes hazard identification, hazard characterization, evaluation and assessment. The three main components of risk analysis (assessment, management and communication) of risk are viewed to be functionally separate (although overlapping) procedures. Risk analysis incorporates scientific and technical information (e.g. it is science based), but is not itself considered to be science.

In the ISPMs for PRA, pest risk analysis is divided into three stages: **initiation, pest risk assessment** and **pest risk management**. Initiation involves defining hazards by identifying the pest(s) and conditions that are of concern and should be considered for risk analysis. Initiation may result from specific pests, pathways (i.e. commodities) or policies. The result of initiation is the defining of particular pests and conditions that will become the focus of the risk assessment.

The second stage is risk assessment. The first part of risk assessment is determining which pests to assess and then evaluating these pests for the likelihood and consequences of their introduction and/or spread. Here, **pest categorization** is the preliminary screening of individual pests to determine whether the pests meet the defining criteria for regulated pests. The risk assessment is the examination and analysis of biological and economic information to estimate the introduction and spread potential and potential economic impact of the pest(s). The resulting risk assessment characterizes the risk to allow for judgments to be made regarding whether the risk is acceptable or what measures may be used to mitigate the risk. The last stage of PRA is risk management that involves identifying options for reducing risk and evaluating them for their suitability. In identifying options to mitigate risk, it may be necessary to return to the risk assessment stage of the PRA to examine the effects of measures on mitigation, i.e. mitigation itself also requires assessment. The available options are evaluated for efficacy, feasibility and impacts.

One of the major outputs of the decisionmaking process is determining the "appropriate level of protection" (ALOP) or "acceptable level of risk" (ALR). The principle of managed risk is introduced in ISPM Pub. No. 1 *(Principles of Plant Quarantine as Related to International Trade)* that states that*: "Because some risk of introduction of a quarantine pest always exists, countries shall agree to a policy of risk management when formulating phytosanitary measures."* In practice, the ALOP should be applied consistently to different pests. For

example, a country may decide that a 0.01% chance of the introduction of a pest that could cause an estimated US$1,000,000 in damage meets its appropriate level of protection. In order to consistently apply the ALOP, the country would then also accept a 0.1% chance of the introduction of a pest that could cause US$100,000 in damage. Bearing in mind the principle of managed risk, all countries have the sovereign right to determine their own ALOP, so long as it is consistently applied to all trading partners and with respect to both imported and domestic products. The principle behind the consistent application of the ALOP is that countries should avoid imposing unnecessarily restrictive measures to protect certain sectors of their agriculture, not from pests, but from fair competition.

APPLICATION OF ISPMs TO LMOs

ISPM Pub. No. 11 *Pest risk analysis for quarantine pests*
This standard describes the PRA process for "quarantine pests". It should be noted that the definition of quarantine pest mentions "economic importance", but environmental concerns are not explicitly distinguished in this definition. The problem arises in the interpretation of "**economic importance**" since this could implicitly, but not obviously, include impacts on non-target organisms. The ICPM has recommended that the term "economic importance" be more fully elaborated in the *Glossary of Phytosanitary Terms* to clarify that environmental impacts are included in the definition. Likewise, a draft supplement to this ISPM which addresses how to include the assessment of environmental hazards in PRAs is schedule to be reviewed and distributed for consultation this year.

Initiation of a PRA for LMOs would be considered to be pest initiated in most cases, in the sense that the LMO itself would be evaluated for its potential to be a pest. Recall that pest risk assessment is defined as "determination of whether a pest is a quarantine pest and evaluation of its introduction potential". This definition is clearly applicable to more conventional plant pests, but may not fully apply to LMOs. One key point of the definition is "quarantine pest", which means that there must be a demonstrable economic harm associated with the introduction of the pest. Likewise, if the LMO being evaluated is the actual commodity or even perhaps a genetically modified biological control agent, then the potential for introduction is already established.

Section 2 contains guidance on various aspects of the assessment stage that would be applicable to evaluating risks of LMOs. **Section 2.2.1.5** discusses the probability of transfer to a suitable host, including dispersal mechanisms (including vectors), intended use of commodity and risks from by-products — each of which may be applicable to examining the pest potential of an LMO. **Section 2.2.2** also provides extensive guidance on evaluating probability of establishment, taking into account such factors as availability of suitable hosts, alternate hosts, and vectors in the PRA area, suitability of the environment, cultural practices, reproductive strategy of the pest, and genetic adaptability. This last factor may be especially applicable to the evaluation of the pest potential of LMOs. Section 2.3 discusses the assessment of potential economic consequences. As with ISPM No. 2, a pest must have the potential to cause economic harm. However, certain indirect effects of an LMO (e.g. effects on non-target organisms) may be difficult to quantify economically. Nonetheless, **Section 2.3.1.1** and **Section 2.3.1.2** discuss direct and indirect pest effects, respectively, and include assessment of environmental effects as well as assessing the potential for crop losses. PRA accounts for uncertainty (**Section 2.4**) in the overall analysis, including in examining risk management options. Where uncertainty is considered in risk management, precaution is considered to be taken into account. However, there remains a significant amount of variation

in the interpretation and application of precaution outside of IPPC contexts. To a certain extent, the discrepancy in interpretation can create conflicting situations at national, regional and international levels. In order to avoid conflicts arising from the interpretation of this concept for LMOs, it may be productive to more fully define the roles of uncertainty and precaution in the conduct of PRAs for LMOs to ensure that the precautionary approach is not misunderstood to be an alternative to PRA but instead is viewed in its proper context as an element of decisionmaking based on the uncertainty expressed by PRA.

Section 3 provides guidance on management options. **Section 3.4.1** discusses risk management options for consignments, including pre- and post-entry quarantine, specified conditions for the consignment and restrictions on end use, all of which may be applied to LMOs.

ISPM Pub. No. 3: Code of conduct for the import and release of exotic biological control agents
In addition to ISPM Pub. No. 11, ISPM No. 3 provides the strongest guidance to contracting parties with respect to the **intentional** importation of exotic organisms, and may provide relevant guidance for the intentional import and release of LMOs into the environment. The scope of the Code applies to those agents capable of self-replication (parasitoids, predators, parasites, phytophagous arthropods and pathogens). Although organisms modified through techniques of modern biotechnology are not explicitly covered in the Code, the Code does state that it can be applied to such organisms.

The **Outline of this Code** states:
Procedures governing the handling and release into the environment of strains of organisms created artificially by genetic engineering are currently being examined by various international organizations and by national programmes. If required this Code could be applied to these organisms.
However, LMOs covered by the standard would be limited to those modified to control pests (e.g. Bt transgenics) but not LMOs modified for other purposes (e.g. "Golden rice"). Likewise, as the code is currently written, organisms modified for sterility (e.g. for SIT) are excluded since by definition the code applies to organisms capable of self-replication. It is possible that (as the code is due for revision) this may be changed in the future to include provisions for sterilized organisms. The **Scope** of the standard could be applied to pest risk analysis processes for LMOs. These types of introductions are in principle addressed through the practice of pest risk analysis and the Code specifically designates responsibilities for certain types of actions associated with intentional introductions to particular national authorities, importers and exporters.

As part of the process of approving intentional introductions, a dossier of information on the organism should be prepared (**Section 4**) by the importer at the time of importation. This information includes biological information on the organism, assessment of its importance, and its known natural enemies. After importation, the importer should prepare dossiers on the organism containing information on identification, biology, host range and specificity, potential hazards to non-target organisms, and any natural enemies of the organism. This is directly relevant to Annex I of the CP which describes information required in notifications under Articles 8, 10 and 13 and Annex III (risk assessment).

Supplement to ISPM Pub. No. 11: Analysis of environmental risks [DRAFT]
The IPPC has recently drafted a supplement to ISPM Pub. No. 11 to provide additional guidance on environmental risks associated with plant pests. The draft is scheduled to be reviewed and distributed for consultation this year, and could be adopted at the 5th session of the ICPM. The supplement is aimed at providing additional detail and clarification on the analysis of environmental risks (including to uncultivated / unmanaged plant species, habitats and ecosystems). Notably, it provides information on methods to assess economic consequences of environmental harm, which has been a difficult concept in terms of practical application.

FUTURE ACTIVITIES OF THE IPPC WITH REGARD TO LMOs

At the 3rd Session of the ICPM in April 2001, the ICPM recommended that the IPPC develop an ISPM to provide guidance on pest risk analysis for LMOs. The ICPM agreed that the current ISPMs for pest risk analysis (PRA) could be applied to analyzing plant pest risks of LMOs, but that countries needed more specific detailed guidance. Following the recommendation of the ICPM, the IPPC conducted another open-ended Working Group including phytosanitary experts and representatives of the CP and the ICCP in September 2001 to draft specifications (a sort of detailed outline) for an ISPM for PRA for LMOs. These specifications were approved (see Annex II *Report of the ICPM Open-ended Working Group on Specifications for an International Standard for Phytosanitary Measures on Living Modified Organisms*) at the 4th Session of the ICPM in March 2002. It is anticipated that an expert Working Group will meet this year (with experts and representatives of the CP) to draft the working document. It is most likely that, like the environmental standard, this draft will be a supplement to ISPM Pub. No. 11. In addition, it is likely that the draft will be primarily aimed at assessing risks of transgenic plants since this was considered to be the major priority of both the Working Group and the ICPM. It was envisaged by the Working Group that this draft could, if necessary, provide the basis for any additional standards on LMOs (e.g. for transgenic arthropods) if contracting parties require additional guidance in the future. In addition to drafting a standard to address pest risk analysis for LMOs, the Secretariat of the IPPC will continue its ongoing collaborative efforts with the CBD and its subsidiary bodies, including the ICCP.

TRANSGENIC ARTHROPODS AND THE STERILE INSECT TECHNIQUE

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Abstract

The Sterile Insect Technique can benefit from transgenesis in three ways by creating; (1) genetically marked strains, (2) genetic sexing strains and (3) strains that induce molecular sterility in the field. Experience with the development of genetic sexing strains based on indicates that caution is required during the experimental evaluation of any potential transgenic strain. Two major scientific concerns involve the overall fitness of transgenic strains and their stability over time. The latter being very important especially when the extremely large numbers of insects that are mass reared is taken into account. Currently transformation events are random and it will probably be necessary to select suitable strains from many that are induced. The success of transformation itself in many insect species will enable many new strategies to be developed and tested.

INTRODUCTION

The Sterile Insect Technique (SIT) is an environmentally friendly alternative to insecticides for the control and/or eradication of insect pests. The SIT is target species specific because it is based on mating, i.e. no other species are affected. It can be terminated at any time and leaves no residues. SIT has been applied with great success against several pest insects, including the New World Screwworm fly, the Mediterranean fruit fly (medfly), the Queensland fruit fly, *Anastrepha* spps and the tsetse fly *Glossina austeni*. At the heart of the SIT is the release of large numbers of insects that can transmit a genetic load into the target population. In all current programmes, this genetic damage/sterility is induced by exposing the insects to radiation. The sterility is transmitted into the target population by the mating of irradiated males with wild females. Irradiated females do not contribute significantly to the sterility in the target population (Knipling, 1955; McInnis at al, 1994).

STERILITY

The principle underlying the SIT is the induction and transmission of debilitating genetic factors, e.g. severe genetic damage (dominant lethality), via sperm to the next generation. When the SIT concept was formulated by Knipling in 1937 it was not clear what genetic factors would be useful or how genetic damage could be generated (for a historical overview see Bushland, 1971). It was the work of Muller on Drosophila that demonstrated that a radiation treatment caused exactly the required effects (Muller, 1927). Later alternatives such as chemical mutagenesis were explored but it became very clear that radiation is by far superior in terms of reproducibility, ease of application and reliability. In addition, most if not all of the chemicals investigated would represent a severe environmental risk if insects treated with them would be released. Consequently, all SIT programs use ionising radiation to induce sterility in the released insects. It has to be stressed that the term "sterility" (or "sterile insect") does not mean that these insects are themselves disabled, e.g. their reproductive system is rendered non-functional due to the treatment. The type of sterility that is referred to here means that the next generation in the field dies because of the genetic damage it has received from its irradiated parent.

It was shown for the medfly that sterility, induced with the appropriate dose and at the correct developmental stage, is irreversible (Anwar et al, 1971: dose 100 Gy, 2 day before or after emergence). This is important in the context that males throughout their life produce new sperm. Anwar et al showed that the radiation treatment destroyed all cells required for the production of new sperm (spermatogonia, primary and secondary spermatocytes, spermatids), i.e. only the mature sperm present at the time of radiation is, and will be, available for transmission. The authors also concluded that irradiating adult males would be preferable because they carry more sperm than pharate adults 1 or 2 days before emergence. However, even males prior to emergence carry sufficient sperm for five to eight matings (Ohinata et al, 1978).

It is widely documented that the radiation treatment also causes some damage to the insect itself (somatic damage). As a result the released insects are less effective in the field, more insects have to be released to compensate for this effect and, as a consequence, programme costs are increased. However, not only the radiation treatment has a negative effect on mating competitiveness but also the mass rearing process. In several sets of field cage tests with equal numbers of wild flies (from eight different populations) and fertile, mass reared GSS flies (four different strains), the GSS males on average took part in 35% of all matings with wild females (Relative Mating Index (RSI) = 0.349, Cayol, 2000). This represents a reduction by 30% relative to a 50/50 ratio expected if both types of males would be equally competitive. In a comparison between wild males from Argentina and sterile, mass reared GSS males (minimum dose 100 Gy, maximum dose 150 Gy), the GSS males participated in 26% of the matings with wild females (RSI = 0.264, Cayol et al 1999). Comparing the two sets of data, one would conclude that the radiation treatment as such reduced the mating competitiveness by an additional 26%, i.e. the magnitude of the negative impact of the mass rearing and the radiation treatment is similar and the combined effect leads to an overall reduction of 48% relative to the expected 50/50 ratio. This figure is in agreement with the results from other studies (e.g. Lance et al, 2000). In addition, there are several aspects to be considered in this context. First, the degree of somatic damage is dependent on which developmental stage is treated with radiation. It was shown for medfly (Ohinata, 1971) and tsetse fly (Mutika et al, 2001) that irradiating adults has only very little, if any, negative side effects. In other words, somatic damage is not an unavoidable problem but it depends very much on the actual procedures, e.g. the most practical stage for irradiation, on how significant the somatic damage is. For example, in tsetse fly SIT adults are irradiated because the sexes can be separated only at that stage (plus the females should not be sterilised because they have to be used to maintain the colony) while in medfly the standard procedure is to treat pupae, 1 to 2 days before emergence. Secondly, it was shown that somatic damage or, more precisely, the negative effects on insect quality can be avoided/reduced by irradiating the insects in an atmosphere of nitrogen (e.g. Fisher, 1997). It is surprising that this technology has not found wider acceptance considering the fact that the reduced quality of irradiated insects is discussed so widely. Thirdly, the radiation dose applied to sterilise, for example medfly, varies tremendously and is in many cases too high. This can have technical (lack of standardisation or the dimensions of the irradiator) or other reasons (requirement for unnecessarily high levels of sterility by the end user).

So far, no case of resistance against radiation induced sterility has been observed. This is not surprising considering the degree of damage that is caused by even moderate levels of radiation, i.e. there is no mechanism known that would repair such damage correctly and would restore the integrity of the irradiated genome to a level where viability is restored. For example, there is no mechanism that can repair deletions either caused directly by the

38

radiation or indirectly by the segregation behaviour of radiation-induced translocations. There were cases where released flies showed a reduced mating competitiveness, i.e. the wild females would prefer to mate with wild males and would accept released males only at a reduced frequency (e.g. McInnis et al, 1996). However, these effects were only observed with specific strains and were easily overcome by changing the release strain.

MASS REARING FOR SIT

SIT has been used, at different levels, against a large number of different species. As shown in Table 1, the highest numbers are produced for SIT against the medfly while in many other cases the production capacities are low because SIT is not yet established (or technically feasible) as a viable control strategy. The number of flies produced and released is directly dependent on the biology of the different species, e.g. reproductive capacity and the resulting population density. For example, tsetse fly females produces only 4-6 offspring over three months which makes the mass rearing of this species very difficult. On the other hand, this low reproductive potential leads to low population densities in the field and, consequently, relatively few sterile males have to be released. The opposite is true for most fruit flies, i.e. large numbers can be produced rather easily but this is also required because the population density of these species is usually very high.

In principle, SIT against all species would require the release of only males because the release of females, despite the fact that they are sterile, usually cannot be accepted. Depending on the species there are three basic considerations. In case of fruit flies, the sterile females sting the fruit attempting to deposit eggs. This damage leads to a reduction in fruit quality and, as a consequence, in market value. In some countries, SIT with bi-sex strains was only acceptable where the fruits were not produced for export and/or where SIT was used only for a limited period of time (e.g. in an eradication program). In case of species like the screwworm fly it is claimed that the attack by sterile females, especially considering the high numbers released, would lead to disturbance of the animals resulting in lower productivity. However, no sexing system exists for screwworm flies so far and this is probably acceptable because the screwworm programme is aimed at eradication and advanced so rapidly that the negative effect of the sterile females can be minimised. In the case of disease transmitting mosquitoes, the release of sterile females cannot be tolerated because they would increase disease transmission. In addition to these three categories of species-specific arguments, there are more general reasons, related to the principle of the SIT, why the production and release of only males would be beneficial. These include lower production costs, higher efficiency in the field and increased safety (Hendrichs et al, 1995).

Extrapolating from the developments of the last decade one can assume with reasonable certainty that the use of the SIT will increase in the coming years, i.e. the number of programmes for existing SIT applications as well as the number of different species where SIT is used will increase. In addition, more programmes will require the constant release of sterile flies over long periods of time. This is the case where SIT is used for suppression rather than for eradication or where SIT is applied proactively to avoid the establishment of invading pests. It follows that also the need for developing genetic sexing systems will increase. However, that would be extreme difficult and time consuming if only the current strategies, classical Mendelian genetics, are available. Clearly, in future more generic methodologies will have to be employed to generate genetic sexing systems for a large number of different species.

	Maximum capacity (millions per week)		Maximum capacity (millions per week)
Ceratitis capitata (Mediterranean fruit fly)	1,400 (GSS) + 900 ⊕	*Cochliomyia hominivorax* (New world screwworm)	500
Bactrocera cucurbitae (Melon fly)	280	*Chrysomya bezziani* (Old world Screwworm)	6
Anastrepha ludens (Mexican fruit fly)	240 ⊕	*Delia antiqua* (Onion fly)	7.5
Bactrocera dorsalis (Oriental fruit fly)	100 ⊕		
Anastrepha suspensa (Caribbean fruit fly)	50	*Glossina austeni*	0.09 (males)
Anastrepha obliqua (West Indies fruit fly)	50 ⊕	*Glossina ssp*	0.04 (males) ⊕
Bactrocera tryoni (Queensland fruit fly)	40	*Anopheles albimanus*	ca 7 (GSS)
Bactrocera philippinensis	20		
		Pectinophora gossypiella (Pink Bollworm)	84
Ceratitis rosa (Natal fruit fly)	3	*Cydia pomonella* (Codling moth)	15
Bactrocera oleae (Olive fruit fly)	1 ⊕		
Bactrocera latifrons (Solanum fruit fly)	<1		Table 1: Present and past mass rearing capacities for SIT applications
Anastrepha serpentina (Sapote fruit fly)	5		
Anastrepha fraterculus (South American fruit fly)	2 ⊕		

GENETIC SEXING STRAINS FOR THE MEDFLY

Current genetic sexing strains (GSS) are generated by linking the wild type allele of a selectable marker (e.g. white pupae, wp) via a Y-autosome translocation to the male-determining Y chromosome (Figure 1) (Franz et al, 1994; Robinson et al, 1999). The first strains that carried such a construct were available in 1980. Although small-scale tests showed no obvious problems, tests under mass rearing conditions demonstrated immediately that these strains were neither stable enough nor was the selectable marker particularly economic. Improved strains were constructed in the following years (Franz et al, 1994) and after intensive testing these 2nd generation GSS were introduced into the mass rearing factory in Guatemala in 1994. Two more years were required to develop the appropriate mass rearing procedures (e.g. Filter Rearing System, (FRS) Fisher & Caceres, 2000) before a true large-scale application was possible. From there on the use of GSS for medfly SIT expanded rapidly reaching a world-wide production capacity of 1400 million males per week by the end of 2001.
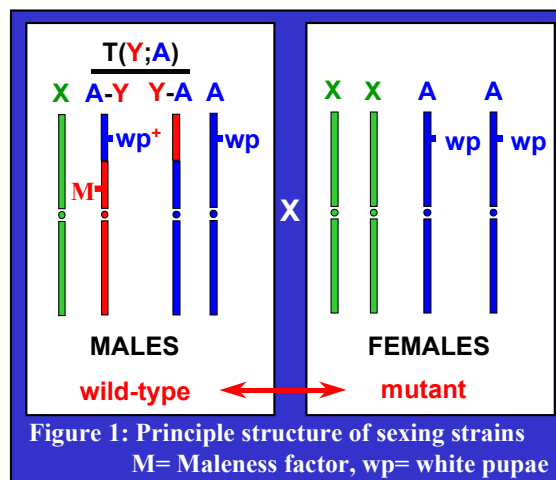

Figure 1: Principle structure of sexing strains
M= Maleness factor, wp= white pupae

One of the primary problems with GSS for medfly was their instability over time. Today, two types of recombination in the translocation-baring males are known that are responsible for this "strain breakdown", i.e. are responsible for the gradual reversal back to a bi-sex strain. They occur at very low frequencies, i.e. 10^{-3} to 10^{-4} (e.g. the most recent GSS: VIENNA 8/D53: 1.5×10^{-4}) and 10^{-6} to 10^{-7}, respectively. However, in both cases recombinant flies are generated that have a selective advantage as compared to their normal, non-recombinant siblings. Especially under the highly selective and stressful mass rearing conditions these recombinants accumulate rapidly in the colony. The current experience is that even additional measures, like the incorporation of chromosomal inversions, will not reduce recombination to zero. In other words, such recombinants will occur, especially considering the extremely high level of production, and they will eventually accumulate in the colony. GSS can only be maintained stable by introducing the FRS (Fisher & Caceres, 2000). Unlike the mass rearing procedure used in the past, the rearing utilising a FRS is non-continuous. It requires maintaining a relatively small colony where recombinants can be removed each generation. Starting from this colony two to three amplification steps are needed to reach the final mass rearing level (release stream). However, no material produced in the amplification steps or in the release stream is ever returned to the clean stream, i.e. recombinants can only accumulate for three to four generations.

TRANSGENIC STRAINS FOR SIT

Three different types of genes/traits have been considered for the development of improved strains for the SIT (Robinson and Franz, 2000):

(a) A marker gene, like the Green Fluorescent Protein (GFP), to allow discrimination of released and wild flies in the field: Currently, released flies are labeled with a fluorescent dye powder. This not only labor intensive and expensive but also not completely safe (e.g. loss of the dye, transfer of the dye to wild flies during mating).

(b) Genes to allow genetic sexing: Genetic sexing systems are basically required for most if not all species. The existing systems are generated using conventional Mendelian genetics but their primary drawback is that they cannot be transferred to other species. An additional problem is that the translocation-based strains are roughly 50% sterile.

(c) Genes that would replace the radiation treatment, i.e. that cause lethality after transmission into the field population: Currently, exposure to radiation is used to cause the required genetic damage/sterility. On average, this reduces the mating competitiveness (in field cage tests) by ca 30%.

All three strategies require the availability of a reliable and stable transformation system. For a long time, no transformation vector was available for non-drosophilid insects (e.g. Atkinson & O'Brochta, 1999; Handler, 2000). Today three different mobile elements can be used to transform a large number of different species of economic or health-related importance. However, the testing of these systems is still in its infancy. All transgenic strains have only been reared at extremely small numbers. Consequently, it is very difficult, if not impossible, to draw conclusions with respect to important characteristics like stability and reproducibility. It could be argued that the transgenic systems are at the same level of development as the GSS were in 1980 when small-scale rearing showed no obvious problems.
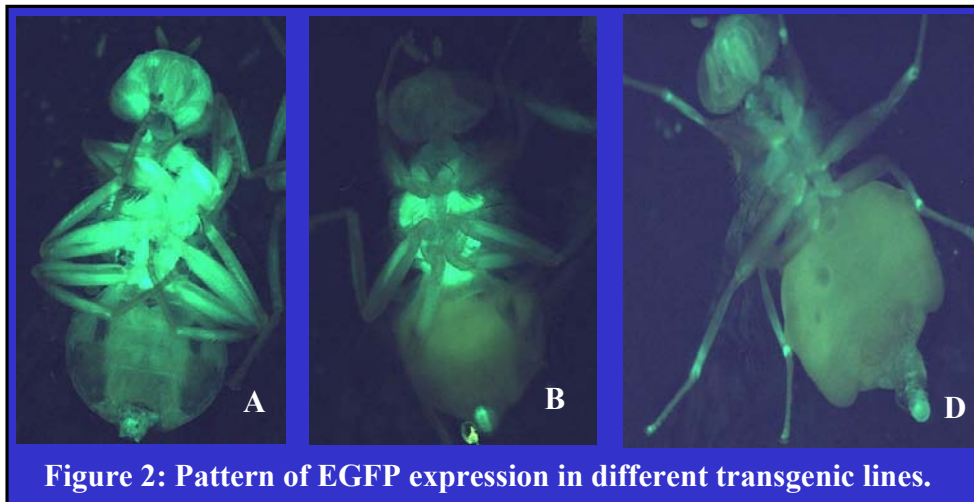
Figure 2: Pattern of EGFP expression in different transgenic lines.

However, already the small-scale rearing of some transgenic strains showed that they have certain undesirable features. Analysis of a sufficient number of lines, transformed with the same construct, shows that the expression pattern of the transgene is usually quite variable. Probably depending on the integration site, different transgenic lines show quite different, but inheritable patterns of expression of the marker, e.g. GFP or DsRed (Figure 2). Furthermore, within each class the overall strength of the expression varies which in turn determines how long the marker can be detected in living or dead adults. This feature would be important if the marker was to be used for labeling released flies. It follows that a large number of strains has to be generated and screened to find those with optimal expression of the transgene. This may become even more complicated when two transgenic strains have to be crossed to obtain the desired combination of genes/traits.
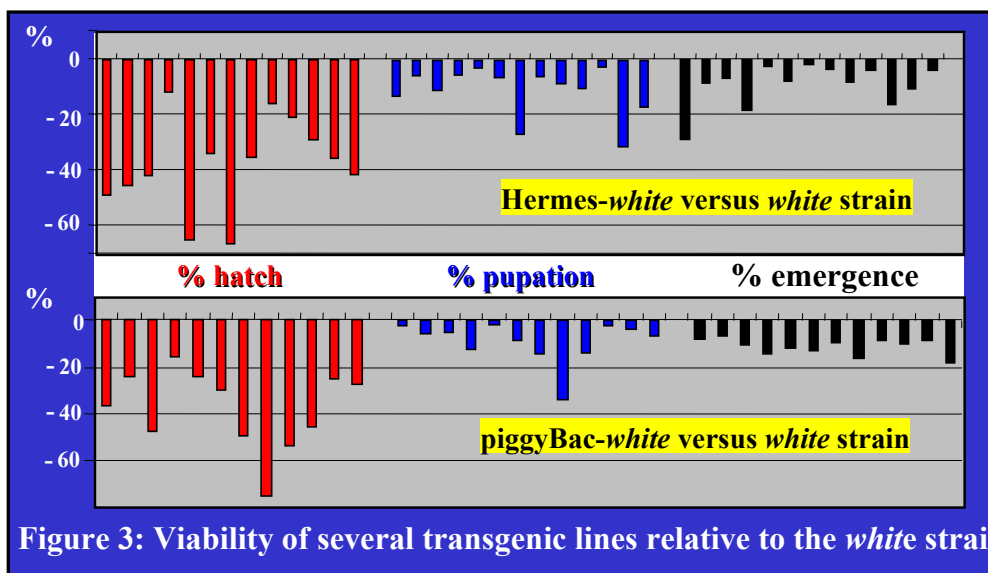
Figure 3: Viability of several transgenic lines relative to the *white* strain

Secondly, one of promises of transgenic technology was that the inherent semi-sterility of current GSS, caused by the presence of a translocation, would be avoided. However, two sets of transgenic lines, transformed with either a *Hermes* or a *piggyBac* vector (both carry the medfly white gene as marker), show on average the same sterility as the current GSS. The sterility is quite variable between lines but in most it is strongest in the embryonic stages

(Figure 3). The underlying reason for this sterility is unknown but it would certainly have a major impact on the mass rearing and, potentially, also on the stability of transgenic strains.

Nothing is known about the behaviour of transgenic lines under mass rearing conditions and, based on the experience with current GSS, it is realistic to expect that yet unknown effects will be discovered. As highlighted above, mass rearing represents a highly selective and stressful condition and even very rare phenomena will be detected and can lead to a breakdown of the respective strain. For example, in the transgenic lines shown in Figure 3 any effect resulting in the occurrence fully fertile insects, for example due to the loss of the transgene, would lead to a rapid degeneration of the strain as a consequence of the accumulation of these insects.

In conclusion, the development of stable and reliable transformation systems appears to have high priority because all other application will depend on the availability of such a system. With respect to the three genes/traits that could be incorporated in strains for SIT, not all seem to have equal weight. The highest priority deserves the testing of existing strategies to separate/kill the females (e.g. Thomas et al, 2000; Heinrich and Scott, 2000). The availability of a generic sexing system that can easily be inserted into a large number of different species would clearly move the SIT forward.

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AREAS OF CONCERN FOR THE EVALUATION OF TRANSGENIC ARTHROPODS

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Abstract

Areas of concern for the release of transgenic insects relate to risks associated with: (1) the host insect involved, (2) the vector used for gene transfer, (3) genes of interest within the vector including markers, and (4) the expected persistence of the transgenic strain in the environment. The transgenic insect must be considered in terms of whether it is a pest or beneficial insect and risks relevant to its use as a non-transgenic insect. The vector used for gene transfer must be considered in terms of its mobility properties in the host insect and its potential for intra-genomic and inter-genomic movement, potentially mediated by a cross-mobilizing system. Intra-genomic movement may influence the expected expression and activity of gene of interest within the transgene, possibly having unanticipated effects on the host and, thus, program effectiveness. Inter-genomic movement is of considerable importance since risks must be evaluated in terms of the effects of the vector system and genes of interest on a multitude of potential host organisms. Risk assessment for transgene stability requires methods for transformant identification and a full genetic analysis of the transformed genome so changes in transgene presence or movement can be rapidly and reliably determined. Genes of interest within the transgene must be evaluated in terms of their affect on the host insect, and the potential influence of their gene products on the environment and other organisms should the transgene be transmitted to another host. These factors must be considered individually, their interaction with one another, and also in the context of transformant strain persistence in the field.

AREAS OF CONCERN

The primary areas of concern for the release of transgenic insects relate to risks associated with four major components of such a program. These are: 1) the host insect involved, 2) the vector used for gene transfer, 3) genes of interest within the vector including markers, and 4) the expected persistence and movement of the transgenic strain in the environment. The host insect must be considered in terms of whether it is a pest or beneficial insect and risks relevant to its use as a non-transgenic insect. The vector used for gene transfer is of primary importance since its behaviour and the ability to control its mobility are critical to stability of the transgene and the safe and effective use of transformant strains. An assessment of transgene stability requires an understanding of transposon vector function including its normal mechanism of transposition, its range of function and existence in other organisms, and the existence of related transposons and their potential for cross-mobilization. Risk assessment for transgene stability requires methods for transformant identification and a full genetic analysis of the transformed genome so that any changes in transgene presence or movement can be rapidly and reliably determined.

Genes of interest within the transgene must be evaluated in terms of their affect on the host insect, and the potential influence of their gene products on the environment and other organisms should the transgene move into another host. These factors must be considered in the context of the anticipated persistence of the transformant strain in the field. This will be a

function of whether the release is for a short term single generation, short term multi-generation (e.g. two to four generations), or for long term persistence where enhanced heritability of the vector may be desired. Short term releases will include those resulting in sterility or lethality to suppress the released insect and its offspring. Some of these will be for immediate suppression such as SIT (see Handler, 2002), or may be for limited multi-generational suppression in systems such as RIDL (Thomas et al., 2000) and ABC (Fryxell and Miller, 1995). For these cases, persistence of the transgene vector DNA in the species and environment will be limited and assessments of strain and vector stability must be considered in that context. Strategies requiring long term heritable release may include the improvement of beneficial insects, or causing pest species to become benign. For such programs persistence of transformant organisms, and possibly replication of the transgene, will be desired, and potential transgene movement into unintended hosts is a more significant consideration. An associated concern is the particular environment where the release will be performed, and other areas of potential invasion. For a final risk assessment, all of these factors must be considered individually, and also considered in terms of negative or positive interaction with one another. Many of these and associated issues for the risk assessment of transgenic arthropods are also reviewed and considered by Hoy (2000).

INSECT GENE-TRANSFER

An evaluation of risk assessment of transgenic arthropods requires an understanding of the systems and processes used for gene transfer. The genetic transformation of a wide variety of insects using several systems has been reviewed extensively in recent years (Ashburner et al., 1998; Handler and James, 2000; Atkinson et al., 2001; Handler, 2001) and thus only a brief overview will be presented here. The transformation systems available include germ-line transformations that result in the stable heritable integration of a transgene, as well as systems that allow the extrachromosomal transient expression of a genetic system, usually mediated by a viral or bacterial system. For the applied use of gene expression systems in released insects, germ-line transformation typically mediated by a transposable-element based system is currently the method of choice.

One the most critical factors for assessing risk factors and strain stability will be a thorough understanding of the behaviour and regulatory properties of the vector used for genomic integration. While all the vectors currently used for non-drosophilid germ-line transformation are Class II transposable elements that share common elements in terms of structure and mechanism of movement, significant differences exist among them, and thus they must be considered independently when assessing risk factors. A primary consideration is that these transposons, along with other mobile genetic elements, are mutagenic by virtue of their ability to integrate into coding and non-coding genomic DNA sequences. Thus they have the potential to disrupt normal gene function resulting in costs to fitness. On the other hand, a large percentage of most genomes are comprised of such mobile elements, and various mechanisms either pre-exist or evolve to regulate transposon function. A major concern relates to how a genome interacts with a transposon-based vector that has been newly introduced.

An important consideration for risk assessment and program effectiveness is the ability of the vector system to replicate. The transposon systems in use generally transpose by a DNA-mediated cut-and-paste mechanism, resulting in simple movement of the transposon from one position to another. Some transposons, however, are subject to template-directed gap repair (TDGR), whereby a transposon present in a homologous chromosome or sister chromatid can

act as a template for repair of the excised element resulting in a replicative event that increases the number of elements. This has been demonstrated clearly for the Drosophila P element (Nassif et al., 1994) which is not used for non-drosophilids, but TDGR has been recently shown to occur in the *mariner* system (Lohe et al., 2000). Thus, the use of *mariner* element vectors, and potentially other *mariner*/Tc family elements such as *Minos*, must be considered in this context.

Transposon-mediated transformation
The first transposon vectors used for insect transformation were P and *hobo* that had been discovered in D. melanogaster, but neither of these has been reported to be used successfully in non-drosophilids (see Handler, 2001). Four other transposon vector systems have been successful in a wide variety of insects, and these include *Hermes* from *Musca domestica* (Warren et al., 1994), the *mariner Mos* element from *Drosophila mauritiana* (Haymer and Marsh, 1986; Medhora et al., 1988), *Minos* from *D. hydei* (Franz et al., 1991), and *piggyBac* from *Trichoplusia ni* (Fraser et al., 1983). All of these systems have short inverted terminal repeats and they transpose via a DNA intermediate.

Regardless of their relative function, all of the transposon vectors are used in a similar fashion that includes a binary system of a non-autonomous vector and helper transposase plasmids. The vector includes the inverted terminal repeat sequences and subterminal sequences needed for mobility, which surround a selectable marker gene and other sequences of interest. The transposase gene within the vector is either deleted or made defective, and vector transposition depends upon a helper plasmid that includes the transposase gene but not the terminal sequences necessary for integration. Thus when transiently expressed in the germ-line, the helper transposase can catalyze integration of the vector, but is lost in subsequent cell divisions allowing the vector integration to remain stable.

Mariner and *Minos*
Of the transposons used as vectors in non-drosophilids, the functional *mariner* element, *Mos*, was the first to be discovered but its ability to transform Drosophila was limited (Lidholm et al., 1993), and it was several years before it was successfully tested in the mosquito *Aedes aegypti,* using the *kynurenine-hydroxylase*-white marker (Coates et al., 1998). Thus far the use of *mariner* for transformation has only been extended to *Musca domestica* (Yoshiyama et al., 2000).

The *Minos* element was the first transposon to be used for germ-line transformation of a non-drosophilid, the Mediterranean fruit fly, (Loukeris et al., 1995) and it has been subsequently used to transform the mosquito *Anopheles stephensi* using a green fluorescent protein (GFP) marker (Catteruccia et al., 2000). Although reports for its further use in transformation have been limited, transposition has been demonstrated in a wide range of insect cell lines and embryos (Klinakis et al., 2000) as well as in mice (Zagoraiou et al., 2001).

Hermes
The existence of *Hermes*, and other members of the *hobo, Ac, Tam3 (hAT)* family, was inferred from the cross-mobilization of *hobo* in species where *hobo* did not exist (Atkinson et al., 1993; see O'Brochta and Atkinson, 1996). *Hermes* and other *hAT* elements were then discovered by PCR amplification of genomic elements using common amino acid sequences in *hobo* (from Drosophila) and *Ac* (from maize) as priming sites. A complete *Hermes* element was first tested for function in *D. melanogaster* by germ-line transformation (O'Brochta et al., 1996), and its function in other species was tested by transient transposition tests (Sarkar et

al., 1997). Subsequently, *Hermes* was used successfully to transform *Aedes aegypti* (Jasinskiene et al., 1998), *Stomoxys calcitrans* (O'Brochta et al., 2000), *Tribolium castaneum* (Berghammer et al., 1999), (Michel et al., 2001) and *Culex quinquefasciatus (*Allen et al., 2001). While most of these transformations resulted from *Hermes*-mediated cut-and-paste transpositions, a curious finding was that the integrations in *Ae. aegypti* and *Culex* were not precise transposon-mediated events, but included rearranged integrations of the entire vector plasmid by some type of recombination event. These unusual integrations, however, were still dependent upon the presence of *Hermes* transposase, and it has been theorized that an interaction between the injected *Hermes* and endogenous *hAT* elements resulted in replicative recombination events (Jasinskiene et al., 1999). Presuming the genes of interest within the vector are not disrupted by the recombination event, transformation by recombination is not necessarily a drawback, especially if it enhances transgene stability (and indeed, "recombination" events are typically used for transformation in plant and vertebrate animal systems). Recombinant integrations are problematic if remobilization of the primary integration is desired for studies such as transposon-tagging and enhancer-trapping, and in applied use where the goal is to have autonomous functional vectors driven into a population (see Handler, 2001).

A more daunting consideration for the use of *Hermes* is related to its interactions with other hAT elements, and this was shown recently by the cross-mobilization of *Hermes* and *hobo* in plasmid and chromosomal excision assays (Sundararajan et al., 1999). Thus the possibility exists that *Hermes* integrations in species harboring functional hAT elements will not remain stable. At present there is some inconclusive evidence that *Hermes* integrations in medfly have been de-stabilized, potentially by a *hobo*-related element. Low level instability may not be problematic for small population experimental studies, especially using efficient visible markers for the transgene. However, even rare instability will be problematic for large mass reared populations, in terms of maintaining strain integrity as well as program efficiency. The potential for mobilization by related elements is of considerable importance to considerations for inter-species horizontal movement.

PiggyBac

The *piggyBac* element was discovered by virtue of its ability to transpose from its host genome within a cabbage looper moth cell line, into an infecting baculovirus (Fraser et al. 1983; Cary et al., 1989). Later molecular analysis of the element (then called IFP2) and its functional characterization by transient mobility assays indicated that it could potentially be used as a vector for germ-line transformation in several orders of insects (see Fraser, 2000). Unlike other vector systems that used heat shock regulated helpers, the first *piggyBac* transformations in (Handler et al., 1998) and *Drosophila melanogaster* (Handler and Harrell, 1999) used an unmodified transposase gene indicating that the *piggyBac* vector has autonomous function in different insect orders. Germ-line transformation with *piggyBac* was then achieved in other dipterans including *Bactrocera dorsalis* (Handler and McCombs, 2000), *Anastrepha suspensa* (Handler and Harrell, 2001), *Musca domestica* (Hediger et al., 2001), *Ae. aegypti* (Lobo et al., 2002), *An. gambiae* (Grossman et al., 2001) and *An. albimanus* (Perera et al., 2002); two lepidopteran species, *Bombyx mori* (Tamura et al., 2000) and *Pectinophora gossypiella* (Peloquin et al., 2000); and a coleopteran, *Tribolium castaneum* (Berghammer et al., 1999). Thus far there is no evidence to suggest that *piggyBac* is a member of a widespread family of elements, though other elements share its specificity for integration into the tetranucleotide site, TTAA. *piggyBac* has also been discovered in a species other than the cabbage looper moth. Control hybridizations for *piggyBac* transformation of B. dorsalis revealed multiple elements in the host genome nearly identical to *piggyBac* (Handler and

McCombs, 2000). Initial hybridization and PCR studies indicated that 8 to 10 elements exist in the genome of wild type and mutant strains, though none have been proven to be transpositionally active. Hybridization studies indicate that *piggyBac* also exists in another lepidopteran, *Spodoptera frugiperda*, though these elements are probably highly truncated forms (A.M.H., unpublished). Thus, the existence of *piggyBac* in both closely and distantly related species indicates that it has recently traversed orders by horizontal transmission and probably exists in other species as well. This movement had to be facilitated by functional *piggyBac* elements or cross-mobilizing systems, and thus concern for vector stability must be extended to *piggyBac* as well.

DEFINITION OF A GERM-LINE TRANSGENIC

Critical to risk assessment evaluation of transgenic insects are reliable, informative, and consistent means of characterizing the transformant line that will allow a definitive determination of changes in transgene position or expression. It is also critical to distinguish the transformant host strain from field insects so that potential intra-genomic movement of the transgene can be distinguished from inter-genomic movement within the same species. It should also be possible to determine whether the transgene has been introduced into related insects or un-related organisms, potentially transmitted by a non-vertical means of inheritance. If necessary, otherwise identical vectors may be marked with unique molecular tags (short DNA sequence) so their origin can always be defined.

Selection marker
The initial means of transformant identification depends on the marker system originally used to select or screen for the transformant line. Fully expressed dominant visible markers, such as wild type eye color genes (Sarkar and Collins, 2000), may be easily detected but difficult to distinguish from wild type non-transgenic insects. Incomplete expression of most transgenic markers due to chromosomal position effects actually helps in this regard since unique phenotypes, or phenotypes distinguishable from wild type are not uncommon, and this allows identification of contaminating insects. Drug or chemical resistance markers (ffrench-Constant and Benedict, 2000) are least reliable since natural resistance may be selected for in non-transgenic insects before fully homozygous lines can be created. If chemical resistance screens are used, it is most valuable to include an additional visible marker to verify transformation and simplify identification. The most reliable markers are dominant-acting visible neomorphs, such as fluorescent proteins, that are easily identified and are clearly transgenic (Chalfie et al., 1994; Higgs et al., 2000). False positives and mis-identification are only problematic when indistinguishable autofluoresence occurs. This can be minimized by using promoters that limit expression to specific tissues.

Southern DNA hybridization
The number and general integrity of transgene integrations requires Southern DNA hybridization using restriction digests and probes that identify the 5' and 3' vector arms independently, and the number of integrations. This requires digests that cut the vector internally and probes that unambiguously hybridize to the vector arms and genomic insertion site DNA. Integration number should be consistent for both arms, but additional digests may be needed to clarify overlapping bands or incomplete digestions. Digests that cut the vector internally close to the termini used with internal probes can generally determine if vector integrity is maintained. Unusual insertions would typically reveal anomalous hybridization patterns in one or more of the described protocols. Some Southern hybridizations may yield banding patterns of varying intensity which could result from imprecise or recombinant

integrations. Such patterns can also result from incomplete restriction enzyme digestion or from a mixed population of transformants having a varying number of unlinked transgene alleles. This results from backcrossing G_1 individuals and inbreeding subsequent generations allowing some alleles to be crossed out. If hybridization intensity varies, the cause must be resolved before Southern analysis can be used as a reliable test for transgene stability.

Insertion site sequencing
The most definitive determination of transposon-mediated genomic integration requires sequencing the 5' and 3' insertion sites. This is achieved most simply by inverse PCR methods that isolate the junction sites independently, or together as part of a single PCR product (Ochman et al., 1993). Independent sequences are most useful when a single integration has occurred; otherwise it is difficult to match the junctions to the same integration. Multiple integrations require a single PCR product (using an enzyme that cuts outside the vector) or screening a mini-genomic library. Although it is usually assumed that a genomic integration has occurred if the insertion site differs from the vector plasmid, given the sensitivity of PCR, it is conceivable that integrations into DNA from symbiont or infectious organisms may be recovered. Definitive determination of transposition is achieved by using primer sites to the proximal genomic insertion site, and performing PCR on transformed and non-transformed host species genomes. The vector should be included in the transformed genome, with only the empty insertion site in the non-transformed genome. These primers then become essential to rapidly defining integration stability in subsequent generations.

Chromosomal in situ hybridization
The most definitive determination of a chromosomal integration is by in situ hybridization, which also allows a determination of the number of genomic integrations and mapping. The most useful information comes from hybridization to polytene chromosomes, but in species where polytenes do not exist or are hard to recover, hybridization to mitotic chromosomes by fluorescent in situ hybridization (FISH) yields useful information.

POTENTIAL FOR INTRA-GENOME MOVEMENT

The primary concern for transgenic risk assessment is transgene stability, which has implications for the integrity of the strain being released or studied, and potential risks if the transgene is transmitted to a non-host organism. In terms of strain integrity, positive or negative effects may result from loss of the transgene, or intra-genomic movement with the potential for transgene replication by TDGR. Both transgene loss and movement would require a mobilizing system that might be the same or similar to the transposon vector. Loss of the transgene obviously destroys the transgenic strain, and would be most devastating if it occurs under mass rearing and is not rapidly detected by screening methods. Intra-genome movement with or without TDGR may result in differing position or dosage effects, or other genomic influences that can change the expressivity of the marker and/or the gene of interest. This could affect defined characteristics of the strain that may be essential to its effective use, and change in the marker may diminish the ability to detect the transgenic for risk assessment and program analysis. Intra-genomic movement may also result in new integrations into lethal or semi-lethal sites that will diminish strain fitness or viability. Movement with TDGR can be a positive event if enhanced heritability is desired, since it would act to increase transgene copy number and presence in succeeding generations. However, a general negative effect TDGR, whether enhanced heritability is desired or not, is that an increase in integration number will also increase the genetic load having a negative effect on fitness. The potential

also exists for movement resulting in positive selection, though this would be rare, as are beneficial mutations.

POTENTIAL FOR INTER-GENOME MOVEMENT

Inter-genome movement implies the horizontal transmission of a transgene between species, or different strains of the same species. The existence of nearly identical transposons in insects from different orders (e.g. *Himar* and *piggyBac*) has suggested that this is not an uncommon natural phenomenon (Robertson and Lampe 1995; Handler and McCombs, 2000). Such movement requires a cross-mobilizing system in the host genome to destabilize the transgene, and some type of mechanism or vector to transmit the transgene into a new genome. As mentioned previously, the mobilizing system may be the same transposon as the gene transfer vector, or a related element. A primary question for risk assessment is how to detect and quantify the activity of such a mobilizing system, and how to determine if it resides in the host genome or a co-habiting or infectious endosymbiont.

The presence of the same transposon as the vector may be simply determined by hybridization or PCR studies. Detecting a related system may be more difficult since, like *hobo* and *Hermes*, transposons may share functional relatedness, but not enough structural identity to make direct comparisons. Detection may rely on functional assays that can detect cross-mobilization. The most sensitive of these are embryonic or cell line excision assays performed in the absence of transposase, such as the *hobo* assays that implicated the existence of *Hermes* in *Musca domestica* (Atkinson et al., 1993). Transposition assays could be similarly useful, but are more likely to detect only identical or nearly identical systems that can catalyze both excision and insertion. A consideration for use of these assays is that the lack of a mobilizing system would be inferred solely by negative data; a lack of excision or transposition. Thus a statistical analysis is needed to determine the number of plasmids that must be tested to give significance to the assessment that a mobilizing system does not exist, or its function is limited to the extent that it is not a concern. An important control for this analysis is a comparison to excisions or transpositions in the same or similar host species having a genomic source of transposase (e.g. testing *piggyBac* mobility in *T. ni* or a jumpstarter strain).

If the potential exists for transgene mobilization in the host species, then an assessment must be made for potential integration into a symbiotic, infectious, or predatory system that could mediate transmission to another host. Such systems can be procaryotic bacteria or viruses, or eucaryotic predators such as mites or wasps. Infectious agents could conceivably harbor a mobilizing system that could destabilize the transgene for intra- or inter-genomic movement. The potential for an infectious virus transmitting a eucaryotic transposon became evident by the discovery of *piggyBac*, which transposed from a cabbage looper moth cell line genome into an infecting AcNPV genome (Fraser et al., 1983). Such transposon movement has been shown repeatedly from insect cell lines into several viral systems, as well as from a larval host into an infecting virus (Jehle et al., 1998). It remains to be determined whether transposon movement can occur from these potential vectors into a eucaryotic host genome, which would be required for horizontal transmission.

RISKS FROM HORIZONTAL TRANSMISSION

If a cross-mobilizing system is discovered, then an assessment must be made for the potential of transgene horizontal transmission, and whether such horizontal transmission presents a biological or ecological risk to potential target organisms. Risk concerns relate to 1) movement of the transgene vector in terms of mutagenicity or genomic rearrangements, and 2)

the effect of transgenes inserted within the vector, including the marker system and genes of interest, on non-target hosts. Vector movement risks relate to the frequency of mobility, which may be genome dependent, and target site specificity. An argument could be made for whether risks from vector mobility are any greater, or add to the risks from mobility of the cross-mobilizing system. However, the potential exists for situations where the cross-mobilizing system is non-mobile itself and simply functions as a non-autonomous helper. Another consideration is the potential (though probably rare) for a non-autonomous vector and helper recombining to form an autonomous functional vector.

The potential for risk from inserted genes depends upon the gene products produced, whether they interact with one another or respond to environmental cues, whether they interact with the local genomic environment, and if they have varying effects on different host organisms. These concerns, generally, should be considered individually for particular vectors and host species, though some may be common to many vectors (e.g. marker systems).

RISKS FROM HOST GENOME INTERACTIONS WITH VECTOR

Risks also relate to vector interactions with the host genome beyond vector instability, and these generally will influence host strain fitness, and expression of the transgene. Transposons have been associated with genomic rearrangements, sometimes localized to breakpoints that have the potential to negatively affect insect fitness, and perhaps viability. This is well-elucidated for some *hobo*-containing strains in *D. melanogaster* (Lim et al., 1981), and a *mariner*-related element is associated with a recombination hotspot responsible for two human genetic disorders (duplication and deletion resulting from unequal crossovers between two elements) (Reiter et al., 1996). Several interactions can result in gene silencing which can profoundly affect the transgene, and thus the strain, from functioning in the anticipated manner. Silencing of a marker system could impede visual identification of the transgenic organism, thus confusing monitoring for program analysis and risk assessment (possibly allowing unintended use or release of transgenic insects). The influence of transgenes on gene silencing in plants is well-documented, though such epigenetic effects are usually mediated by CpG methylation of promoter sequences. Methylation does not occur in Drosophila and it remains to be determined whether methylation has a role in other insects. Beyond the influence of position effects on transgene expression, gene silencing may also occur by opposite strand enhancers proximal to the insertion site promoting transcription of antisense RNA resulting in RNA inhibition - a type of reverse enhancer trapping. Various outcomes could be envisaged for silencing of the marker and/or genes of interest relative to developmentally-dependent enhancers.

SUMMARY

The areas of concern for the use of transgenic insects relates to risks associated with four primary components. These are (1) the host insect involved (and whether it is a pest or beneficial), (2) the vector used for gene transfer, (3) genes of interest within the vector including markers, and 4) the expected persistence of the transgenic strain in the environment. The latter concern relates to whether the release is for a short term single generation, short term multi-generation (e.g. two to four generations), or for long term persistence where enhanced heritability of the vector may be desired. An associated concern of significant importance is the particular environment where the release will be performed, and other areas of potential invasion. For a final risk assessment, all of these factors must be considered individually, and also considered in terms of negative or positive interaction. As we contemplate the release of transgenic insects and arthropods, it would be highly advantageous

for a standardization of tests and analysis to quantify potential risks where possible, and for the support of basic research that might mitigate risks in general or for specific programs. An example is to develop vectors that have enhanced stability or can be rendered immobile after genomic integration to minimize or eliminate potential inter- and intra-genomic movement.

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CAN THE TECHNICAL ISSUES RELATED TO RISK ASSESSMENT OF TRANSGENIC ARTHROPODS BE SOLVED?

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Abstract.

The recent progress that has been made in the development of transgenic technologies for non-drosophilid insects now leads to discussions about both how best to use this technology and the types and magnitudes of risk that are arise from the use of transgenic non-drosophilid insects. We discuss the principal issues of risk and conclude that, for the majority of them, techniques already exist that enable the quantification of risk. For others, such as the possibility of horizontal transfer of transposable elements, assessments of risk can still be made however these are indirect measures. Perhaps what is really remarkable is that a true characterization of any transgenic strain of any insect species with respect to genetic fitness and viability is still yet to be made, even though many of these strains have existed for several years. The need for these types of data is becoming urgent as schemes for the use of transgenic insects start to attract public attention and we suggest that support be made for these types of experiment.

INTRODUCTION

Since the first report of P element mediated transformation of *Drosophila melanogaster* by Rubin and Spradling (1982), progress in the production of non-drosophilid insects can be divided into two periods. Between 1982 and 1995, little progress was made as the majority of investigators initially focused on using the P element as a gene vector in insects. Limited success was achieved with the reports of genetic transformation of three mosquito species, *Anopheles gambiae* (Miller et al., 1987), *Aedes aegypti* (Morris et al. 1989), and *Aedes triseriatus* (McGrane et al. 1988), however the transformation frequencies were low and it now seems unlikely that *P* element transposition was necessarily involved in the generation of these transgenic lines. For example, Biessmann et al. (1996) showed that integration of the *P* element into *An. gambiae* had involved integration of both P element and plasmid backbone sequences and that duplications of some of these sequences had also occurred. This type of integration is not characteristic of the many *P* element integrations seen in *D. melanogaster*. For the first half of the 1990s attention was diverted to the identification of other insect transposable elements that, unlike *P*, possessed broad host ranges and, during this time, the *Hermes, Minos, Mos1* and *piggyBac* elements were discovered. In contrast to the preceding 13 years, the period from 1995 to 2001 has been marked by rapid progress in which all four of these elements have been used to genetically transform insect species from three orders (Diptera, Lepidoptera, Coleoptera) and, in the case of some species, such as the yellow fever mosquito *Ae. aegypti* and the Mediterranean fruit fly, *,* transgenic lines have been created using more than one of these transposable elements (for reviews see Atkinson et al., 2001; Handler 2001). As a consequence, questions of risk assessment arising from the generation, maintenance and even release of genetically engineered insect strains have quickly assumed key importance in determining the future application of genetic engineering-based technology in insects of medical and agricultural importance. These discussions are held in an atmosphere of increased public concern over the use of genetic engineering in agriculture where questions

of increased benefit resulting from the application of this technology are weighed against risks, real or perceived, associated with generating and releasing new genetic strains of plants and animals.

THE BENEFITS AND RISKS OF GENETICALLY ENGINEERED INSECTS.

The benefits of applying genetic engineering to arthropods of agricultural importance are obvious. It increases our ability to quickly generate new genetic strains that can, for example, be used in sterile insect technique programs. Genetic sexing strains can be created in only a few generations with only small, discrete changes being made to the insect genome. These can be made in the context of a largely wild type genome and so disadvantageous effects arising from inbreeding over many generations can, in theory, be eliminated. Insects produced in this manner will therefore be far more competitive than genetic sexing strains made by conventional breeding technologies. New approaches to the creation of genetic sexing strains can also be applied. Sex specific promoters can be used to direct the repression or induction of conditional lethal genes as has recently been demonstrated in *D. melanogaster*. Similar strategies using RNA interference (RNAi) will most likely be applied to insects to produce new strains that are deficient, in a sex specific way, in one or more key gene products. For insects of medical importance, such as mosquitoes, genetic engineering technology has already produced strains, which, in principle, should be less capable of vectoring pathogens that cause human disease. One common theme in these applications of recombinant technology is the lessening of our dependence one the use of broad spectrum insecticides. Indeed, one benefit arising from the release of genetically engineered insects for sterile insect technique programs is the reduction, and often elimination, in the use of environmentally damaging chemical insecticides.

What then are risks associated with the generation, rearing and release of genetically engineered insects and can these risks be measured in realistic terms? Will these risks out weigh the benefits resulting from the application of this technology? And, just as importantly, are there some risk factors that cannot be measured by experiment?

Hoy (2000) identified 22 risk factors that required resolution before a transgenic strain of arthropod could be released into the environment. These 22 factors were divided into four classes of enquiry: 1) the attributes of the organism being identified, 2) the nature of the genetic change, 3) the phenotypic difference between the modified and unmodified organism and 4) the nature of the environment into which the transgenic organism is released. These lines of enquiry are little different in principle to those that have confronted practitioners of biological control when they have sought to introduce new organisms, including insects, into an environment in order to control or eradicate a pest animal or plant species. In the case of biological control, procedures have been established for the testing of candidate control organisms, first in their county of origin, then in quarantine laboratories in the county where the release will occur and finally in limited field trials before large scale release is undertaken. For genetically modified organisms, initial experiments would also be performed under containment conditions (but not necessarily in the same county or location where the final release will occur) followed by limited field trails under some level of containment (or strain 'callback' or eradication) if necessary before a final large scale release is commenced.

In examining Hoy's (2000) risk issues, two classes stand out as presenting unique challenges to experimental assessment: the nature of the genetic change and the phenotype of the genetically modified organism relative to its unmodified, wild-type form. The remaining two,

the attributes of the target organism (that is; it's position in the environment) and the attributes of the environment into which the genetically modified organism is released require attention but are often easily tested using existing protocols. Indeed the site of release can be selected, in part or wholly, on the ability to recall the released strain most likely through an area wide control program.

THE NATURE OF THE GENETIC CHANGE.

The biological means by which transgenic strains of insects are generated are transposable elements that transpose by a DNA intermediate. Questions of risk associated with the creation of these strains are therefore inevitably imbedded in the behaviour of these transposable elements in their new insect host genomes. We know very little about either what controls the behaviour of these transposable elements or about how interactions with new host genomes modulate transposable element activity. We can, however, in the absence of this knowledge devise experiments aimed at determining the role that the behaviour of these elements might contribute to risk assessments of genetically modified insects containing these elements. These address questions of a) transposable element mobility, b) transposable element stability, c) horizontal transfer.

Transposable element mobility
The mobility properties of the four transposable elements used to genetically transform non-drosophilid insects are the very feature that has led to the development of moderately robust transformation technologies for these species. Not surprisingly, assays for transposable element mobility are very well developed since, in several cases, the outcomes of these assays led to these elements being used as gene vectors in these species. Insect transposable element excision and transposition assays have been used for the *P* element (O'Brochta et al., 1991), the *hobo* element (O'Brochta et al. 1994), the *Hermes* element (Sarkar et al., 1997a,b), the Mos1 element (Coates et al., 1995, 1997), the *Minos* element (Catteruccia et al., 2000; Shimizu et al., 2000; Klinakis et al., 2000) and the *piggyBac* element (Thibault et al., 1998; Lobo et al., 2001; Grossman et al., 2000). These assays are simple to perform and provide reliable information concerning the mobility of these elements in the target species. In general they have proven excellent indicators of transposable element activity in these species even though they primarily target somatic, rather than germ-line nuclei. Perhaps the most elegant example of demonstrating mobility of any transposable element has been the *Sleeping Beauty* element of fish (Ivics et al., 1997). The transposase gene of this inactive element was broken down into its several domains and each domain tested assayed for optimal activity. The transposase, and the element, were then reconstructed and the resurrected Sleeping Beauty element found to be active not just in fish but in other species as well (Izsvak et al. 2000; Dupuy et al., 2002).

The pertinent conclusion is that, using these simple approaches, any transposable element can be examined for mobility in a target species, provided there is an efficient means to introduce the plasmids used for the assay into, typically, the developing embryos of each species. Moreover existing elements can be modified and tested for hyperactivity in the target species with the aim being to develop even more robust transformation systems for insects. This approach has worked for the *Sleeping Beauty* element and for the synthetic *Himar* element (Lampe et al., 1999) and should be applicable to the four elements currently used for non-drosophilid insect transformation since each is based on a naturally occurring element. Each of these, most likely, has been selected for reduced mobility in order that it not becomes too effective a mutagen in the original host species.

For Class II elements used for the genetic transformation of non-drosophilid insects, there are obvious targets for mutagenesis experiments aimed at increasing mobility. These are the inverted terminal repeat sequences, small repeated sequences located adjacent to the inverted terminal repeats, the DNA binding domain of the transposase, the active site of the transposase and regions of the transposase that are involved in dimerization of the transposase in order to produce an active enzyme. These experiments are simple, if not time consuming, but should lead to more efficient transposable elements.

Transposable element stability
Unlike transposable element mobility, where the role of specific DNA sequences is clear, little is known about the actual mechanisms that lead to transposable element instability. These can be classified into two groups. One is instability caused by illegitimate recombination between the transposable element vector and DNA sequences located either on the same chromosome or on homologous or non-homologous chromosomes. These can lead to the formation of apparently complex and partially complete transposable elements, most of which are unlikely to be functional since those sequences necessary for mobility have been lost or rearranged. These types of rearrangements are difficult to predict and also difficult to develop meaningful assays for. Furthermore the level of illegitimate recombination may vary between insect species and orders and may also be dependent on genome size and the complexity of the genome. Insects that possess large genomes composed of large amounts of mid- to highly-repeated DNA may be more likely to rearrange newly introduced DNA, particularly if it is part of a transposable element. Perhaps the only solution to this may be to constantly apply selection for the transgene (or a closely linked gene) while eliminating most, if not all, of the flanking transposable element sequences, perhaps by employing the FLP recombinase or cre/lox recombination systems. The applicability of these systems to each target species can be determined by appropriate excision assays performed beforehand.

The second cause of instability is caused by interactions between the introduced transposable element and host factors, typically related transposable elements, within the target genome. Evidence that this can occur has been obtained by Sundararajan et al. (1999) who showed, using both somatic and germ-line transposable element excision assays, that the related *hobo* and *Hermes* elements can interact and cross-mobilize one another in transgenic lines of *D. melanogaster*. These types of interactions might be predicted to occur within the many different families of transposable elements and we remain ignorant of three important components of these interactions. These are a) the DNA sequences of the vector element that interact with the host factors, b) the amino acid sequences of the host factors that interact with the DNA sequences of the vector element, and c) the distribution of transposable elements, or transposable element-like sequences, related to the vector element within insects. For example, as described above, some limited progress has been made in determining that insect hAT elements can cross-mobilize and several insect *hAT* elements or related but inactive sequences have been described. *Mariner* elements have a wide distribution in insects and can be present in thousands of copies, the majority of which are most likely inactive. Recent work by Handler and McCombs (2000) show that the distribution of the *piggyBac* element in insects is most likely wider than originally thought, leaving open the likelihood that this element too, may be affected by specific interactions with host factors in new genomes.

Can assays be developed for these interactions? In principle the relevant excision and transposition assays are no different to those already described above. They can be performed in the absence of helper plasmid and any mobility observed could be attributed to host factors. The difficulty arises in isolating the genes responsible for producing these enzymes and

determining that these are the principal cause of the instability. This has proven successful for the *Hermes* element of *Musca domestica*, which was discovered through the use of *hobo* element excision assays performed in this insect in the absence of *hobo* helper transposase (Atkinson et al., 1993). The question remains, however, whether other proteins that may interact with the vector element can also be identified, isolated and tested. For example, a host-encoded protein such as the inverted repeat binding protein (IRBP) of *D. melanogaster*, which directly interacts with part of the inverted terminal repeat sequences of the *P* element, may play a role in the stability of this element (Beall et al., 1996). It is unlikely that a protein such as IRBP would be identified using existing transposable element mobility assays and so these assays at present can have only a very limited role in assessing all aspects of instability. Another factor that could confound assessments of cross mobility is that the vector element DNA sequences responsible for this type of instability may be different from those required or necessary for mobility. At present there are no clear examples of DNA sequences located on insect transposable elements that are used as gene vectors in insects that play essential roles in transposable element stability as opposed to mobility.

Horizontal transfer
Horizontal transfer of transposable elements between species has become an accepted fact based on circumstantial evidence. No direct, measured experimental evidence of incidental horizontal transfer has been documented although examples of forced horizontal transfer of the *Mos1* element across kingdoms have been demonstrated when Mos1 was used as a gene vector to genetically transform Leishmania (Gueiros-Filho and Beverley, 1997). The use of *Hermes, Minos, Mos1* and *piggyBac* to genetically transform a range of insect species is another example of directed horizontal transfer of insect transposable elements across families, and sometimes orders, of insects. The ability of a transposable element to transfer itself horizontally across species is an important trait that is selected for and retained by these elements in order to perpetuate themselves through as many species as possible. Within the time frame of modern genetic studies two transposable elements, P and *hobo*, have invaded one of the most important genetic model organisms, *D. melanogaster*. This suggests that, provided we have the genetic tools and mental preparedness to identify horizontal transfer, then we will see it and also indicates that, based on the invasion of *P* and *hobo* into *D. melanogaster*, horizontal transfer may be more frequent than initially thought.

How then to test for horizontal transfer? Protocols designed to create a microenvironment that approximates the field environment in a rearing container and then populate it with the genetically modified insect and a range of other species in order to examine if the transposable element is ever transferred to the other species are seriously flawed. These experiments are neither empirical nor complete and consist simply of a "wait and see" approach without any knowledge of the components and conditions required for transfer to occur. During the time course of the experiment, no evidence of horizontal transfer is detected leading to the conclusion that it is unlikely to occur, at least in the experimental system used. Yet horizontal transfer might be a very rare, and fluky, event but catastrophic for the recipient naïve population should the transposable element be able to move rapidly and unhindered through the genome of the new species. Without complete, or near to complete, knowledge of how horizontal transfer actually occurs, it is highly problematic to design experiments that will detect it in any reasonable amount of time. Yet the consequences of horizontal transfer demand that a calculation of the risk of it occurring be at least attempted.

One approach to this problem, proposed by David O'Brochta (personal communication) is not to look for horizontal transfer to occur, but to assume that it will occur, and then assess the

consequences of this on the new host organisms. This can be measured by performing conventional transposable element excision and transposition assays in these species and by determining whether compatible and related transposable element systems that may cross-mobilize the target transposable element already exist in these species. Comprehensive ecological data can be assembled listing species that, based on their relationships with the transgenic insect species in question, might be likely targets of horizontal transfer. These can range from bacteria located in the gut of the insect, to other arthropods, plants and vertebrates and the final list may consist of as many as 10-20 species. The physical presence of endogenous transposable elements that are related to the element in question can be determined by southern blots or by PCR using degenerate primers designed to conserved regions of the transposable element. The ability of the transposable element in question to move in each of these species can be determined by deploying the appropriate excision and transposition assays. For bacteria this might be achieved by transformation (if feasible), for insects it can be achieved by embryo microinjection if sufficient numbers of embryos can be obtained (or by transfection of related cell types maintained in culture), for plants it can be achieved by biolistic delivery or by the transformation of protoplasts, and for vertebrates it might best be achieved by transfection of cells in culture of a related species (for example monkey, rodent or human cell lines). The implied, but real, presence of related elements, or other enzymatic activities that can cross-mobilize the transposable element in question can be determined by performing the same excision or transposition assays but without the presence of the appropriate helper plasmid containing the corresponding tranposase gene. As mentioned above, helper-less excision assays performed in the house fly, *Musca domestica,* using the *hobo* element were the key experiments in identifying the presence of the *Hermes* element in this species (Atkinson et al., 1993). In a similar vein, the ability to detect the insertion of sequences into the genome of engineered baculoviruses, were of fundamental importance in uncovering the presence of *piggyBac* elements in the genome of Trichoplusia ni (Cary et al., 1989).

The experiments outlined here do not detect horizontal transfer of transposable elements. The outcomes of these experiments would, however, provide:

- knowledge that a related transposable element system did or did not exist in these species,
- quantitative estimates of excision or transposition frequencies in many of these species, and
- quantitative estimates of cross-mobilization in these species, should it occur at all.

As such they would enable a real measure of risk should horizontal transfer occur and so could be employed to enable scientists and regulators determine which particular transposable element vector would be the safest to use in a given ecosystem.

THE NEW PHENOTYPE OF THE TRANSGENIC INSECT; THE TRUE COST OF TRANSGENESIS REMAINS UNKNOWN.

For transgenic insect strains destined for field release, the resulting phenotype of the strain determines its success as an agent for genetic control. An advantage of genetic engineering over conventional genetic strategies, such as the creation of translocation strains, is that precise and small changes are made in the genome through insertion of the transposable element. As such, the effect on the fitness and viability of the genetically engineered strain should be reduced compared with strains that have undergone significant genomic rearrangements. This, in turn, will influence any calculations of the risks arising from releasing these transgenic insects since viable, competitive strains (if they are not used for SIT

experiments) might be predicted to persist in the field and so perpetuate the genes, and transgenes, they are carrying.

This crucial question — the true effect of transgenesis on phenotype — remains unexplored in the transgenic insect strains so far generated. Life table analyses on multiple, independent transgenic lines will yield comprehensive data concerning their fitness and viability. Should transgenic strains contain toxins (for example a gene encoding a toxin that would selectively kill one sex only) then the effects of this toxin on likely predators can be determined. It is remarkable that, some six years after the production of the first transgenic medfly, no published data exist concerning the viability of these insects relative to the wild type strain and relative to translocation strains currently used for the SIT. Similarly, there is a paucity of data for transgenic mosquitoes, even though the long term goal is to produce transgenic lines of mosquitoes that are capable of spreading transgenes through wild populations. These studies are simple to perform and can be undertaken by population geneticists and/or ecologists and the outcomes of these will be of profound importance in assessing risk issues.

CONCLUSIONS

The recent progress that has been made in the development of genetic transformation systems for non-drosophilid insects has been extraordinary. Emphasis has now shifted from problems associated with the simple development of these techniques to issues concerning the use of this technology. These are welcome developments and challenge us to address the nature of transgenesis itself. What is clear is that, at present, we are very ignorant of the precise genetic and biochemical mechanisms that lead to non-drosophilid insect transgenesis and are equally ignorant of the fates of these transgenes once they are inserted into the insect genome by transposable element mutagenesis. These issues are of particular importance in this area of applied entomology since we seek to use these transposable elements as genetic tools in the laboratory but, more importantly, we also seek to use these elements as gene vectors in transgenic insects that may be used as a component of an area-wide control strategy. Timely answers to these issues are thus important and of some consequence to future research strategies.

Fortunately most of these issues can be directly addressed experimentally using existing techniques. Questions of transposable element mobility and some aspects of transposable element stability can be answered using the appropriate assays. Questions of fitness and the consequences of transgenesis on the phenotype can also be addressed using standard techniques even though little effort has been made to do so as yet for any of the transgenic lines so far generated in agriculturally or medically important insect species. Likewise, questions relating to transposable element mobility and spreading through field populations can be addressed using simple population cages and procedures that have been established to track transposable elements through populations.

The issues that are perhaps intractable to direct analysis are horizontal transfer and cross-mobility leading to instability. Both can be approached indirectly leading to at least some quantitative measure of risks that arise from the rearing, and perhaps release of transgenic insects.

If these questions are approached in a logical and stepwise manner, there seems to be justified hope that real estimates of these risks can be calculated. We would argue that the risk of ignoring these questions is simply that we may lose the opportunity to truly harness these

powerful genetic technologies for the purpose of improving agriculture and human welfare. A few ill-conceived and presumptuous experiments with the release of transgenic insects may cause significant damage to this late-blossoming field of research. The emphasis must now be on not just developing novel genetic strategies but on utilizing existing procedures in order to arrive at meaningful assessments of risk arising from the use of transgenic pest insects. The majority of techniques required to undertake this already exist.

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PINK BOLLWORM: TRIALS AND TRIBULATIONS

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Abstract
Preparations for a limited field test/release of a genetically modified strain of Pink Bollworm are described. The insects to be released in a field cage have been transgenically marked with a *piggyBac* transposable element coding the fluorescent protein EGFP. Certain biological characteristics of the transformed strain are detailed. The release experiment is described, as are events associated with obtaining a permit for release of this genetically modified insect. Descriptions and responses to the comments and criticisms received during the public commentary and the comment period are detailed and analyzed. Certain of the critiques are summarized, as are the responses to those critiques.

INTRODUCTION

Lepidopteran species are key pests on food and fiber crops worldwide. The development of resistance to pesticides, along with heightened public distaste and ecological concerns driving a movement away from chemical control methods presents an increasing challenge to pest control. Our ability to manipulate insect species at the molecular level will help advance our understanding of insect biology and to we hope promote the development of novel field applications consistent with reduction of ecologically undesirable pesticide use. Germline genetic transformation and genetic engineering can immediately and directly contribute to pest control efforts.

An example of the immediate utility of this technology would be through indelible genetic marking of insects produced for Sterile Insect Technique (SIT) programs. The SIT strategy can be summarized as follows. The target insect is colonized and methods for mass rearing are developed. A certain proportion of the mass colony is retained for the next generation. The rest are "sterilized". They are exposed to a dose of a mutagen, most often ionizing irradiation, to the point where every gamete carries at least one induced dominant developmentally lethal mutations. Mutagenized colony insects are then released to mate with wild population. Because each progeny of the matings with the colony insects receives a dominant developmentally lethal gene, embryos from such matings will not develop[17, 47].

Biological risk assessments, Pink Bollworm as a study subject
Significant questions about general biological safety of transgenic arthropods can be effectively addressed by studies on a model organism such as transgenic PBW (pink bollworm), *Pectinophora gossypiella*. PBW has many characteristics that enhance the safety of transgenic studies with this species compared to studies in other organisms. Species with close sympatric non-target relatives, especially if related closely enough to allow hybridization and fertile offspring pose a special risk. In such a case the potential for the transgene to become introduced into a non-target population would be significant. Such a scenario resembles the controversial assertion that transgenic maize interbred with untransformed Mexican landraces and thereby transmitted the introduced transgenes into the latter's populations[36]. As PBW is a species introduced to North America with no known indigenous close relatives, transgene escape from PBW into non-target populations through interspecific can be considered extremely unlikely. Additionally, PBW have restricted

geographic and host ranges in North America. They are incapable of surviving harsh northern winters and feed only on cotton and Malvacea relatives. Methods for rearing this insect are well developed, automated to a great extent, and reliable enough to produce billions of insects on a regular basis. Unlike transgenic biting flies, PBW have minimal or no potential to directly injure humans or animals, much less transfer their transgenes to them through trophic or other interactions. Finally, there is an immediate benefit to be obtained from studies on genetically marked transgenic PBW. Genetic marking of SIT insects has been proposed as a crucial first experimental step in the use of genetically modified arthropods and as an important subject for hazard identification and risk assessment in transgenic arthropods [2].

TRANSGENIC PBW: THEIR PRODUCTION

The utility of transposable elements to manipulate an insect genome has been clearly demonstrated in Drosophila with the *P* element [37, 44]. However, the host range of *P* is restricted [32, 33]. Elements with broader host ranges such as *mariner* [7], *Minos* [28], *Hermes* [31, 38, 39]; and *piggyBac* [14–16] have led to the development of vector systems for transformation of more insect species including the medfly, [19, 20], the mosquito *Aedes aegypti* [5, 6, 24] and now silk worm and pink bollworm[34, 45]. Successful germline transformation events have typically relied upon a marker system in which incorporation of a wild-type gene product rescued the phenotype in a mutant strain or dominant marker genes such as fluorescent proteins [5, 6, 18–20, 24, 29, 34, 48]. For many insect species, as is the case with pink bollworm, mutant recipient strains that can be rescued and cloned copies of the corresponding wild-type genes are unavailable. The use of recombinant DNA technology for manipulation of most pest insects was problematic for a number of reasons until recent years. Fluorescent proteins [18, 22] as markers for identification of transformed insects allow the use of transgenic technology in organisms where little genetics is known, other than the identification of a integration-competent transposable element [22]. The dominant phenotype of this small gene and concomitantly small protein makes expression of fusion constructs in the transgenic construct more flexible. Additionally, non-destructive detection technology and the relatively non-debilitating/non-toxic protein encoded by the gene allow putative transformants to be identified early, before effort is expended in mating and rearing untransformed insects. The animal husbandry required for expansion of a transgenic strain is perhaps the major impediment for development of transgenic insects. Though visible means for fluorescent protein detection are preferable, they are not necessarily suitable for all situations. For example, dead transgenic insects captured in surveillance traps may not be readily or correctly identified as transgenic insects. Thus, alternative methods for identification of the transgene may be needed, both for screening purposes and for additional confirmation of transgenic status. Fortunately, Enhanced Green Fluorescent Protein (EGFP) protein is detectable by immunochemistry and it is not present in untransformed insects. Finally, fluorescent proteins are, as far as is known, environmentally benign and are metabolised similarly to other proteins of similar size and composition.

Plasmid-based mobility assays demonstrated that *piggyBac* is mobile in pink bollworm (PBW) embryos [46]. Therefore, we constructed a *piggyBac* vector containing Enhanced Green Fluorescent Protein (EGFP) as a marker for transformation. Green Fluorescent Protein's (GFP), its derivatives', and other fluorescent protein's (e. g. DsRed) utility as dominant, visible, non-destructive markers in a variety of insect [3], mammalian [35], and plant systems [21] made us confident of its utility in PBW, which was borne out in our successful transformation of PBW [34].

We transferred strain #35 of transgenic strains produced in our work to Phoenix as per our USDA/APHIS permit for movement of transformed insects between labs in Riverside and Phoenix. This strain was chosen because of its clearly recognizable phenotype. The integrated constructs have thus far proven stable under laboratory rearing conditions, though further investigation under mass-rearing conditions is continuing. Tests of mating competence of the transgenic strain will also be performed with attention to containment, release and biosafety issues [4, 23].

LONG TERM GOALS OF PBW TRANSFORMATION

A general principle in the genetic control of insects is that the quality of colony insects is paramount to the Sterile Insect Technique. Small increases in the "vigor" of insects released for SIT result in disproportionately greater effectiveness of the technique.

Autocidal strategy resembles SIT and can be summarized as inundative release of reproductively inert insects: whereby the inert insects, who carry a dominant conditional lethal gene that expresses lethally outside of the colony contribute to their own population reduction by mating with conspecifics. Advantages of autocidal genetic control include the following: The effect is highly specific. Only insects that mate with the control insects are targets essentially restricting control to the target insect. Because there is no need for toxic chemicals to be broadcast, etc. the procedure should be environmentally benign with effects not much different from traditional SIT. Behavioural and other resistance to SIT insects has been reported, but this can be circumvented through practices like introgression of fresh genetic material and with new technology reliant on engineering of the insects for characteristics that enhance competitiveness. Thus, the use of transgenic technology can provide a dynamic response to SIT and related resistance in the pests. Like SIT, autocidal strategy should complement existing strategies for pest control, as little or no change to additional control methods should be required for autocidal control to function. Additionally, when all contingent costs are considered, the methods are potentially inexpensive to employ.

The autocidal genetic control method may be less expensive than traditional SIT The more competitive the sterilized insects are in mating compared to the wild mates, the fewer sterilized specimens will need to be released to obtain a certain level of control. Mating competitiveness of radiation sterilized insects is typically compromised; hence, genetically sterilized insects may be superior in this respect. Additionally, transformation technology may offer possibilities for enhanced production in colony. Finally, no costs would be associated with purchase, maintenance and quality assurance of the radiation source used to sterilize colony insects in traditional SIT.

PERMITS AND INITIAL RELEASE EXPERIMENTS

Although transgenic technology for insect control is promising and beginning to show concrete results [26], the biological safety of this approach is a topic of considerable debate. Addressing some of those concerns will require well-controlled and appropriately contained release experiments. Initially such study must be restricted to low risk transgenes in low risk insects. We contributed to this effort with a series of experiments employing a benign marker gene. Besides being of considerable academic and scientific value, this marker gene is directly usable in an SIT program to identify colony-reared insects. Precisely this type of experiment was suggested as one of the first release experiments of transgenic insects [2].

Hence we have performed a limited version of such a field release of genetically modified Pink Bollworms. We began by pursuing a permit for free release of EGFP marked transgenic pink bollworms. Our first step was to submit a proposal to the Arizona State Department of Agriculture in which plans for the release were detailed along with the purpose for the release. Comments were obtained from within the department. After this initial inspection and go-ahead from the state secretary of agriculture, we entered the permitting process at the federal level. This process involved a public comment period and a detailing of concerns and objections to the proposed experiments. We replied to the comments to help allay concerns and minimize perceived hazards as discovered during the public comment period. Additionally, we redesigned the experiments and containment protocols in accordance to stake holder suggestions to further minimize the risk of transgene escape into the wild. Subsequent to this redesign and the necessary permit, we performed the first series of releases and are now analyzing the data.

The issues that were important to the people who volunteered to comment were interesting and significant as they were not entirely the concerns we expected. We take this as additional validation of the permitting process involving public comment. Selected reviewer's technical questions suggested that we should have more clearly stated aspects of the release and pertinent issues. Reviewers at all levels wanted specific details of transforming constructs. We replied to this by providing DNA sequence information from the plasmids used. Although this information was readily obtainable and we anticipate it to be in the future, there may be situations in which details of such information are either unavailable, or a matter of commercial or trade secret. There should be some mechanism in the permitting process to allow for retention of trade or commercial secrets and the protection of proprietary interests, while allowing proper stakeholder scrutiny of the proposed transgenic release.

At the state level of release-permit, the reviewers wanted to know if there was "read-through expression" and wanted to know the exact transcription termination. Other than the unusual cases of certain viruses and viral derived elements, eukaryotic genes do not operate in this manner. Though this question would be of interest in a prokaryotic system, the post-transcriptional processing of mRNA in eukaryotes suggests that this information would not be pertinent or available. Questions were raised regarding the rate for transgene movement. Since we didn't see any indications for transgene movement, and have not after 20 + generations, we can only say that this rate must be very low, and thus we could not provide any estimate for this rate. The state level review asked "Can intragenic movement be masked by lethal gene disruptions"? We read this question to be "may the reason for not seeing any transgene movement be that, for some reason transgene movement is lethal to any insects in which this happens". If this hypothesis were true, then the transgene movement would result in a dead end without any further biological risk, since the animal in which transposition occurred would die. The net effect would be that no such movement could take place due to lethal effects.

The question of potential insertional inactivation or enhancement of expression of PBW genes was addressed by the reviewer's question, "Is the transgene inserted in a gene?" Our reply was that no genes were seen in a homology search of the databases. The flanking DNA sequences did not align with anything using Blast comparisons with GenBank. The reviewer responded that our search sequence must have been too short to find a match in the database. We replied that the Blast documentation [1, 30] suggests that sequences of only 25 bases or less may very well provide negative results. However our query sequence was the entire sequence available

from the PBW DNA flanking the transgene, 516 base pairs. This should be clearly quite long enough for finding matches in the even they exist in the database.

The mobilization of transgenes by endogenous transposase activity is a further concern. The reviewers addressed this in part with their question, "Are there other mobile elements in PBW?" Our answer was that we did not detect *piggyBac* homologs in PBW with low stringency Southern blots nor was any endogenous *piggyBac* transposase activity detected in transposition assays [46]. However, this work did not satisfy at least one of the reviewers. A reviewer at the federal level did not address nor reference the appropriate peer-reviewed literature dealing specifically with this finding [46], preferring to refer to work done with Bombyx rather than the readily available literature on PBW. Even in that reference, this reviewer interpreted data from the Bombyx to indicate instability in the transgenic silkworms, which is inconsistent with the conclusions drawn by the authors reporting transformation and *piggyBac* stability in Bombyx. This last oversight by the reviewer was rather disconcerting to us, as we expected that reviewers would be sufficiently familiar with the extant literature or at least choose to refer to the most pertinent literature in making their conclusions. We had hoped that proper consideration of evidence would have been acceptable to that reviewer.

Public statements during the public comment phase were against any release of a transgenic insect. However, at least one of these negative commentators accepted that both GFP and the EGFP transgene were benign. The major solicited criticism of transgenic insect technology centered on the discovery of the original autonomous *piggyBac* element by insertional mutagenesis of a baculovirus in cell culture. Because of this observation that an autonomous *piggyBac* element could insert into a virus, claims were made that this indicated a biological hazard. Nevertheless, it is known that *piggyBac* is not alone in this behaviour and that a variety of transposable elements will insert into DNA of viruses, including relatively unrelated Tc1-like element s[25].

SIGNIFICANT TIME POINTS ON TIMELINE TO RELEASE EXPERIMENTS

March 1998:	transgenic PBW made
Sept 1998:	applied for transfer permit to move transformants to Phoenix
March 1999:	transfer permit issued and insects transferred to Phoenix USDA/APHIS
Jan. 2000:	release permit draft submitted
May 2000:	solicitation of Public comments for permit request
July 2000:	revision to confinement protocols- in light of critiques and comments and permit resubmitted for a confined field cage release
Feb–April 2001:	Environmental Assessment (EA) of proposed permit written
May–June 2001:	Review of EA, APHIS counsel waives review
June–July 2001:	EA made available to public and comments on EA solicited
July–Aug 2001:	Public comments analyzed; with a finding of no significant impact, the EA is again rewritten.
Sept 2001:	rewritten EA and analysis of comments are reviewed by APHIS OGC prior to publication in the US Federal Register
Oct 5–16:	releases

The release was performed to gain information and to compare these biological responses:

- Compare EGFP and non-EGFP male response to pheromone in the field
- Compare EGFP and non-EGFP male longevity in the field

- Compare EGFP and non-EGFP female's ability to solicit and mate with EGFP and non-EGFP males in the field

The releases were performed in a 3 acre cotton field located 1 km from the PBW rearing facility. It is important to note that this release was not made freely into the environment. Containment precautions were made that included:

- Chain link fence surrounding field to limit access by humans and animals
- The location was guarded to prevent vandalism etc.
- The releases took place in large mesh field cages that excluded insects and retained the PBW released within the cage.
- Pheromone traps were placed at edges of field to capture escaped insects.
- The field was treated with 100 Sterile PBW/acre 3 times a week.
- EGFP female wings were clipped.
- EGFP females kept in "mating stations" within the cages which prevented them from moving from their locations
- At the end of the release, cotton bolls from release field were destroyed.
- Only irradiated transgenic males were released

PUBLIC COMMENT REQUESTED ON GM BOLLWORM RELEASE

USDA's APHIS announced its intention to prepare an Environmental Impact Statement (EIS) on the proposed release of genetically modified (GM) pink bollworms. The public is invited to comment on what issues should be addressed in the EIS.

In addition to the scientific goals of initial release these releases were made to assess public commentary, particularly with a benign transgene. This experiment was inherently low risk, and was the type of study recommended as a "first release" experiment. Perhaps most important of all, it allowed proof of effective containment protocols and would, we hoped, assuage the public's fears of GMOs in general as we hope to be seen to be making good faith efforts to accommodate concerns of the interested public related to this matter.

Public comment, horizontal transmission
Horizontal transmission of the transgene, that is transmission by means other than sexual, was of considerable importance to the critics of this work. We operated on the assumption that horizontal transmission of the *piggyBac* transgene was rare or possibly non-existent. Therefore, how does one estimate frequency of such an extremely rare event? Without unlimited resources it may indeed be impossible to obtain reasonable estimates of such rare frequencies. Perhaps, the risk associated with horizontal transmission should be assessed in terms of the possible damage that could result from this event. Some questions that come to the fore resemble questions asked about exotic pests. These include the following:

- Would horizontal transfer of a given transgene result in a more dangerous or virulent pest due to the nature of the transgene?
- Would such a transgene negatively impact natural enemies?
- Would the transgenic process result in a more virulent pest?
- Would the transferred transgene negatively impact natural enemies?

STATISTICAL ANALYSIS ESTIMATION OF FREQUENCY OF RARE EVENTS AND THE IMPORTANCE OF CONFIDENCE LIMIT LEVEL.

Of course to make estimates of risk of horizontal transfer, it would be best to have some estimate of the likelihood of such a transfer. Such an estimate requires quantification of the frequency of such transfer. Examples from studies of horizontal transfer from prokaryotes and some GMO crops suggest the horizontal transmission rate is low, but these examples may not be good models for insects. The flow of transgenes among prokaryotes and between GM crops and their weed relatives is most often by sexual or conjugative mechanisms, unlike the horizontal transmission scenarios proposed for transfer of transgenes in insects.

To predict horizontal transfer levels from PBW, it will be necessary to assay for movement of transgenes from PBW. Essentially, one would look for the transgene in organisms where we would not otherwise expect it through some sort of assay. Of course, no test is perfect. Thus, one must consider various, and possibly a priori unknown levels of test error before one can get an estimate of the numbers of animals to be screened.

The appropriate statistics to use for this analysis are an important issue. The error rate of the test in examining a particular species for the transgene can be determined by testing known positives and known negatives for the transgene and comparing the results from the test to the known and expected values. This would provide the error rate for this particular insect and transgene combination. However, such an analysis requires that there be available bone fide examples of transformed insects. Of course this will not be available for the majority of the likely candidates for HT from transgenic insects including PBW. Investigating transfer to other organisms will require methods to deal with the test reliability that do not rely on known transgene positive and negative insects. In the case where multiple tests from each single individual can be performed, test reliability may be estimated by means of probabilistic mixture distribution modeling. This "mixture problem" is analogous to a series of Coin flips with two types of differently biased coins (e.g. high probability of heads versus low probability of heads) where one wants to estimate the probabilities of heads (or tails) in each coin type, and additionally estimate how many flips were done with each type of coin. The appendix outlines the mathematics for such an approach. In the case at hand, where an organism either has the transgene or does not, the problem approximates to a mixture of two binomial distributions with the associated statistics.

Figure 1 is based on data resulting from a hypothetical experiment with two treatments: (1) organisms which have never been exposed to a transgene (we assume none of them has the transgene) (2) organisms which have been exposed to conditions of possible horizontal transgene transmission (e.g. predators having fed on transgenic prey). A sample of specified size of each treatment is tested for the presence of the transgene. The test is imperfect with a certain rate of false positives and a certain rate of missed positives. The test results are such that in the treatment that has been exposed to potential transgene transmission, no more positives have been detected than in the treatment without exposure. The question to be asked is thus "How sure can we be that the rate of transgene transmission is below a certain threshold?" Graphs displayed in Figure 1 suggest several things. Most importantly, a huge numbers of insects must be assayed for even 95% confidence that the transgene was not present above a given level. With increased sample size we gain certainty, but there are diminishing returns. The desired confidence level has perhaps the most significant influence on the necessary number of assays. If 95% confidence is acceptable; an upper limit on the event (HT in this case) can be placed at a much lower value. As more restrictive confidence

levels are employed, the upper limits of frequency are considerably higher. Missed positives (false negatives) in the range below 5% are not as important as one might intuitively expect; as increasing the incidence of missed positives from 0.5% to 5% decreased the discriminatory power only slightly. False positives, however, have a more severe effect on increasing the upper limit of HT. False positives basically add noise to the data, and thus add uncertainty. Hence optimization of test procedures should primarily focus on reducing the rate of false positives. Although test reliability can be empirically established for PBW itself, given the above, whether test reliability data can be extended to other insects is open to debate.

Figure 1.
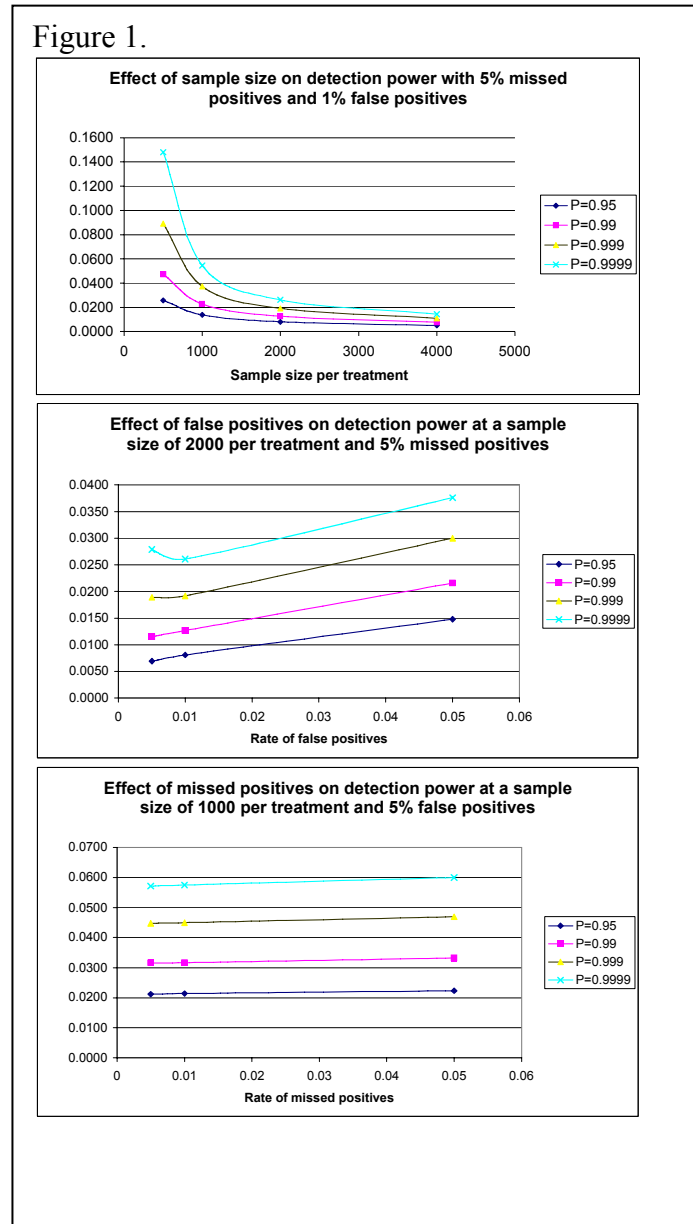
The end point for numbers of organisms to be assayed for transfer of the transgene is difficult to determine. Perhaps the best that can be done is to put a ceiling on HT frequency within the traditional 95 % confidence limits. If the true rate of horizontal transmission is very low, say 10^{-8}, or less, and then the resources needed to detect such a rare event would make this a very difficult study indeed.

An additional complication to assay of horizontal transmission into any animal is that transfer to facultative prokaryotic symbionts associated with the animal host is possible. Gut bacteria are nearly ubiquitous in insects. These prokaryotes could potentially pick up a transgene, multiply and increase the effective concentration of the transgene and thereby serve as conduit to gene transfer between PBW and other organisms, including natural enemies. However, the analysis for HT will be heavily reliant on PCR with attendant problems. In Figure 1, graphing confidence in results versus the necessary number of observations to have a given level of confidence in the results demonstrates that huge numbers of assays must be done.

ASSESSING HORIZONTAL TRANSMISSION (HT) FROM PBW, AN ECOLOGICAL PERSPECTIVE

First, is important to assay biological risk in ecologically sensible systems. For example, the hazard of transgenes in PBW being incorporated into an infecting virus has been noted. What risk this represents is open to debate, not in the least because of the specific biology and behaviour of the transformed insect, PBW. Baculoviruses are not a significant source of morbidity of PBW in the field. Thus the likelihood of any of these viruses infecting PBW may actually be very low. Secondly, the original discovery of *piggyBac* was as the result of an insertional mutation. This mutation rendered the host baculovirus plaque-defective, and thereby a less competitive virus, perhaps even less infective- working against the virus's potential as a vector for a transposable element. In general, the ecology and the host range of baculoviruses, particularly with respect to PBW, suggest that this would be ecologically unimportant pathway for HT of a transgene.

As it is possible that HT, if it occurs at all, is rare, looking for evidence of such transfer should be concentrated on organisms that have an ecological, especially a trophic relationship with the transgenic insect. Good examples of this are PBW natural enemies, scavengers and saprophytes on the corpses of the insects. As movement of the transgene is largely if not entirely dependent on a source of transposase, these ecologically sensible organisms should be examined for the presence of autonomous related elements as a source of transposase. It is important to note that in *Mariner*, the best studied of the wide host range DNA mediated transposable elements, it is necessary for the transposon's sequence to be closely matched, if not identical, to a given canonical transposase recognition sequences at the termini for excision and integration to occur at all [27]. So, though *piggyBac* biology is still not completely understood, the evidence from *Mariner* suggests that distantly related elements may not provide transposase activity necessary for movement. PBW DNA when ingested may quickly be broken down into oligonucleotides through the digestive process of the natural enemies etc., thus lessening the likelihood of HT. Therefore, an important ecological consideration would be transgene persistence in predators etc. The same would pertain to transgene persistence in PBW corpses etc. These phenomena are accessible to analysis.

Subsequent to the determination of ingested transgene survival, tests for HT can be made in the suspected recipients of the transgenic DNA. Though transgene survival in natural enemies of PBW is not expected, there is a body of work from mammalian studies on the fate of ingested DNA. According to these studies, DNA does indeed survive ingestion and furthermore integrates as fragments of less than about 1000 bases into the somatic cell genomes of the animal fed the heterologous DNA [8-13, 40-43]. Whether similar processes take place in non-mammalian organisms is unknown, though this should be an avenue of investigation in determining biological risk on transgenic insects.

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THE CHALLENGE OF DEVELOPING AND UTILIZING TRANSGENIC ARTHROPODS IN THE CARIBBEAN

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Abstract
To date transgenic arthropods are not being utilized within the Caribbean to control agricultural pests. Bio-control in the region is largely focused on the control of the Pink mealybug since infestations of this pest in any region can result in restrictions being placed on the export of fresh produce into non-infected countries. Other pests controlled by use of conventional bio-control agents include citrus blackfly, citrus leafminer, sugarcane stemborer, coffee berry borer and coconut whitefly. Control of these economic pests is of great importance as crops such as sugarcane, coffee and citrus are major foreign exchange earners in some countries. Applications for the importation, movement and release of these organisms are done through the Pesticide Control Board of the Ministry of Agriculture. Generally guidelines of the FAO code of conduct for the import and release of bio-control agents are used. It is almost inevitable that with the need to increase productivity in the agri-food sector, combined with the need to reduce negative impacts of agri-chemical use, regional research and development efforts will be focused on developing cost effective means of controlling pests common to the region. Such research and development efforts must combine conventional control with genetic engineering methods. Several countries in the Caribbean region are currently examining their regulatory mechanisms to address the trans-boundary movement and environmental release of genetically modified organisms specifically for agricultural purposes. Although most attention has been focused on crop and food regulations some attention will be placed on developing regulations for the use of transgenic arthropods used to control common economic pests.

INTRODUCTION

The role of insects in agriculture production is two fold, they can have either a beneficial or detrimental impact on crops and ornamental plants. Insects are beneficial from the perspective of being pollinators and from their activity in controlling other insect infestations in crops. Insect damage to crops is generally as a result of their direct feeding activities and their ability to act as vectors to pathogens whose activities can have an adverse effect on crops. Of major concern for the agricultural sector regionally is the control of insect pests that either directly or indirectly cause millions of dollars of crop losses annually. An additional concern is the use of chemical pesticides as a means to control insect pests. Use of such chemicals is costly as well as harmful to human and animal health and the environment.

In addition to the use of chemical pesticides, conventional methods used to limit insect damage include the use of biological control techniques. Bio-control utilizes natural enemies of pests to limit their detrimental impact on crop yields and over all profitability. More recently, efforts have been focused on the use of genetic engineering methodologies to produce more effective insect predators, less virulent insect pests and ineffective disease carriers (Pew Initiative on Food and Biotechnology, 2001).

The major objectives of the application of genetic engineering techniques to produce transgenic arthropods have been to minimize the adverse impact on the environment as a result of agri-chemical use and to maximize profits at the farm level. Research and development activities have therefore focused on the potential for limiting insect interactions

with other pests; controlling insect survival in natural habitats and target specificity (Pew Initiative on Food and Biotechnology, 2001).

The Caribbean perspective: Biological control activities
The Caribbean consists of the group of Small Island Developing States situated between Florida and Guyana. Most of the islands are independent and self-governed. Agriculture production is generally for local consumption except for field crops such as sugarcane, cocoa, and cotton and some root crops and fruits. Trade in agricultural commodities regionally is governed by protocols set out by Trade agreements established under the Caribbean Community (CARICOM) and also by other international trade agreements such as the World Trade Organization.

The major crops grown in the region include sugarcane, rice, cotton, banana and plantain, citrus, coffee, vegetables and onions. Some of the pests that cause economic damage to these crops are listed in Table 1 while Table 2 gives a summary of successfully implemented biological control programs in the Caribbean region (Bennett et al. 1985)

Table 1. Some pest of Economic importance to the Caribbean region	
Crop	**Pests**
Citrus	Citrus blackfly, whitefly, citrus mealybug, fruit fly, citrus weevil, cottony cushion scale
Coconut	Coconut whitefly, coconut mealybug, coconut scale, palm castniid
Cotton	Green stink bug, pink boll worm, cotton leafworm
Sugarcane	West Indian cane fly, sugarcane froghopper, sugarcane mealybug, sugarcane root borer, yellow sugarcane aphid, sugar cane moth borers
Vegetables and field crops	Phytophagous snails, locusts and grasshoppers, mealybug, thrips, tomato flower midge, sweet potato leaf roller, armyworms, leaf miners,
Trees and ornamentals	Various mealybugs including pink mealybug, various scale insects, cocoa thrips, banana weevil, coffee leaf miner, coffee berry borer.
Livestock	**Pests**
	Amblyomma variegatum, Boophilus tick (Pergram, personal communication)

Table 2. Summary of successful biological control programs in the Caribbean region		
Insect pest species	**Effective control agents**	**Countries**
Aleurocanthus woglumi Ashby	*Encarsia opulenta* (Silv.) *Eretmocerus serius* Silv.	Bahamas, Barbados, Cayman Is, Jamaica
Aleurodicus cocoas (Curt.)	*Encarsiaella noyesi* Hayat	Barbados
Aspidiotus destructor Sign.	*Cryptognatha nodiceps* (Mshl.)	St. Kitts & Nevis
Diatraea saccharalis (F.)	*Apanteles flavipes* (Cam.), *Lixophaga diatraeae* Tns., *Metagonistylum minense* Tns., *Paratheresia claripalpis* (Wulp.)	Barbados Antigua, Barbados, Dominca, St. Kitts Guyana, St. Lucia Dominica
Icerya purchasi Mask.	*Rodolia cardinnnalis* (Muls.)	Bahamas, Barbados, Cayman Is, Jamaica, Montserrat, St. Kitts and Nevis
Plutella xylostella (L.)	*Apanteles plutellae* Kurd.	Barbados
Spodoptera frugiperda (J.E. Smith)	*Telenomus remus* Nixon	Barbados

Insect pests cause substantial losses to the regional agricultural sector per annum. These losses are not only due to loss in overall crop yield but also due to loss in trade since restrictions are placed on imports of fresh produce from countries infested with some pest. A classic example

within the region is the trade restrictions placed on countries infested with hibiscus mealybug (pink mealybug) (Kairo et al. 2000).

Regionally, it is estimated that the value of annual crop losses due to insect infestation and from diseases carried by some insects can be in excess of US $150 million. The reduction of yields per crop is not clearly documented, although figures do exist for some crops. For example, the froghopper pest in sugarcane is estimated to cause a 30% reduction in yields per annum (Kairo et al. 2000). Dr. Pegram of the Caribbean Amblyomma program estimates a 7% loss due to death and losses in weight gain of about 2.5 kg every two to three weeks during infestation of small ruminants with *Ambylomma variegatum* (tropical bont tick). Likewise the Boophilis tick is estimated to result in a 1-2 kg per month loss in weight gain during infestation. Although data is not readily available to provide a clear picture of overall losses due to trade restrictions, regional figures for hibiscus mealybug are estimated to be greater than US $500,000.00 per annum.

Within the region insect pests are generally controlled by use of chemical pesticides and in some cases by biological control methods. Figures for the cost of insect pests control are not available on a regional basis but some data can be found for some countries. For example, the estimated cost of control for hibiscus mealybug in Grenada between 1995 and 1998 is estimated to be US $1.1 million (Kairo et al. 2000).

Agricultural production within the region is on a relatively small scale, although in most countries it plays a major role in the economy. As a consequence, the value of crops grown is not substantial enough to support research and development (R&D) activities funded from within the industry. One of the major challenges the Caribbean face in controlling insect pests is funding for R&D. This has meant that bio-control R&D initiatives are heavily reliant on activities initiated in developed countries. The last comprehensive report conducted on biological control in the region was that of Bennett and colleagues in 1985. Very little has changed since then regarding R&D or technology adaptation to enhance the effectiveness of biological control agents used to control pests of economic importance in the region.

Regional R&D institutions are beginning to realize that they must take the initiative and begin to develop research projects that seek to produce methodologies for the effective control of insect pests. Such projects should combine conventional methods of biological control, search for new natural enemies and the use of genetic engineering techniques.

Internationally more and more R&D initiatives are being focused on the control of insect populations by employing gene technologies. Some of these research initiatives include:

- Work with transgenic mites, with future research objectives being to produce transgenic pesticide resistant mites for enhanced predator activity.
- Working towards the development of transgenic pink bollworm to assist in monitoring and assessing the distribution of pink bollworm used in bio-control in cotton.
- Working towards the development of transgenic nematodes for easy detection of transformants and also for heat tolerance in nematodes (Pew Initiative on Food and Biotechnology, 2001).

Traditionally, the Caribbean region use either biological control or chemical pesticides to control insect infestations among crops and is currently not actively involved in R&D

activities to control insect pests using genetic engineering methodologies. Imports of biological control agents within the region are governed by the FAO code of conduct for the import and release of bio-control agents and are generally executed through the pesticide control board and quarantine departments of the Ministry of Agriculture or other relevant ministries. The limited activity in the application of genetic engineering techniques to assist in the management of insect infestations may be a result of several factors including (i) the lack of appropriate regulatory or legal framework, (ii) limited human resource and institutional capacity and (iii) limited funds.

Very few Caribbean countries have national regulations in place to deal with research activities involving genetic engineering. Institutions such as the University of the West Indies that conduct transgenic research do have in place guidelines for safe handling of transgenic material for research purposes. These guidelines are in keeping with those of other international universities who collaborate on these R&D initiatives and are not guided by national or regional policies.

Most countries within the region have signed onto the biosafety protocol, although few have ratified the protocol. Countries are therefore now in the initial stages of developing regulations to deal with the transboundary movement of living modified organisms (LMOs). Countries such as Barbados, Jamaica and Trinidad and Tobago are leading this process but are still a long way from having legally instituted policies and an institutional framework to deal with movement of LMOs across regional and international borders.

Progress in this regard is impeded by the limited human resource capacity in some countries. To address this problem there may need to be a regional approach where regulatory policies and legislation are harmonized and a regional 'team of experts' established to process applications for import or export of LMOs. Only after having the appropriate regulatory, legal and institutional framework in place can the region proactively participate in research activities that require the use and development of transgenic material and also proprietary technology.

Regional R&D institutions will need to develop more collaborative projects with other international institutions to work on identifying projects of relevance to the region. For example work on major insect pest problems in sugarcane, cotton and ornamental plants. Such collaborations should compel regional institutions to upgrade their regulations, especially to include guidelines for managing transgenic arthropods since there are currently no regulations in place to do this.

The region must also be actively involved in international meetings, conventions and dialogues that address regulation of transgenic materials. Such involvement is necessary so that the views of small island states can be expressed and considered. There is also a significant challenge to regional policy makers to put issues of biosafety, science and technology and R&D in genetic engineering high on their agendas. This way greater priority will be placed on these issues, more human and financial resources will be committed and faster progress will be made in developing the required regulations.

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BIOSAFETY REGULATIONS AND GUIDELINES IN ZIMBABWE

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Abstract
Living organisms obtained from modern biotechnology are subject to varying guidelines and regulations at national and international levels. There are different interpretations of the Biosafety Protocol adopted in January 2000. The provisions of the Protocol could be misused to create trade barriers. Worldwide, opinions differ on the agricultural use of genetically modified organisms including crops, livestock and arthropods. Heated debates on the issue continue in both the developed and developing countries with the consequence, that negative attitudes have been created amongst policymakers, scientists and consumers. Concerns have been raised about human, animal and environmental safety with the release and utilization of genetically modifies organisms. In view of the concerns regarding biosafety of GMOs, Zimbabwe has developed "Biosafety Regulations and Guidelines" aimed at regulating the preparation and implementation of programmes in relation to research, production, importation and release of genetically modified organisms including arthropods. The paper highlights the application of the Biosafety Regulations, Guidelines and the role of policymakers and stakeholder institutions in the country. It also suggests areas where genetically modified arthropods are likely to make an impact in agriculture. Genetically modified arthropods are a product of a new technology and in Zimbabwe's context are likely to stimulate new approach to research and development (R &D) in the protection of stored products against insect pests. Zimbabwe has established a Biosafety Board already with a mandate to advise the Government through the Research Council of Zimbabwe (RCZ) on the development, production, importation, application and release of GMOs. The author believes that the introduction of genetically modified arthropods is likely to impact positively on plant protection, especially in post harvest losses due to insects once risk assessment on the new technology has been undertaken. Currently there is limited use of genetically modified crop varieties in research and development but no release for commercial use has been approved.

INTRODUCTION

Biotechnology is a new and powerful technology for sustainable agricultural research and development. With the even increasing world population, estimated to double 5.5 billion by the year 2050, while more land cannot be created by man, biotechnology seems to hold the solution to World Food Security in the future. Biotechnology is likely to contribute considerably to the alleviations of poverty and hunger for the majority of developing countries if only biotechnology food products could be made safe to humans, animals and the environment. Although biotechnologies differ in techniques and application, FAO has defined biotechnology as biological process or processes aimed at producing materials and services beneficial to mankind. There are many aspects of biotechnology that have attracted little or insignificant controversy yet there are others like genetically modified organisms (GMOs) that have raised concerns worldwide about human and animal safety. Debate on GMO continues to be very sensitive and emotionally charged.

There is high potential for biotechnology to increase agricultural productivity and prevent the teaming masses in the developing world from poverty and starvation. However, there are potential risk to human, animals and environment. Mankind must strive to avert the risks as far as possible in the process of gene manipulation, gene transfer, DNA typing and cloning of

crops and livestock. Like most countries in the world, Zimbabwe is concerned about the possible adverse effects of introducing or releasing GMOs to people, animals and the environment. However, Zimbabwe recognizes the role that biotechnology could play in increasing agricultural productivity. Amongst the various aspects of biotechnology in agricultural research and development, transgenic arthropods could find application in plant protection such as the protection of stored products against insect pests. It is in this view that Zimbabwe is currently but cautiously undertaking agricultural research and developing involving GMO.

APPOINMENT OF BIOSAFETY BOARD

Zimbabwe developed biosafety regulations and guidelines in relation to introduction of genetically modified organisms and products from outside the country. Zimbabwe is therefore one of the few African countries that have developed Legal binding code of conduct for institutions and individuals dealing with genetic engineering, release and application of GMOs. As concerns for the safety of people, animal and the environment turned into an outcry, voluntary code of conduct was drafted by individual in 1992 who latter lobbed the Research Council of Zimbabwe (RCZ) to be actively involved in monitoring and controlling the development of biotechnology. Recognizing the potential risk and the ethical issues in relations to biotechnological research and development including the impact of GMO release into the environment, an interim Biosafety Committee was appointed while the Research Act was undergoing amendment. In 1997 the first meeting of the Boisafety Committee was convened and set up a subcommittee with the mandate to draft biosafety regulations and guidelines (Mupawose,) 2000. The Research Act was amended in 1998 and RCZ was co mandate to monitor and control research considered potentially harmful, covering research activities suspected to be a risk to the health of the people, animal and the environment. Although the decision of RCZ is final, it must be based on the reasonable grounds. The amended Research Act empowered RCZ to sep up a biosafety board to ensure safety in research and development. RCZ appointed an Interim Biosafety Board, drawing membership from relevant disciplines as follows:

- Agriculture
- Health
- Institutions of higher learning e.g. University of Zimbabwe
- Standards Association of Zimbabwe
- Institute of Environmental studies

After approval by the Boisafety Board, the draft regulations and guidelines were submitted to the Office of the Attorney General for revision and development of a statutory instrument. The Cabinet approved the "Regulations and Guidelines and published them in the Government Gazette on 4th February, 2000 as Statutory Insurance 20 of 2000. Members of the Board, officially appointed, meet once every three months.

BIOSAFETY BOARD FUNCTIONS

The Board advises the RCZ and Government of Zimbabwe on matters related to the development, productions importation, utilization application and release of GMOs. Functions may be categorized into regulatory and promotional.

Regulatory role

The regulatory functions include the following:

- Reviewing project proposals concerning high risk category organisms and controlled experimental trials involving them and approve, prohibit or restrict such trials;
- Reviewing reports of all ongoing approved projects and controlled experimental trials involving the high-risk category organisms;
- Monitoring and approval of the discharge of GMOs or cells or waste from laboratories and hospitals into the environment;
- Ensuring that biosafety guidelines and standards are adhered to generally and in the execution of project or controlled experimental trials involving high risk category organisms;
- Identifying, prioritizing and proposing areas for standardization of products or recombinant DNA technology to various bodies such as the Standards Association of Zimbabwe, the Medicines Control Authority of Zimbabwe and the Hazardous Substances Control Board;
- Approval of safety aspects of the import, export manufacture, processing and selling of any GMOs or cells, including substances, foodstuffs and additives containing products of genetic engineering; and
- Advising customs authorities on the import and export of biologically active material and GMOs, substances or products.

Promotional role

- The promotional functions include the following.
- Evolving a long term policy for a safety in biotechnology in Zimbabwe;
- Actively promoting biotechnology in Zimbabwe;
- Approving deliberate releases of properly evaluated genetically modified organisms;
- Assisting in the clearance of applications for setting up industries based on genetically modified organisms;
- Recommending a training programmed for biosafety officers;
- Collection and dissemination of information pertaining to safety procedures associated with work on or research into genetically modified organisms; and,
- Establishing contact and maintaining liaison with bodies in other countries and international organizations concerned with monitoring work on or research into genetically modified organisms in order to perform its function and Board may, amongst other possibilities.
- Co-operating or entering into agreements with any other person or institutions upon such conditions as the parties may agree on, and
- Promoting co-operating between Zimbabwe and any other country with regard to research, development and technology transfer in the field of genetically modified organisms.

GUIDELINES

The Biosafety Board developed a set of ethics, which constitute Biosafety Guidelines. These guidelines give the researchers or workers procedures that must be followed when dealing with genetically modified organisms but at the same time minimizing risks and complying with legislation. The guidelines spell out all aspects of research and development covering organisms likely to undergo genetic engineering in the country. Recombinant DNA technology in microorganisms and their use in large-scale production, genetic transformation of plants and animals, deliberate or accidental release of such organisms are covered. The guidelines also give detailed containment and safety measures.

It is believed that full adherence to the procedures, risks related to recombinant DNA could be minimized or prevented. Biosafety Guidelines protect people, animals and the environment by minimizing potential risks that are associated with recombinant DNA and promoting biotechnology to the benefit of mankind.

CURRENT STATUS

The use of genetically modified technology in Zimbabwe is still in its infancy. The private sector has interest in testing some GMO crop innovations in the country with the hope of improving agricultural product competitiveness. There have been 3 applications to test genetically modified crops.

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THE INTERMINISTERIAL COMMISSION ON BIOSECURITY AND GENETICALLY MODIFIED ORGANISMS IN MEXICO

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Abstract

With the development of transgenic technology related to agriculture, Mexico has taken the initiative to develop a regulatory framework to deal with issues related to risk assessment, biosecurity and biodiversity. An Interministerial Commission has been formed which oversees all aspects of genetically modified organisms (GMOs). This paper describes in detail the responsibilities of the different bodies involved in the Interministerial Commission, and discusses some of the issues related to the introduction of genetically modified maize.

INTRODUCTION

The last few years have witnessed incredible technological advances in genetic engineering and molecular biology. These advances allow us to combine existing DNA sequences in completely new and innovative ways, thus allowing for the creation of transgenics or genetically modified organisms (GMOs).

It is important to note that GMOs did not previously exist in their current forms. Their characteristics have been purposefully modified so as to have a positive impact on agriculture, human and animal health, the environment, and international trade. Unfortunately, the beneficial contributions of GMOs are often overlooked. Rather, lack of information regarding the creation and effects of GMOs has bred anxiety about possible risks to human health and the environment. Because the potential positive and negative effects of GMOs are so widespread, nations throughout the world have taken an unprecedented interest in this developing technology. It is clear that every country will need to develop well defined norms regarding the regulation of GMOs in order to help reduce potential risks of this technology while allowing for its maximum utilization.

Mexico is already blessed with great diversity with respect to her agricultural produce. Mexico is the center of origin of the following crops: corn, beans, cotton, chili, avocado, squash, tomato, and amaranth. The Mexican government's commitment to agricultural has resulted in two clearly identified goals. First, Mexico is committed to guaranteeing an adequate supply of agricultural goods to meet domestic and international needs. Second, Mexico is committed to safeguarding the wide variety of plant species currently existing in Mexico, as well as their "savage relatives," to make sure they are still available as a genetic resource for future generations.

Mexico participated in the first trial GMO plants in the 1980s because it was enthusiastic about the many benefits available through this biotechnology. However, there has been concern about the effects of GMOs on the national ecosystem. To help ensure Mexico's rich biodiversity the Ministry of Agriculture, Livestock, and Water Resources founded the National Committee of Agricultural Biosecurity to study the impact of each GMO and advise the government on any relevant scientific aspect of the trial. However, realizing that the effects of GMOs would extend well beyond plant diversity and would impact ultimately on human and animal health, the Mexican government decided to create a higher-ranking

government organism with greater jurisdiction and decision-making authority. Thus the Interministerial Commission on Biosecurity and Genetically Modified Organisms was born.

THE CREATION OF THE INTERSECRETARIAL COMISSION OF BIOSECURITY AND GENETICALLY MODIFIED ORGANISMS (CIBIOGEM)

The Interministerial Commission on Biosecurity and Genetically Modified Organisms (CIBIOGEM) was created by Presidential Decree on the 5th of November, 1999. The Commission is comprised of six Ministers of State as well as the General Director of the National Council for Science and Technology (CONACYT). The Presidential Decree mandates that the Presidency of the CIBIOGEM will rotate every 6 months among the Ministers of Agriculture, Ecology and Health. The main objectives of CIBIOGEM, as defined in this Decree, are to coordinate federal policies regarding biosecurity and the production, import, export, movement, propagation, liberation, consumption, and use of GMOs.

CIBIOGEM has been operational since early 2000 and its mandate has been integrated by the heads of the Ministries of Agriculture, Ecology, Health, Finance, Commerce, and Education, as well as by the Council for Science and Technology. Additionally, this Decree establishes the Executive Secretary, the Technical Committee, and the Consultative Council on Biosecurity.

Under Mexican federal law, CIBIOGEM has the following functions:

- to elaborate and present for the consideration of the President of the Republic national policies regarding biosecurity and GMOs. This includes policies regarding the incorporation of CIBIOGEM´s mandate throughout the ministries. CIBIOGEM must also periodically update the President of the Republic as to any developments in the arena of biosecurity and GMOs;
- to suggest a legal framework to each of the ministries so that they may conform to the mandate of CIBIOGEM;
- to present to the National Normalization Commission suggestions about Mexican official norms for the research, production, trade, import, export, movement, commercial use and consumption of GMOs;
- to determine, in conformity with applicable legal principles, uniform criteria to be required of commercial producers for licenses and permits from the different ministries regarding the GMOs;
- to promote the establishment of a National Register for GMOs;
- to promote, together with the National Commission for the Knowledge and Use of Biodiversity (CONABIO), the establishment of a data bank on the presence and distribution of savage species related to GMOs, and monitor mechanisms and evaluate the environmental impact and the impact on human and animal health resulting from the production and consumption of GMOs;
- to ensure that the resources and capacities of the public and private sector, including existing research institutes, are fully utilized with respect to biosecurity and GMOs;
- to set up a uniform program for the inspection of GMOs research and production plants;
- to recommend methods for the dissemination of information regarding the benefits and possible risks of the use and consumption of GMOs to the public.
- to recommend research projects of national interest related to GMOs;
- to suggest programs for safe technology transfers;
- to organize consultations on issues within the competence of the Commission;

- to, with the participation of the Ministry of Foreign Affairs,
  (1) designate participants in Mexico's delegations to events organized by international organisms regarding biosecurity and GMOs,
  (2) make recommendations regarding national positions on biosecurity and GMOs to promote the systematization of national and international information relevant to the functions of the CIBIOGEM;
- to systemize information regarding GMOs and ensure their accessibility; and
- to further elaborate on CIBIOGEM´s operational rules and on the statutes of the Consultative Council of Biosecurity.

THE EXECUTIVE SECRETARY

The Executive Secretary is responsible for:

- ensuring that the laws regarding biosecurity and the regulations of CIBIOGEM are followed by government institutions;
- registering GMOs and their products and sub-products;
- inviting members of CIBIOGEM to any ordinary and extraordinary meetings;
- formulating and submitting proposals for the order of the day regarding any ordinary and extraordinary meetings for the approval of the President of the Commission;
- communicating to corresponding institutions and entities of the Federal Public Administration, rules and regulations of the CIBIOGEM;
- broadcasting to the public, social, and private sector of the country, the policies and regulations of the CIBIOGEM;
- executing the decrees of the Technical Committee;
- establishing a link between the CIBIOGEM and the Technical Committee;
- establishing the mechanisms of coordination and communication between the Commission and the Council;
- establishing and maintaining an up-to-date registry of GMOs;
- establishing and maintaining an up-to-date data bank regarding the presence and distribution of savage species related to GMOs; and
- informing the leadership of CIBIOGEM about the activities of the Technical Committee.

THE TECHNICAL COMMITTEE

The activities of the Technical Committee are coordinated by the Executive Secretary of CIBIOGEM. The responsibilities of the Technical Committee include:

- to keep track of the implementation of the laws and CIBIOGEM regulations;
- to prepare and suggest to the Executive Secretary issues and regulations that have to be submitted for the consideration of the CIBIOGEM;
- to transmit to competent institutions, recommendations made by CIBIOGEM;
- when suggested by CONABIO, to reach agreements with the responsible institution regarding the performance of risk analyses for GMOs, and their products and sub-products;
- to recommend the activities or performance of necessary studies to aid in the adoption of national positions regarding biosecurity and GMOs; and
- to recommend to CIBIOGEM the creation of any specialized subcommittees considered necessary.

CONSULTATIVE COUNCIL ON BIOSECURITY

The Consultative Council on Biosecurity is made up of 13 scientists of known reputation and experience. The Council designates the coordinator amongst its members, whose functions are determined by statute. The Consultative Council is a mandatory consultative body to CIBIOGEM. Its responsibilities include, giving technical and scientific opinions regarding various issues in biosecurity and GMOs.

When more information is needed for a competent analysis of risk in a given situation, the Council may ask CIBIOGEM to gather further evidence and submit its own risk analysis for a particular GMO. The Council also, through CONACYT, has jurisdiction to ask for the collaboration of any national or international research center, when it considers it relevant for the resolution of the issues within its competence.

RULES OF THE CIBIOGEM

The objective of the Rules of Operation is to regulate the organization and functioning of the Commission, as well as its interaction with the Council, the Technical Committee and the Executive Secretary. The rules also establish a process by which commercial producers will be authorized to work with GMOs:

- The authorization for the production and use of GMOs, or its products and sub-products, will be granted by competent authorities if the application follows the legal regulations established by CIBIOGEM precedent.
- CIBIOGEM will create a uniform time period for replying to requests for the production or use GMOs. Once the time period established by the CIBIOGEM has lapsed, CIBIOGEM will be considered to have no objection to the application.
- CIBIOGEM can create working or counseling groups to help understand how the government should respond to novel issues raised in applications for licensing. These groups will be directly responsible to CIBIOGEM and their activities will be exclusively of an internal character.

Requests for the production and use of GMOs must be presented to the head of each ministry. The application must submit a statement of possible side effects on the subject matter of each ministry to that ministry (e.g. a statement on the impact to the environment to the Ministry of the Environment). The ministries must then turn over a copy of the application to CIBIOGEM and the Technical Committee. CIBIOGEM must reasonably weigh the opinion of the Consultative Council regarding any technical and scientific impact of license approval before formulating a recommendation, which it delivers back to the ministries. CIBIOGEM has the jurisdiction to ask the competent branches of office, for inspection and verification visits, which will have to be performed in accordance with current legislation.

When the conditions, to which the authorization is subject to, are not observed by the interested party, the party will be subject to sanctions established in the law. CIBIOGEM has jurisdiction to ask competent branches of office to dictate contemplated security measures in order to prevent or avoid accidents or improper handling of substances that represent a danger or affect the physical integrity of humans or the environment.

MAIN ACCOMPLISHMENTS OF CIBIOGEM

During the eight months following the Presidential Decree creating CIBIOGEM, involved ministries worked hard to create a legal and administrative infrastructure that would allow CIBIOGEM to quickly and responsibly respond to the demands of Mexico's biosecurity needs. During this time period, the Technical Committee, the Consultative Council on Biosecurity and the subcommittees specializing in agricultural and legal affairs were established and made fully operational. Moreover, the constitution for a Trusteeship of Financial Support has been approved, so that CIBIOGEM has the financial support to fulfill its mandate.

CIBIOGEM has held sessions on three occasions; the Technical Committee, five; and the Consultative Council, three. There has also been a joint meeting of the Technical Committee and the Consultative Council. Consensus has been reached regarding the Rules of Operations of CIBIOGEM, as well as the statutes of the Consultative Council and the Internal Rules of the Technical Committee. An expedited communicative mechanism has been developed to allow for the timely response to requests for licenses to produce and use GMOs. Additionally, consensus has been reached regarding Mexico's position with respect to the Cartagena Protocol on Biosecurity discussed below.

THE PROTOCOL ON BIOSECURITY

CIBIOGEM representatives actively participated in negotiations leading to the Agreement on Biological Diversity held in Montreal, Canada. It was during these negotiations that the Project for the Protocol on Biosecurity of Cartagena was adopted. The Cartagena Protocol's main objective is to establish an international legal instrument regulating the trans-border movement of GMOs, taking into account concerns for the environment and human health. In May 2000, CIBIOGEM advised the President of the Republic to sign the Cartagena Protocol, as it would help establish an international risk assessment and monitoring mechanism that would reinforce Mexico's domestic regulation in this arena. The recommendation was followed, and in Nairobi, Kenya, the Minister of Ecology, acting on behalf of the President of the Republic, signed the Cartagena Protocol on Biosecurity along with 67 other nations.

ACTIVITIES FOLLOWING MEXICO'S RATIFICATION OF THE CARTAGENA PROTOCOL ON BIOSECURITY

Once a nation-state had signed an international instrument, it has the obligation to take all necessary domestic legal and administrative measures to conform to the requirements of the international instrument. Mexico has, through the creation of CIBIOGEM, met all overt obligations to conform its domestic agenda to that of the Cartagena Protocol. Mexico still needs to revise preexisting laws, regulations, and international agreements that affect the subject matter of the Cartagena Protocol. To help accomplish this CIBIOGEM and its supportive organs have developed a strategy for a coordinated and efficient revision of nonconforming laws and regulations. CIBIOGEM has created a technical sub-commission specializing in legal issues, which will be integrated into the offices of the Legal General Directors of each Ministry. This sub-commission and the Legal General Directors will assume responsibility for ensuring that all legal codes reflect the requirements of the Cartagena Protocol.

CIBIOGEM´s Current Priorities

In order to more efficiently respond to Mexico's biosecurity needs, the Technical Committee, the Consultative Council and the Commission developed a list of issues that needed to be prioritized and given CIBIOGEM´s immediate attention. The following list resulted from their discussions and is now being used to determine CIBIOGEM´s allocation of economic resources:

General Priorities
- Training and Expert Groups
- Public Perception
- Code of Ethics
- Human Health
- Regulation/Legislation

Specific Priorities
- Transgenic Corn (Maize)
- Labeling (Commodity Grains and Food)
- Animal Vaccines
- Bioremediation
- Genetic Transformation of Potato
- Expert Lists
- Genetic Transformation of Squash
- Genetic Transformation of Perennials (e.g. Papaya)
- Genetic Transformation of Fish

THE NEED FOR RESEARCH REGARDING TRANSGENIC CORN (MAIZE) FOR MEXICO

One of the most important current concerns of CIBIOGEM is the production and use of transgenic corn, or maize, in Mexico. Research is needed to show how genetically engineered corn can be produced safely in the country. Corn has been given specific priority because:

- Mexico is the center of origin of corn and consequently contains the greatest level of genetic diversity regarding this species in the world.
- The country is an important producer, consumer, and importer of corn.
- Mexican society has a significant socio-cultural relationship with corn. There are also a number of communities in Mexico for whom corn is the main source of food and income.
- Various forms of transgenic corn, already produced by leading biotechnology companies, constitute a significant portion of the 40 million hectares of transgenic crops grown throughout the world.
- Additionally, with respect to scientific research, Mexico is the site of the International Center for the Development/Improvement of Maize and Wheat. There are also a number of eminent national scientists working on corn genetics in the various Mexican research institutions.

The Consultative Council determined that to fully research the effect of transgenic corn in Mexico, a number of ad hoc Working Groups would be needed to fully study its effects on Mexico's biodiversity as well as Mexican society. For this reason, the following ad hoc

committees were created: agriculture, society, culture, public opinion, economy, health and biodiversity.

CHALLENGES FOR THE IMMEDIATE FUTURE

In the global context, it is expected that biotechnology, along with other technological advances in agriculture, will allow for an increase in food production. Hopefully, this will allow us to meet the demand for food production from populations currently suffering from malnutrition. Such technology is considered by some to be more important to developing countries than to the industrialized countries because yield per crop in developing countries is often significantly lower as a result of disease, plague, weeds and susceptibility to a number of adverse factors.

Scientific research may offer a cure for the food crisis faced in many parts of the world. Unfortunately, the study of many agricultural species, important to the socioeconomic development of some countries, is not prioritized by leading biotechnology companies. Consequently, these countries have to develop their own national capacities to ensure their specific priorities will be met. Because of the many ecological, economic and ethical complexities involved with GMOs, Mexico finds that its own response has to be multifaceted enough to respond to this technology in an intelligent and sophisticated manner.

First, as was previously mentioned, Mexico is the center of biological diversity for many agricultural crops. This gives Mexico access to a great diversity of genes, which constitute the raw material of biotechnology. It also requires that Mexico be more environmentally sound in the development and manufacture of GMOs. Second, Mexico has already acquired, through the work of eminent scientists and scholars, a scientific and ethical framework for the regulation of GMOs. Lastly, and in contrast to many other countries, Mexico has established legal instruments regulating GMOs.

In short, Mexico will continue to integrate biotechnology into its agricultural policies. But we will do so only after evaluating fully the implications of each GMO on human health, the environment, and the national economy.

PROPOSED DRAFT PERMIT GUIDANCE FOR GENETICALLY MODIFIED ANIMAL DISEASE ORGANISMS AND THEIR VECTORS

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Abstract

This paper consists of proposed draft guidance and represents the author's opinions only. They are presented below solely for the purpose of open discussion and comments on the subject of genetically modified arthropod regulations and should not be construed as representing actual or current regulations or opinions of the USDA, Animal and Plant Health Inspection Service (APHIS).

INTRODUCTION

The U. S. Code of Federal Regulations (CFR) 9 Part 122 does not currently include genetically altered forms of pathogenic disease organisms of livestock and poultry in its definition of organisms. Technology in this area has progressed to the extent that genetic modification of these organisms could potentially affect their infective ability, or virulence, by posing new risks to animals and the environment in the following ways: (1) increase in animal host range, which may include humans; (2) increases in rate of infection within animal populations; (3) changes in transmission cycles from limited to multiple vectors and other means of disease transmission; (4) increased mortality rates; (5) pesticide, antibiotic, or other drug resistance, since antibiotic resistance is commonly used as a marker gene; (6) increased bacterial or fungal toxin production; (7) change or enhancement of toxin characteristics; (8) impaired ability to detect disease; and (9) need for new treatment development.

9 CFR Part 122 also does not currently include arthropods and other invertebrates, their biological associates, and genetically modified forms that carry or transmit diseases of livestock and poultry in its definition of vectors. Biological associates are commensals (animals and microorganisms that live in association of two or more species where one derives a benefit, but neither harms the other) or symbionts (animals and microorganisms that live in association of two or more species where all usually derive some benefit). The importation into the United States, transportation from one State or Territory or the District of Columbia to another, and release of these vectors into the environment could result in establishment of a new species or variety of vector in an area resulting in increase of animal disease. Genetically modified vectors may be altered in ways that pose new risk to animals that include: (1) more efficient vectors with increased ability to transmit diseases, and (2) vectors with a biological selective advantage resulting in larger numbers of vectors.

Therefore, we propose expanding the definitions of vectors and organisms in 9 CFR 122 to include vectors, their biological associates, animal pathogen disease organisms, and all genetically modified forms of these as regulated articles. A permit from the APHIS, Veterinary Services, National Center for Import and Export would be required for importation and transportation within the United States, research under quarantine provisions, and release into the environment of these regulated articles. To meet this proposed requirement, the vectors, their biological associates, animal pathogen disease organisms, and all of their genetically modified forms (regulated articles) would have to be new or uncommon to an environment and pose risk of new animal diseases, increase in the frequency of existing

diseases or change in characteristics of diseases. To facilitate identification of which vectors and disease organisms may require a permit, lists of vectors and disease organisms will be made available to the public. The lists would be as comprehensive as possible and would be revised periodically. Application would require submission of sufficient information to the Agency for appropriate risk assessments to be made. The kind and amount of information required for risk assessment would depend on the intended purpose of the permit request and the biological nature of the regulated article.

APHIS has existing authority under 21 U.S.C. Chapter 4 and 5 to regulate the importation, interstate movement, testing, and release of pathogenic organisms, their vectors, and their genetically modified forms that transmit and cause animal zoonotic diseases of livestock or poultry. APHIS does not have statutory authority to regulate the importation, interstate movement, testing, and release of disease organisms, vectors, and their transgenic forms that transmit diseases that only affect humans (anthroponoses) and not other animals. These diseases include certain types of malaria, filariasis, onchocerciasis, Carrión's disease, dengue, yellow fever, African sleeping sickness, and epidemic and scrub typhus. However, CDC requires import permits for human etiological agents, infectious biological materials, and vectors. The importation of etiologic agents is governed by the following federal regulation: 42 CFR 71, Foreign quarantine and 71.54, Etiologic agents, hosts, and vectors. The following website contains the Department of Health and Human Services (HHS) regulations on interstate shipment of etiologic agents, laboratory registration and select agent transfer program, and importation permits for etiologic agents:
http://www.cdc.gov/od/ohs/biosfty/biosfty.htm

If a genetically engineered form is developed to have pesticidal properties as defined under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) of 1947, 7 U.S.C., *et seq*., the genetic material and the pesticidal substances produced are subject to regulation by the Environmental Protection Agency, Office of Pesticide Programs, and would not require an APHIS permit. However, if the intended pesticidal use is outside the United States, its territories and possessions, and not subject to FIFRA, but subject to Executive Order 12114 as it applies to environmental affects abroad of major Federal Actions under the National Environmental Policy Act (NEPA), it may require an APHIS permit.

Genetically modified vectors
Although the field of research on transgenic arthropod vectors of animal diseases is less than ten years old, there has been a variety of developments. Some of these include the following: gene modeling; control of hormonal and tissue-specific gene expression in mosquitoes; genetically engineered mosquitoes that to do not transmit or are refractory to diseases such as malaria and dengue through immunity, or melanotic encapsulation of disease agents and parasites; genetic engineering of an insect gut symbiotic bacteria to prevent transmission of Chagas disease, repressible dominant lethal genes for autocidal biological control; marker genes that express green fluorescent protein from a jellyfish; use of *Wolbachia* bacterial cytoplasmic symbionts to affect host insect reproduction and as transformation vehicles; use of transposon-based gene transfer systems such as *piggyBac*, *Hermes, hobo, Mariner*, and *Minos*; RNA mediated gene interference for manipulating gene function; and use of densoviruses (parvoviruses) and SindBIS virus as carrier agents for transduction of foreign genes into insects. The products of this new research may pose potential risks to animals and the environment that would need to be identified, prevented, or mitigated.

PERMIT PROCEDURES AND GUIDANCE

The following section provides guidance for submission of risk assessment information that the Agency is proposing to require for the issuance of permits for vectors, biological associates of vectors, animal disease organisms, and any of their transgenic forms that are intended for importation, transportation, testing, and release into the environment. The Agency may issue a permit upon evaluation of all or appropriate portions of the information that is described below.

APHIS VS permit Form 16-3 is located at:
http://www.aphis.usda.gov/forms/ under Veterinary Services with application information at: http://www.aphis.usda.gov/forms/info-n.txt. The internet "On-line" application is at: http://www.aphis.usda.gov/ under: Import Authorization System (On-line Permit Application). A signed and dated copy of the application form will be required. When an application for a permit is received, it will be evaluated for sufficient information to do a risk assessment appropriate for the purpose of the permit and nature of the regulated article. This should take no longer than 30 days. If more information is required, the applicant will be requested to provide what is needed and sufficient time will be allowed to produce and submit that information. If the risk assessment indicates that a release into the environment may adversely affect under the Endangered Species Act, consultations with the U.S. Fish and Wildlife Services will be required before a permit is issued.

Before a permit is issued, a qualified Agency officer may conduct an inspection to determine adequacy of biosafety level containment under quarantine. A second inspection may by made after the permit is issued to determine whether the conditions of the permit are being met and if any other containment methods or procedures could result in unexpected risks. Quarantine conditions of the permit may then be amended by the Agency as needed to prevent or mitigate risks that become apparent. The applicant may also submit amendments to the Agency for review and approval. The Agency should be notified within 24 hours of any accidents, vandalism, or other events that could adversely affect activities under the permit or result in risks to animals or the environment. A permit would be valid for the specific time period of the activity of up to two years following issuance, but may be renewed for up to three additional years, depending on the nature of the activity. A summary report of the activity conducted under the permit should be submitted within a year of its issuance and renewal and would be required prior to renewal. Records of activities conducted under the conditions of the permit should be maintained for at least seven years after the permit expires and should be made available to the Agency within 30 days of request.

Information Requirements for a Permit
APHIS Form VS 16-3 should be completed and an appendix attached for additional information not specified on VS 16-3, but required for a permit. It is requested that this information be accompanied by electronic copies in MS Word on 3 ½" disks or CDs, and that a signed and dated application form be submitted.

Proposed additional information requirements for contained research under QUARANTINE of livestock and poultry disease organisms
(1) taxonomic description, synonyms, and common names of the disease organism and genetically modified forms (the regulated article); (2) names, addresses, e-mail address, and telephone numbers of persons who supplied or developed the regulated article; (3) description of packaging for security during transport and package labeling for identification; (4) location

and description of the intended destination, including final and intermediate destinations (e.g., laboratory or growth chamber location); (5) applicable biosafety level; (6) summary description of the proposed research; and (7) other Federal, state, or local government agencies notified and respective permits applied for or received.

Proposed additional information requirements for contained research under quarantine for vectors of animal diseases
(1) taxonomic description, synonyms, and common names of the vector or its biological associate and genetically modified forms (the regulated article); (2) names, addresses, e-mail address, and telephone numbers of persons who supplied or developed the regulated article; (3) description of packaging for security during transport and package labeling for identification; (4) location and description of the intended destination, including final and intermediate destinations (e.g., laboratory or growth chamber location); (5) applicable biosafety level; (6) summary description of the proposed research; and (7) other Federal, state, or local government agencies notified and respective permits applied for or received.

Contained research guidance
The National Institute of Health (NIH) has developed Guidelines for Research on Recombinant DNA Molecules and Gene Transfer, http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html, that would apply to vectors, biological associates, animal disease organisms, and their genetically modified forms. The Centers for Disease Control and Prevention (CDC), Office of Health and Safety (OHS) and NIH have made recommended biosafety levels for infectious diseases of humans available at: http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

They include agent summary statements for bacteria, fungi, parasites, prions, rickettsia, viruses other than arboviruses, arboviruses, arenaviruses, and filoviruses. Import permits are required by CDC, OHS for etiological agents, infectious biological materials, and vectors. Importation of all living flea, fly, lice, mite, mosquito, or tick adults, eggs, larvae, pupae, and nymphs requires a CDC import permit, regardless of infection status. Any other living insect or arthropod, known or suspected of being infected with any disease transmissible to humans requires a CDC import permit. Laboratories or facilities that transfer or receive select agents are also required to register with CDC.

The American Committee of Medical Entomology of the American Society of Tropical Medicine and Hygiene has also developed Arthropod Containment Guidelines available at: http://www.astmh.org/subgroup/acgdraft.pdf, that apply to contained studies with vectors. Contained studies should provide multiple levels of biological, physical, or chemical containment, as well as precautions against weather, animal damage, and human vandalism, to insure against escape. There should be remedial monitoring and control measures that are fully operational and ready for deployment should escape or damage to the containment facilities occur.

An APHIS permit would be required for contained research on vectors, their biological associates, animal disease organisms, and genetically modified forms of these that may be exotic or uncommon to the USA and pose risk of new animal diseases, increase in the frequency of existing animal diseases, or changes in disease characteristics. If containment and security measures and facilities of the research laboratory are unknown or not verified by the Agency, an inspection may be made by a qualified Agency officer to determine adequacy of containment and security measures. Permit issuance would be contingent upon adequate

containment measures commensurate with the risk posed by the disease organism or vector. Vectors may be typically contained at Biosafety Level two.

Proposed additional information requirements for release into the environment of livestock and poultry disease organisms
The guidance stated above for contained research under quarantine for disease organisms would apply, with the following additions: (1) known geographic and seasonal occurrence; (2) species of animals infected and economic significance; (3) taxonomic description and distribution of the organism's vectors or description of other means of disease transmission; (4) nature of transmission cycle between hosts of same and different species; (5) field or pilot project location; production, propagation, release, or distribution location; (6) invasiveness consideration that is relevant to sites and surroundings of a proposed release, including proximity to sensitive ecological areas and the presence of susceptible threatened and endangered species and indigenous relatives of the regulated article; (7) development and validation of mitigation measures to reduce identified or potential risks upon release into the environment; (8) development, validation and deployment of monitoring measures to track releases and potential unintended adverse effects; (9) consideration of human health implications, particularly occurrence of new human infection; (10) development and validation for deployment of emergency management plans and control measures, should the need arise; (11) when relevant, development of technology transfer, education, and training plans.

The following additional information would be submitted in support of application for a permit for a genetically modified animal disease organisms: (1) gene map or description of the genetic construct, associated regulatory sequences, and marker genes; (2) description of the expression of the altered genetic material in the regulated article and any differences between it and the unmodified parental type (e.g. physical characteristics, physiological activities and processes, number of copies of inserted genetic material, and the physical state of this material inside the recipient organism, growth characteristics, and any associated toxins); (3) description of the genetic engineering method (e.g. donor to recipient and involving any transduction, transformation, or other methods used to produce the transgenic form) and analytical methods employed; (4) phenotype stability over multiple generations; This may involve serial passage in suitable media or living hosts under containment and laboratory analyses to evaluate expression in subsequent generations; (5) evaluation of horizontal gene movement to other possible recipient species of animal disease organisms or other organisms, such as those closely related to and associated with the regulated article; (6) evaluation of potential host range changes; (7) evaluation of potential changes in site of infection, such as from inhalation to dermal, and changes in rate of disease development in a host; (8) evaluation of potential changes in transmission cycles to include additional vectors and other potential means of increased disease transmission than that of the parent organism; (9) evaluation of increases or decreases of disease induced mortality rates; (10) evaluation of changes in the susceptibility or resistance to antibiotics or other drugs compared to the parent organism; (11) evaluation of changes in microbial toxin production and changes in toxin characteristics, such as changes in toxicity and changes in mode of toxic action; and (12) evaluation of changes in disease symptoms and concurrent ability to detect disease in an animal.

Proposed additional information requirements for release into the environment of vectors
The guidance stated above for contained research under quarantine for vectors of disease apply, with the following additions: (1) field or pilot project location and production, propagation, release, or distribution location; (2) invasiveness consideration such as relevant

details about sites and surroundings of a proposed release, including proximity to sensitive ecological areas and the presence of threatened or endangered species and wild relatives of the regulated article that may be sexually compatible with the regulated article; (3) known geographic occurrence or distribution of the regulated article including habitats and climates; (4) biology and life cycles in various habitats, climates, and ecology of occurrence; (5) kinds of diseases vectored and economic significance; (6) parasites, predators, pathogens, symbionts, and commensals of the vector; (7) development and validation of mitigation measures to reduce identified or potential risks upon release into the environment; (8) development, validation and deployment of monitoring measures to track releases and potential unintended adverse effects; (9) consideration of human health implications, such as potential effects on humans including disease transmission; (10) development and validation for deployment of emergency management plans and control measures, should the need arise; (11) when relevant, development of technology transfer, education, and training plans.

The following additional information concerning genetically modified vectors should also be submitted to support application for a permit: (1) gene maps and description of the genetic construct, associated regulatory sequences, and marker genes; (2) expression of the altered genetic material in the regulated article and any differences between it and the unmodified parental or wild-type (e.g. morphological or structural characteristics, physiological activities and processes, number of copies of inserted genetic material, and the physical state of this material inside the recipient organism [integrated on chromosomes or extra chromosomal], and any associated products, secretions, and growth characteristics); (3) description of the genetic engineering method (e.g. donor to recipient and any transduction, transformation, or transposable elements used to produce the transgenic form) and analytical methods employed; (4) phenotype stability over multiple generations; This may consists of maintaining several generations of a multivoltine species in confinement, with the actual number of generations depending on species' biology and determining Mendelian inheritance characteristics and stability of the phenotype over those generations. The rate of appearance of a phenotype known to result from a given genotype or penetrance would need to be determined, as well as expressivity (range or degree of variation in the expression) of a given trait or combination of traits that is produced by a gene. Anticipated biotic or abiotic interactions a given gene product or trait may have in the environment should be considered. Additionally, (5) Transgene stability information should be required for transposable elements employed in gene transfer, as well as stability of the genes of functional interest. This could be part of a multiple generation study and, when applicable, laboratory assays may be done to define expression over multiple generations. For example, the host could be assayed for the presence of active transposable elements similar to the one used to transmit the genes of interest. (6) The effects of genetic modification on any potential changes in the vector's ability to transmit diseases should be addressed as fully as possible. (7) The following biological and ecological factors that may affect the success or fitness of the transgenic form compared to its parent should be addressed: altered host range, enhanced or reduced vector characteristics, movement ability, behaviour, juvenile viability, age at sexual maturity, female fecundity, male fertility, mating advantage, and adult viability or longevity. (8) An evaluation would be made of the possibility of horizontal movements of transgenes through transposons, feeding, and other biological means to closely related species, parasites, predators, viruses, culturable symbionts, or other such microorganisms that may be associated with the genetically modified form. (9) Information could be provided on changes to susceptibility or resistance to pesticides for genes that encode for resistance or susceptibility, as well as those genes that may not. (10) Development and validation of mitigation measures to reduce identified or potential risks upon release into the environment. Changes that decrease fitness in the

transgenic organism may be considered for biological risk mitigation. Other mitigations may include lethal genes, and conventional chemical, biological, and cultural control measures.

Parasites

Although parasites are animal disease organisms, some are arthropods or other invertebrate metazoans with complex life cycles and free living stages. Therefore, some of the proposed information requirements for genetically modified vectors listed above may also apply to parasites. The additional information listed in the section above on Genetically Modified Vectors that may be required for animal parasites would depend upon the nature of the regulated article, the environment to which it may be introduced, its intended purpose, and commensurate potential risks. The Agency would make that determination after evaluation of the permit application and discussion with the permit applicant.

Risk assessment studies should provide comparative data between transformed and nontransformed forms of the same species or parental strain of the regulated article. The studies should also provide data to aid in the development of predictive risk assessment models, if appropriate, of the probability and consequences of establishment and invasiveness. It is desirable that the studies be done according to the principles of Good Laboratory Practice Standards as set forth in 40 CFR Part 160 or, at minimum, good scientific methods as required for publication in established, peer reviewed, scientific journals. Measurable changes in characteristics from the above studies that could possibly increase risk to animals and the environment may lead to further research to determine the nature of the changes with the purposes of identifying, understanding, preventing, mitigating, or eliminating adverse risks.

National Environmental Policy Act

An Environmental Impact Statement (EIS) as specified by the National Environmental Policy Act (NEPA), 42 U.S.C. 4321, et seq., 40 CFR Parts 1500-1508, may be required for a release or other activity when they involve novel technology that could affect the environment. Applicants for APHIS permits should become aware of NEPA requirements that may apply to their work. However, an Environmental Assessment (EA), which is much shorter than an EIS, may be sufficient if a Finding of No Significant Impact (FONSI) can be made. The applicant may be requested by the Agency to prepare and submit a draft EA that the Agency would review, verify, possibly change, approve, and adopt for Agency purposes. Similarly, an applicant may be requested to draft an EIS or consider retaining a third-party contractor for preparation of an EIS that the Agency would review, verify, possibly change, approve, and adopt. Alternatively, the Agency itself may prepare an EA, FONSI, or EIS with information requested from the applicant and obtained from other sources. An EIS would require the following parts to be completed: (1) summary; (2) table of contents; (3) statement of purpose and need; (4) alternatives, including the proposed action, relationship of alternatives to purpose and need, and level of analysis; (5) list of other Federal permits; (6) affected environment description; (7) environmental consequences, including direct effects, indirect effects, cumulative effects, and other types of effects; (8) mitigation measures for adverse environmental impacts, including a definition of adequate mitigation, feasibility of mitigation measures, and level of commitment to implement mitigation measures; (9) list of preparers; (10) list of agencies and organizations involved; and (11) an index. A comprehensive scoping process is also done as part of the preparation of an EIS and often includes public meetings that are advertised in advance in the Federal Register and other appropriate public media to insure that interested parties have the opportunity to participate and comment.

40 CFR Part 1508.27(b) concerns the following actions and environmental issues that must be addressed by documentation in an EIS: (1) impacts that may be both beneficial and adverse; (2) degree to which the proposed action affects public health or safety; (3) unique characteristics of the geographic area; (4) degree to which the possible effects are likely to be highly controversial; (5) degree to which the possible effects are uncertain or involve unique or unknown risks; (6) degree to which the action may establish a precedent for future actions; (7) whether the action is related to other actions with individual insignificance, but cumulatively significant impacts; (8) degree to which the action may cause loss or destruction of significant scientific, cultural, or historical resources; (9) degree to which the action may adversely affect an endangered or threatened species, or its habitat; and, (10) whether the action may be in violation of Federal, State, or local law or requirements for the protection of the environment.

THE GUIDELINES PROJECT OF THE IOBC GLOBAL WG AND RISK ASSESSMENT OF TRANSGENIC ARTHROPODS

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Abstract

The guidelines project 'Development of International Scientific Biosafety Testing Guidelines for Transgenic Plants' is an international initiative of public sector scientists organized within a global Working Group on 'Transgenic Organisms in Integrated Pest Management and Biological Control' under the umbrella of the International Organization of Biological Control (IOBC). The project aims to:

- develop comprehensive, transparent scientific guidelines for pre-release biosafety testing of transgenic plants, which could serve as an international standard.
- extend the guidelines for possible use in post-release monitoring.
- facilitate the development of the scientific capacity in developing and developed countries to implement the guidelines
- test the application the guidelines in real policy contexts to assist in the evaluation of particular transgenic crops.
- publish the guidelines and periodically revise them in response to new developments thereby providing for their up-to-date, long-time use.

INTRODUCTION

The Guidelines will give a series of questions and methodologies by which any particular GMO issue can be evaluated scientifically. The questions will be structured to start with broader questions and will get more specific. In the whole, the Guidelines can be seen as a series of modules that link between each other. The guidelines will also be tested in real policy contexts using case studies from countries in Africa, Asia and South America. This will take place in three workshops over the next two years.

The development of the guidelines will be an open process, which will include scientific and technical capacity building and communication between scientists and policy makers in developed and developing countries. Obviously, any particular authority cannot be required to use any section of the Guidelines (no regulatory guidelines). They are provided so that the authority can choose as they desire or need and have confidence in the scientific soundness associated with evaluations of the issues. An advisory committee will accompany the process, critique constructively the products and advice on their improvement. It will be comprised of representatives from various international and national organizations The scientific work is divided into five sections; needs analysis or good agricultural practices, transgenic plant characterization, non-target and biodiversity effects, pest resistance management, and gene flow and its effects.

Needs analysis/good agricultural practices
This section sets the context for the rest of the analysis. It will provide a framework for evaluating the need for the transgenic plant in specific crop production contexts. This includes

providing an approach to evaluate projected changes in crop production practices (tillage, insecticide use etc.)

Transgenic plant characterization
This section will assess (1) how a transgene should be described to enable evaluation of its stability and inheritance; (2) how the phenotypic effects of the transgene in the plant should be specified to facilitate assessment and management of environmental effects (what, how, what plant parts, and when product concentrations should be measured in transgenic plants). Typically, transgenes are comprised of integrating elements, a marker gene and its promoter, a target gene and its promoter, and possibly other genetic elements. Questions arising here are: How many copies of the transgene elements are incorporated into the plant? Where is the transgene integrated in the genome? Is it on a chromosome, organelle DNA, or somewhere else? Where might the transgene break into parts (by recombination or other genetic mechanism)? How is stability evaluated or proved? Is it dependent on the inheritance mechanism?

Nontarget effects and biodiversity
In this section, there are two main tasks to accomplish for each of the identified categories of organisms. (1) specify a procedure to determine the non-target species or function/processes that should be tested (= selection procedures) and (2) Specify scientific procedures for testing these species/functions/processes (= Testing procedures). Seven categories of organisms have been identified that need to be addressed: (a) natural enemies, (b) pollinators, (c) soil organisms, (d) species of conservation concern, (e) species of cultural significance, (f) non-target pests, (g) other non-target species. Routes of exposure need to be identified. Exposed organisms are determined through suspected causal chains of impact. Based on this information, protocols and methodologies for appropriate testing can then be developed.

Pest resistance management
Determine the resistance risk, and management responses needed to reduce this risk. It will be important to address the feasibility of implementation. In addition, approaches for developing a practical monitoring and response system to detect resistance and to adapt management appropriately should be considered. While this is primarily addressing resistance development in pests, also resistance development of weeds as a result of commercial production of transgenic crops will be considered.

Gene flow and its effects
Gene flow is the route along which transgenes can spread genetically into populations of related species and geographically into other regions including protected areas of sensitive ecological value. Gene flow is considered a risk because of the great uncertainties that are associated with the consequences this might induce in the recipient ecosystems. Successful transgene flow will affect simultaneously both recipient plants and their associated organisms.Protocols need to be developed for establishing

[1] The likelihood of intra- and interspecific gene flow
[2] The possibility of subsequent geographic and genetic spread of transgenes
[3] The potential ecological effects resulting from gene flow
[4] The effectiveness of sterility mechanisms, their breakdown and management

108

RISK ASSESSMENT OF TRANSGENIC ARTHROPODS

(Excerpt from summary of report on 'Transgenic Animals: Use, Risks, and Possibilities for Risk Prevention', Commissioned by the German Environment Agency, Berlin (for complete report (in German) contact Dr. Mathias Otto Umweltbundesamt F6 IV 2.5 Seecktstr. 6–10 13581 Berlin mathias.otto@uba.de)

Today, there are no commercial applications of transgenic invertebrates yet because effective transformation technologies are still under development. However, this field is advancing fast and the commercial production of transgenic invertebrates expressing novel traits can be expected in the near future. In contrast, biosafety research concerning the risks and containment of transgenic invertebrates lags behind. Because of the unique combination of several characteristics of invertebrates, possible risks are even more complex and difficult to assess than for transgenic plants. With few exceptions, invertebrates are non-domesticated animals. They are small in size and have highly variable life cycles. Some invertebrates have a relatively long or a very short lifespan with life stages that are resistant to environmental stresses. Many are highly mobile allowing them to spread and move quickly. This may additionally be enhanced by a high reproductive capacity. All these characteristics make risk assessment of transgenic invertebrates a very challenging task. Until today, comprehensive criteria and methods for the assessment of the short- and long term risks or the containment of transgenic invertebrates are still at an early stage of development.

Containment strategies for transgenic invertebrates are essential for two applications: Firstly, for planned, spatio-temporally limited releases of transgenic invertebrates in open or semi-open systems where their establishment in the environment is undesired. Secondly, for the use of transgenic invertebrates in closed systems where their escape into the environment is to be prevented. This is necessary if the intended end-use of the transgenic invertebrate is in a closed system or for pre-release, biosafety testing of transgenic invertebrates. Currently, it is being discussed whether or not all experimental, transgenic invertebrates should be subjected to strict containment, like for example introduced biological control organisms. Alternatively, they could be placed into different categories with different levels of containment depending on the novel traits and type of transformed invertebrate species (Atkinson et al. 2001). In the early days of classical biological control, no strict, uniform regulation regarding containment was in place for introduced exotic natural enemies to be used as biological control agents. Entomologists felt that a thorough assessment based on existing knowledge combined with the experience gained during pre-release experimentation would be sufficient to anticipate potential adverse effects. It was further believed that the benefits would outweigh the risks. But our increasing historical experience with increasing introduction of biological control organisms has shown that while the majority of them did indeed not inflict sustained, environmental damage, but in fact were beneficial or neutral (i.e. didn't work), it was the rare, unexpected events that did (Stiling 1993, Bin & Bruni 1997, Howarth 1991). And while some of these adverse effects may have been outweighed by their benefits in the short run, more and more ecologists are now realizing that in the long run they may not and suggest that precautionary measures are taken (Lockwood 1996, Samways 1988, Nafus 1993). These few, unexpected adverse events arose because of our gaps in knowledge of the ecology of the biological control organism and our lack of understanding and inability to forecast their highly complex interactions with other organisms in the environment. This has lead to the regulatory authorities of several countries in the world, including the US, to apply a precautionary approach and impose containment rules to any to-be-introduced organism, including biocontrol agents (Knutson & Coulson 1997). This is in full understanding that for most of

them it would probably not be necessary. Similarly, transgenic invertebrates are a novelty for the recipient ecosystem as well as for biologists and ecologists alike. No long term experience exists today as to what the unexpected side effects of the application of this new technology to invertebrates are or about the resulting environmental implications. This is not necessarily only due to insufficient knowledge about the performance, inheritance, ecology and interactions with biotic and abiotic factors of the transgenic invertebrate, in fact, our knowledge about the transgenic organism may even be fairly complete. However, what is lacking is a similarly complete understanding of the recipient ecosystems and their organisms, with which the transgenic invertebrates will interact, that makes the outcome unpredictable. It seems therefore reasonable and responsible that such a great deal of uncertainty in face of potential sustained harm should trigger the application of precautionary measures until sufficient experience and knowledge has been accumulated and a generally accepted level of confidence has been achieved. This will likely be the case in the EU where the precautionary principle has been inscribed into the new Directive 18/2001 regulating the release of transgenic organism into the environment.

For these reasons, we recommended that at such an early stage of the development of a new technology, the first planned field releases should be carried out under containment with transgenic invertebrates with limited mobility (immature life stages or immobile species) and that live above ground. The first releases should be of short duration and the number of released organisms should be kept so small that they still can be completely recaptured and counted. The data collection on the effectiveness of the applied containment strategies should be a scientific priority objective of the study. Further, post-release monitoring programs should be developed and established that allow the detection of unnoticed malfunctioning of the applied containment strategy, i.e. detect escaped transgenic invertebrates. This information will be essential for the continuing improvement of containment strategies.

As an example of the complex problems and the potential risks regarding the application of transgenic invertebrates, some of the problems that are raised by scientists regarding the use of transgenic mosquitoes designed to control malaria are summarized in the following. One goal in the field of research concerned with medical parasitology is to equip mosquitoes with a defense mechanism using gene technology that enables the mosquitoes to destroy the malaria pathogens in their own body. Through such a defense mechanism the vector competence of the mosquitoes would be reduced. Released transgenic mosquitoes are expected to displace natural mosquito populations and with their reduced vector competence, they may disrupt the transmission of the malaria pathogens. However, information on biology and ecology of the pathogen and the vector, the mosquito, is incomplete. For example, there are few reliable estimates of the prevalence of malaria vector competence in mosquitoes (Spielman 1994). Therefore, the assumption that a reduced vector competence will automatically reduce malaria disease needs to be verified before relying on this strategy (Snow & Marsh 1995, Spielman et al. 2002).

In order to ensure that an anti-malaria transgene spreads rapidly through a mosquito population, the transgene will be coupled to a transposon (James 2000). To date, little is known about the stability of transgene-transposon-constructs in a genome (Spielman et al. 2002). However, the stability of the transgene-transposon-construct will be important in determining the pace at which a natural mosquito population will be replaced by a transgenic strain and how long a transgenic mosquito strain will be vector incompetent.

So far, only a one-pathogen-strategy has been considered for the control of malaria diseases using transgenic mosquitoes that are vector incompetent against one malaria pathogen. However, a mosquito species may act as a vector for several different malaria pathogens and usually several mosquito species are present in one area (Spielman et al. 2002). Therefore, an effective control of malaria would require the release of several transgenic mosquito species incompetent to several malaria pathogens in one area, or of one transgenic mosquito species that is simultaneously incompetent to multiple malaria pathogens.

The use of transgenic mosquitoes for human health purposes raises also a number of novel ethical issues that have to be resolved (Spielman et al. 2002). First of all, the effectiveness of transgenic mosquitoes could only be tested with release trials. Theoretically, the people present at a release site would all have to give their consent to be part of the experiment. To terminate such release trials all transgenic mosquitoes should be recaptured. However, this is probably a rather impossible task and no working strategies for a recapture have been presented so far. Therefore, it will be difficult to determine the size of the group of people that need to give their consent.

Even if a transgenic mosquito strain may be able to reduce malaria disease in an initial phase after release, the unexpected, sudden loss of the vector incompetence in a mosquito population, e.g. due to the instability of the transgene in subsequent generations, might lead to a new spread and increase of the disease (Spielman et al. 2002). People living in an area with malaria disease that have experienced a reduced selection pressure during the time of the effective use of the transgenic mosquitoes may have a reduced immune defense and, therefore, be more susceptible after new exposure to the pathogen. During a period of absence or low prevalence of malaria in an area, people may change their behaviour (e.g. do not use bed nets anymore, do not take anti-malaria drugs, etc.) because they may acquire a feeling of safety. Without continuous education and understanding by the people, which requires their consent, many people may not be prepared well enough anymore and a potentially recurring prevalence of the disease might hit them harder than before (Spielman et al. 2002). We propose that this risk scenario needs to be thoroughly assessed and, if found to have merit, effective communication methods, continuous educational programs and reliable efficacy monitoring systems must be in place prior to a field release trial.

The complex situation in the case of transgenic mosquitoes shows that the use of transgenic invertebrates can be associated with complex problems and may impose potential short- and long term implications. There are still great gaps of knowledge and uncertainties regarding the ecological and, in case of mosquitoes for malaria control, human health implications of the transgenic organism with its recipient ecosystem. Therefore, careful risk assessment prior to any release, reliable containment strategies, effective governmental regulations and continuous oversight of the transgenic invertebrates are mandatory for a responsible and sustainable application of gene technology to invertebrates.

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REGULATION OF GENETICALLY MODIFIED ORGANISMS IN NEW ZEALAND

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Abstract

In New Zealand the Hazardous Substances and New Organisms Act (1996) (the "HSNO" Act) was created to "… protect the environment, and the health and safety of people and communities, by preventing or managing the adverse effects of hazardous substances and new organisms." (from Section 4 of the HSNO Act). Genetically modified organisms (GMOs) are defined as new organisms in this Act. The Environmental Risk Management Authority (ERMA New Zealand) was established to implement the Act and regulate the importation, manufacture, development, field-testing, or release of hazardous substances and new organisms. (An exception is the genetic modification of humans, which are covered by other legislation). The Minister for the Environment has responsibility over the HSNO Act. All proposals (regardless of the type of genetic modification or potential risk) to develop, import, field test, or release GMOs in New Zealand require applications to be made and approvals given. In addition, consideration must be given to potential adverse effects on the traditions of Māori (the indigenous people of New Zealand) or their relationship to native or valued species. These features make the New Zealand approach to regulation of GMOs probably the most comprehensive in the world. Approvals are given on an organism basis rather than a project basis, so in the case of GMOs all types of GMOs developed in a project need to be described and approvals given to them. This paper gives an overview of the risk management framework implemented by ERMA New Zealand, as well as noting some of the outcomes of a recent general inquiry on the issue of genetic modification in New Zealand.

THE ROLE OF ERMA NEW ZEALAND

ERMA New Zealand has three components:

- The decision-making Environmental Risk Management Authority ("the Authority"), which consists of eight members appointed by the Minister for the Environment. Three or more members make up decision-making committees for individual applications. Members of the Authority have a range of backgrounds and skills and are not an expert scientific panel.

- ERMA New Zealand staff provide administrative and technical support for the Authority. ERMA New Zealand staff prepare "Evaluation and Review" reports on applications to assist the Authority in it's decision making process, but staff do not provide recommendations for the approval or declining of applications. These reports evaluate the information provided by applicants, particularly in regard to the identification and assessment of potential adverse effects and benefits associated with the proposal. In some cases people from outside of ERMA New Zealand with particular expertise are contracted to contribute to specific evaluation and review reports or to provide additional information or advice to the Authority. ERMA New Zealand staff can also provide guidance to applicants on preparing applications.

- Ngā Kaihautū Tikanga Taiao is the Māori advisory committee set up by the Authority to provide it with advice on issues relevant to Māori, such as the involvement of native flora and fauna, and potential adverse cultural effects of proposed applications.

There are several functions that the HSNO Act requires the ERMA to undertake, of which making decisions on applications is one of the more important. For developments of GMOs that meet defined "low risk" criteria, the decision-making can be delegated to research institutions. ERMA New Zealand must process applications within statuary time frames, although these vary depending upon the type of application. It is government policy that ERMA New Zealand recovers a large proportion of the costs of the application process. Consequently, in the case of large or complicated applications there may be significant time and financial costs associated with making an application.

Applications that involve field-testing or release of new organisms, as well as any other applications that the Authority may consider to be of significant public interest, are required to be publicly notified. In these cases anyone can make a public submission on the application, noting whether they approve or oppose it, and can request a public hearing. The decision-making committee considers all the evidence presented and some time later issues its written decision.

A field test is generally conducted in the environment in which the organism would normally be released, but is contained such that all heritable material can be recovered. Field tests are to evaluate the performance of the organism(s) before general release. So far there have been no applications to release a GMO in New Zealand, although several approvals have been given to release other organisms (primarily biological control agents). While applications to field test GMOs have attracted considerable public interest (in some case resulting in hundreds of submissions opposed to the field test), applications involving non-genetically modified organisms have resulted in few public submissions (10 or fewer, and equally spilt in support or opposition).

RISK MANAGEMENT FRAMEWORK

The HSNO Act deals with hazards (a source of potential harm), risks (the magnitude of an adverse effect and the probability of its occurrence), and effects (areas of impact). The core of the HSNO process relies on the identification, assessment, and management of risks. For the assessment of applications the HSNO Act requires five categories of effects to be considered:

(1) Environmental
(2) Human health
(3) Cultural
(4) Social and community
(5) Economic

There are four key steps in the risk management framework: identification, analysis, evaluation, and management of risks (see Figure 1). A new organism is the hazard and it has one or more associated risks. These risks must be identified and assessed to determine risk management options. The decision-maker has the primary responsibility for the overall evaluation of potential risks and the imposition of controls to manage the risks if the application is approved.

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Figure 1. The risk management framework from the Australian and New Zealand risk management standard (AS/NZS 4360:1999 Risk Management Standard. Standards Australia). Note that risk assessment involves both the analysis and evaluation of risks.
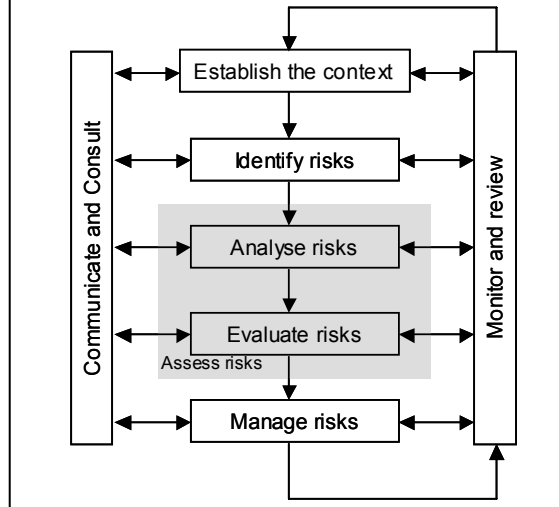
Risk identification
Risk identification needs to be undertaken in a systematic and robust way. Identifying risks requires identifying the sources of risk (such as an escaped animal and/or pollen), the areas of impact (such as adverse impact on the environment or public health), and the exposure pathways (such as wind, water, people, and/or animals). Identifying the source of risk requires addressing the hazard and examining the incidents that may release the intrinsic potential of the hazard (e.g. a broken fence or unintended flowering). ERMA New Zealand has produced a technical guide on identifying risks to help applicants in risk identification.

In the ERMA New Zealand application process risks are identified, assessed and analysed to varying degrees by the applicant, submitters (if the application is publicly notified), ERMA New Zealand staff, and the decision-making committee of the Authority. The HSNO Act requires that the applicant has primary responsibility for identifying, assessing and analysing risks. In practice, however, we have found that applicants for GMO approvals do not comprehensively identify or assess risks. Submitters may identify other risks or assess the risks differently from the applicant. Staff at ERMA New Zealand, and any external experts contracted for a specific application, evaluate the information provided by the applicant and submitters and provide advice to the Authority members who consider that application.

Risk assessment
Applicants are expected to consider both the **likelihood** and **magnitude** of an effect occurring (risk analysis), and from these to determine what are the risk management priorities (risk evaluation). Analysis and evaluation of risks are based upon professional judgement and information derived from other sources (such as previous studies). In most situations, even when the available information is relevant and accurate, there will be some residual uncertainty attached to the assessment. In its reports ERMA NZ attempts to identify the causes and degree of these uncertainties. Cultural and social risks can be particularly difficult to evaluate.

In relation to risks associated with new organisms, qualitative descriptions of risk are generally used because there is insufficient information available for quantitative assessments. We use a set of qualitative scales for characterizing risks (Tables 1, 2 and 3). The magnitude or consequences of an effect are generally given more weight that the likelihood of that event occurring. For example, while the likelihood of transfer to another species of an introduced gene could be very low, if that transfer would result in an obvious significant adverse effect on the environment then the Authority would take a more cautious approach. This could be achieved either by additional controls or by declining the application if the Authority were not satisfied that controls would be effective in managing the risk(s).

Risk management

The management of risks will be influenced by the approach to risk. For example, whether only insignificant risks are acceptable, or whether higher levels of risks may be tolerated (see Table 3). The HSNO Act currently does not provide the ability to impose controls or conditions upon releases of new organisms, but this is currently being reconsidered by the government. There are, however, minimum standards set by the HSNO Act that must be met for an approval of a release to be given. These minimum standards mean that an application will be declined if the new organism is likely to cause significant displacement or adverse effects on native biota, natural habitats, human health and safety. It is the Authority's task to decide what is significant.

Table 1. Qualitative scales for likelihood of effect used by ERMA	
Descriptor	Description
Very Unlikely	Not impossible, but only occurring in exceptional circumstances.
Unlikely	Could occur, but is not expected to occur under normal conditions.
Possible	Equally likely or unlikely
Likely	Will probably occur at some time.
Very Likely	Is expected to occur.
Table 2. Qualitative scales for magnitude of adverse effects used by ERMA	
Descriptor	Description
Minimal	Insignificant (repairable or reversible) environmental impact.
Minor	Reversible environmental impact, limited adverse cultural effects.
Moderate	Slight effect on native species, adverse cultural effects to wider area.
Major	Irreversible environmental effects but no species loss, remedial action available.
Massive	Extensive irreversible effects, adverse widespread cultural & health effects.

Table 3. Calculating the level of risk. ERMA risk levels.					
Likelihood	Magnitude				
	Minimal	*Minor*	*Moderate*	*Major*	*Massive*
Very Unlikely	Insignificant	Insignificant	Low	Medium	Medium
Unlikely	Insignificant	Low	Low	Medium	High
Possible	Low	Low	Medium	Medium	High
Likely	Low	Low	Medium	High	High
Very Likely	Medium	Medium	High	High	High

Under the HSNO Act it is the Authority that determines whether and how the risks can be managed. The Authority must consider controls or risk management options and determine whether the remaining residual risk (i.e., the risk remaining after inclusion of management options) is negligible or not. For non-release applications the Authority determines whether

appropriate controls and conditions can be imposed that will manage any risks to a negligible level. Such controls include having a registered facility of an appropriate level of containment for the new organism, use of special equipment or procedures, a comprehensive monitoring regime, and having contingency plans in place to minimise the risk of escape of the organisms or any viable genetic material (such as gametes or spores). To date the Authority has declined all or part of two applications where there were uncertainties over the likelihood and consequences of adverse effects.

ERMA New Zealand does not have sole enforcement responsibility for controls imposed on decisions. Another government agency, the Ministry of Agriculture and Forestry has the responsibility for checking that the controls are adhered to.

THE ERMA PROCESS SO FAR

The new organism's part of the HSNO Act has been operational now for nearly four years. In that time there have been 13 approvals to field test GMOs (mostly plants) and four approvals to release non-GMOs. One application to release a new organism has been declined. Our experience has shown that a formal risk assessment approach is unfamiliar to many biologists, and the applications that ERMA New Zealand has received so far for field-testing or release of new organisms have not covered all of the major issues in sufficient depth. These deficiencies have been addressed during the evaluation of the application and at the hearing for the application. It is important for applicants to clearly and simply explain the purpose, scope, and nature of the project, and to take a step back from the project and comprehensively identify risks. A key component of risk assessment is the justification of conclusions, and this applies to all contributors in the process; applicants, submitters, those providing input to the ERMA New Zealand evaluation and review report, and the Authority itself. In particular the assertion that there are no risks needs to be substantiated just as rigorously, if not more so, as risks that are identified.

Where the applicant has not undertaken comprehensive identification and analyses of risks, additional information is usually requested during the evaluation and consideration process. This increases the processing time and expense of the application. As familiarity with the process develops such requests should become less common. ERMA New Zealand often provides pre-application advice on what level of detail is necessary for specific applications. With respect to containment and field test applications for new organisms the Authority to date has determined that in nearly every case risks can be managed to low levels by the imposition of controls, and so residual risks were deemed negligible. In most applications for release of new organisms the Authority has also concluded that risks are negligible. If risks are non-negligible then the proposed benefits of the new organism are also taken into account.

ERMA New Zealand considers that it is desirable to be able to impose controls on releases in some situations for example, to monitor the spread of the new species and impacts on other species. Such information could be useful in the case of failed introductions to understand the reasons for the failure, which may ensure success the next time. In addition, the ability to enforce such monitoring may also allow early warning of unexpected effects and the possibility of implementing additional control measures.

One of ERMA New Zealand's roles is to assist with informing the public about issues to do with the HSNO Act. ERMA New Zealand has a "generic issues" program to produce general reviews of some key issues to help inform everyone involved in the process. So far only one

such project has been completed (a report on the use of antibiotic resistance marker genes). Funding for analysis of additional topics is being sought. In its evaluation and review reports ERMA New Zealand also provides detailed analyses of the key issues. In addition, ERMA New Zealand has also held some well-attended public seminars and debates on key issues such as the precautionary approach, and horizontal gene transfer.

Public hearings for applications
Public response to applications for releases of non-genetically modified new organisms (mainly biological control agents) has been very low, despite the potential long term environmental impacts such releases may have. Only a few individuals and organizations have made public submissions on these applications, often with similar numbers supporting or opposing the application. In contrast, very large numbers (in some cases hundreds) of people have made submissions in response to field trials of GMOs, with the vast majority of submissions being opposed. This reflects public concern over genetic engineering. Common themes of submitters opposed to field trials of GMOs are the risks of horizontal gene transfer (especially, but not exclusively, in relation to antibiotic resistance genes), the development of new viruses or diseases, other unknown effects of such modifications, adverse environmental and/or economic effects for organic production systems or tourism, and an abhorrence of "playing with nature". Opposition to field trials can often focus on issues that are outside the scope of the application (such as future commercialisation of the GMO), or are of a fundamental opposition to GMOs irrespective of whether it is a field trial. Many submitters focus on the need to prevent adverse effects and appear unwilling to accept that management of the risks is achievable or desirable. In other words, it is their view that the only risk management option is to decline the application. For release of biological control agents the key issues have been the potential for non-target effects of the new organism, and also the environmental consequences if the control agent is successful.

Public hearings provide the opportunity for all sides to provide the Authority with more detail on the issues and to become better informed of alternative views. From our observations most submitters opposed to an application involving GMOs are not persuaded by responses to their concerns. This can reflect differences in approach to risk, as well as a distrust of or unfamiliarity with the science or the regulatory process. Whether the views of submitters to ERMA applications reflect the wider public view of genetic modifications is unknown.

A central and unique requirement of the HSNO Act is to take account of Māori concerns. Concerns from Māori have ranged from the use of genetic material from native species to beliefs that it is spiritually offensive to introduce human genes into livestock. The latter has been a particularly difficult issue, but applications have not yet been declined on this point. Some applications may require consultation with Māori or other sectors of the community. In these cases early in the process the applicant should inform the relevant groups what they intend to do and why, and be willing to discuss possible community concerns and how these can be reduced. Agreement does not necessarily have to be achieved, but areas of concern need to be clearly identified and there must be both a commitment and a possibility of taking action as a result of the responses to consultation. If the community requests that special conditions be imposed for valid reasons then, as far as is practicable, those requests are expected to be complied with. The Authority considers the consultation process and its outcomes very carefully before making decisions.

THE ROYAL COMMISSION OF INQUIRY ON GENETIC MODIFICATION

During 2000 and 2001 a Royal Commission of Inquiry on Genetic Modification was established in New Zealand. This Commission heard submissions from a wide range of people and organizations, and many contributors from other countries presented evidence or points of view to the Commission. The Commission reported in 2001 and made 49 recommendations covering the following areas: research, field uses, food, medicine, intellectual property, the Treaty of Waitangi, liability, the HSNO Act, and biotechnology strategy. The full report is available at http://www.gmcommission.govt.nz/RCGM/index.html.

The overall conclusion of the Commission was to "preserve opportunities" with respect to all forms of agriculture, and proceed with caution with respect to genetic modification. The Commission considered that the regulatory framework in New Zealand was generally sound, but recommended some changes to the system.

The Government has responded to the Commission's recommendations by adopting, at least in principle, many of the recommendations. In addition it has adopted a more cautious approach by imposing a two year "period of constraint" during which time release of genetically modified organisms is not permitted, unless for special circumstances. It is hoped that during this time research will be started or continued to address some of the concerns about genetic modification. However, some of the concerns about genetic modification require considerable research so that it is unlikely that significant results will be available in the short term.

The Government is also currently legislating what conditions should be imposed on field tests, rather than leaving it to the Authority's discretion. However, this is unlikely to result in significant differences in the decision-making. The Government has also undertaken to establish a Bioethics Council to "advise, provide guidelines and promote dialogue on the cultural, ethical and spiritual issues associated with biotechnology". This Council will be separate from ERMA and not involved in decision-making for specific applications.

The Commission was also useful for focusing national attention on genetic modification issues and encouraging debate. There is, however, still a great deal of mistrust by some groups towards genetic scientists and a reluctance to accept information that may address their concerns. On the other hand, researchers still need to pay more attention to listening to these concerns, understanding what the real issues are, and consulting with potentially affected groups early in their research program.

CONCLUSIONS

The HSNO Act is a far-reaching and comprehensive piece of legislation. It is taking time for all parties to come to terms with its requirements and implications. ERMA New Zealand has endeavoured to provide the science community with information and processes to enable the law to be effectively and efficiently implemented.

Release of a GMO into the environment will necessitate a larger amount of information on potential adverse effects than for contained applications, and in particular it must be relevant to New Zealand environments. This information could be obtained by initial laboratory and perhaps field test research, but will mean that obtaining approval to release a GMO is likely to be a long process.

In relation to the release of genetically modified insects in New Zealand, the approval process would require considerable evidence that the organisms would not result in significant adverse effects. Testing for non-target effects on relevant New Zealand flora and fauna would be a prerequisite. The application would also need to show significant benefits in relation to the environment, human health, and/or the economy. Widespread consultation with communities where the organism would be released or is expected to spread to would also be required. Consultation involves both informing and discussing, and is a two-way process. It requires every reasonable effort on the part of the party consulting (the applicant) and a willingness to respond reasonably on the part of those consulted.

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EVALUATING POTENTIAL RISKS OF TRANSGENIC ARTHROPODS FOR PEST MANAGEMENT PROGRAMMES

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Abstract

Genetic modification using recombinant DNA methods can now be used, almost routinely, to transform pest and beneficial arthropods and such genetically engineered insects and mites could be used to improve pest management programs. Genetic manipulation with recombinant DNA techniques may generate concerns about risk, requiring additional time and resources to resolve. Risk assessments must be conducted prior to releasing transgenic arthropods into the environment for either short term experiments or permanent establishment. Potential risk issues to be resolved include whether: the inserted gene(s) (trait) is stable; the traits can be horizontally transferred to other populations or species; released arthropods will perform as expected (especially with regard to their geographic distribution, host or prey specificity; released arthropods will have unintended environmental effects; and, in the case of short term releases, the released arthropods can be recovered from field sites. If the transgenic arthropods strain(s) perform well in preliminary, short term releases and risk assessments are completed satisfactorily, permanent releases into the environment may follow. Many pest management programs, especially those involving replacement of pest populations by the transgenic population, will require permanent establishment in the environment and the use of 'drive mechanisms', have been proposed to achieve this. Because efficacy can be severely compromised by 'transgene silencing', plant molecular biologists are now attempting to stabilize gene expression by building in 'insulators'. Transgene silencing occurs in Drosophila and will no doubt be a factor in other transgenic arthropods.

INTRODUCTION

Recent successes in transforming a variety of arthropods with transposable element (TE) and viral vectors indicates we now have several relatively rapid systems for introducing genes into pest and beneficial arthropods (for recent reviews see Ashburner et al. 1998, Handler and James 2000). Such genetically engineered insects and mites could be used to improve pest management programs. Goals include modifying mosquitoes, and other arthropods that transmit human and animal diseases, so that they are unable to transmit the causal pathogens (Crampton et al. 1990, Eggleston 1991, Crampton 1994, Beatty 2000, Collins et al. 2000, Curtis 2001, James 2001). Transgenic methods could improve genetic control programs, in which males are mass reared, sterilized by irradiation, and released to mate with wild females (Heinrich and Scott 2000, Robinson and Franz 2000). Producing sterile males or producing only females by transgenic methods could improve the efficiency and effectiveness of such programs. Other goals include producing honey bees and silk moths that are disease resistant, or have other desirable traits, through transgenic methods (Gopinathan 1992, Robinson et al. 2000). Natural enemies used in biological control programs could be modified to enhance their effectiveness in several ways (Beckendorf and Hoy 1985, Hoy 1990, Heilmann et al. 1994).

A number of steps are involved in any program designed to modify pest or beneficial arthropods using transgenic methods. After a transgenic strain has been developed, it must be evaluated in the laboratory for fitness and stability. Eventually the transgenic strain may be

released into small plots in the field for evaluation. Permission to release a transgenic arthropod will have to be obtained from the appropriate regulatory agencies in the United States of America. Short term releases initially will be made into small plots, but if the strain(s) perform well and risk assessments are completed satisfactorily, permanent releases into the environment may be allowed. After permanent releases, evaluation of efficacy will be needed.

Initial releases of transgenic arthropods into the environment are intended to be short term experiments and current regulation of such releases in the United States of America by the US Department of Agriculture require the researcher to retrieve all transgenic arthropods from the environment at the end of the experiment (USDA-APHIS 2001). Many pest management programs will require that the transgenic arthropods become permanently established in the environment if they are to perform effectively. At present, there are no guidelines as to what types of evaluation will take place before transgenic arthropods can be established permanently (Hoy 2000).

POTENTIAL RISKS AND TRANSFORMATION METHODS

Transposable element vectors
Initial efforts to engineer *Drosophila melanogaster* were rarely successful until Spradling and Rubin discovered that the P transposable element (TE) could be genetically manipulated to carry exogenous genes into the chromosomes of germline cells (Rubin and Spradling 1982, Spradling and Rubin 1982). This pioneering work elicited immense amounts of research aimed at using the same, or similar, methods for other arthropods (Handler and James 2000). From a risk assessment viewpoint, there are advantages and disadvantages to using a TE vector. Typically, only a portion of the *P*-element vector inserts into the chromosome, primarily of the inverted repeat sequences and the transgene(s). The plasmid DNA outside the inverted repeats should not insert and should be lost subsequently. Once transformed fly lines are obtained, the lines typically are stable unless transposase is provided by endogenous or exogenous sources.

Elements such as *mariner, piggyBac*, *Hermes,* and *hobo* have been isolated and genetically modified for use as vectors for transforming arthropods (Robertson 1993, Atkinson et al. 1993). However, the use of these TE vectors could have some disadvantages if the transgenic arthropods are released into the environment (Table 1). First, the inserted gene should be stable within the transformed arthropod, which means that the TE vector should not be active. Second, the TE vector should be incapable of moving horizontally to another species (Hoy 1992a, b, 2000). Because TEs are found in diverse organisms, including bacteria, yeast, plants, nematodes, most arthropods, mice and humans (Berg and Howe 1989), it is unclear if TE vectors used in arthropods could move horizontally to other organisms. Ideally, a TE vector would have a very narrow host range—as, apparently, *P* has.

The *piggyBac* element can transpose in a *Spodoptera frugiperda* cell line (Fraser et al. 1995, Elick et al. 1996) and in embryos of *D. melanogaster*, *Aedes aegypti*, and *Trichoplusia ni* (Lobo et al. 1999). It has been used to transform *Ceratitis capitata* (Handler et al. 1998), *Pectinophora gossypiella* (Peloquin et al. 2000), and *Bombyx mori* (Tamura et al. 2000), suggesting it has a broad host range, which raises possibilities that the transgene could be unstable or move horizontally if the vector were activated by endogenous transposases or if the transgenic population mated with a wild population that contained active elements.

122

Table 1. Issues or concerns potentially needing evaluation prior to permanent release of transgenic arthropods into the environment	
Transposable element vectors	
Transposable elements with a broad host range could make horizontal movement to diverse species in some cases including humans	*piggyBac, Hermes, Minos, mariner,* others
Cross mobilization of transposable element vectors is feasible with endogenous transposases and could lead to unstable lines, especially after field releases	*Hobo, Hermes,* others
Lack of precise integration could result in the insertion of antibiotic or other resistance genes into the genome that would need to be removed prior to release into the environment	*Minos*
Lack of stability due to heat shocks could stimulate endogenous transposase activity	*hobo*
"Drive elements"	
Potential resistance over time could result in loss of efficacy of the 'driver' element	*mariner,* other elements
Horizontal transfer to other species feasible through unknown mechanisms	transposable elements
Potential resistance over time to *Wolbachia* could result in loss of efficacy of the 'driver'	*Wolbachia*
Gene silencing	
Gene silencing could eliminate the transgene function if appropriate insulator elements are not used to preserve transgene function	observed in *Drosophila* and plants
Transgenic arthropod symbionts	
Transgenes from transgenic gut symbionts could move horizontally to other microorganisms associated with arthropods	

Hermes was discovered in *Musca domestica* (O'Brochta et al. 1996) and is functional in four dipteran families (Drosophilidae, Calliphoridae, Tephritidae, and Muscidae) (Atkinson and O'Brochta 2000). Interestingly, two *M. domestica* strains tested exhibited low rates of transformation (Sarkar et al. 1997a), perhaps because *Hermes* is endemic in housefly populations and some form of resistance has been selected for. Resistance to TEs could reduce the effectiveness of a program in which arthropods containing active TEs were released to 'drive' genes into wild populations. *Stomoxys calcitrans* (Muscidae) (O'Brochta et al. 2000) and *Aedes aegypti* (Jasinskiene et al. 1998, 2000) have been transformed with *Hermes*. However, integrations of *Hermes* into *A. aegypti* did not occur precisely, suggesting that the *Hermes* vector could have integrated into the genome by general recombination or through a partial replicative transposition (Jasinskiene et al. 2000). Jasinskiene et al. (2000) concluded this insertion mechanism by *Hermes* in *A. aegypti* "precludes its immediate use in experiments that involve field release of transformed animals into the field."

Minos can transpose in cells and embryos of Drosophila, *Bombyx mori*, and *Anopheles stephensi* (Catteruccia et al. 2000a, Klinakis et al. 2000a, Shimizu et al. 2000) and also induces stable germline transformation (Catteruccia et al. 2000b). Surprisingly, Klinakis et al. (2000b) found *Minos* could transform human cell lines, making it a useful tool for mutagenesis and functional analysis of the human genome. Thus, *Minos* potentially has a very wide host range, which would elicit concerns if *Minos* is used to transform arthropods destined for release into the field in pest management programs or if active elements were released as "drive mechanisms".

mariner initially was isolated from *Drosophila mauritiana*, but is extremely widespread among arthropods (Robertson 1995). *mariner* and other *Tc1* elements have been highly active in trans-order horizontal transfers through unknown mechanisms (Robertson 1995, Robertson

and Lampe 1995). The host range of *mariner* is amazingly broad; it has been found in nematodes *Heterorhabditis bacteriophora* (Grenier et al. 1999) and Meloidogyne (Leroy et al. 2000)], flatworms (*Dugesia tigrina, Stylochus zebra, Bdelloura candida*) (Garcia-Fernandez et al. 1995), hydras (*Hydra littoralis* and *H. vulgaris*) (Robertson 1997 and mammals, including humans, mouse, rat, Chinese hamster, sheep and cow (Auge-Gouillou et al. 1995, Oosumi et al. 1995, Robertson and Martos 1997).

A *mariner* vector was used to transform the chicken (Sherman et al. 1998), the zebrafish *Danio rerio* (Fadool et al. 1998), and the protozoan *Leishmania major*, which indicates that *mariner* has a general ability to "parasitize the eukaryotic genome" (Gueiros-Filho and Beverley 1997). At least two different subfamilies of *mariner* have been isolated from the human genome and Oosumi et al. (1995) suggested that *mariner*s could be used as transformation vectors of humans. A *mariner* from fish was genetically engineered to make it 25-fold more active in human cells (Plasterk et al. 1999). Thus, *mariner* elements potentially could enter and damage the human genome. So far, all *mariner*s discovered in humans are "molecular fossils" (Robertson and Martos 1997), but Robertson and Zumpano (1997) estimated there are thousands of copies of several types of *mariner* fragments, suggesting that *mariner* had "a considerable mutagenic effect on past primate genomes". *mariner* is not alone in invading the human genome; at least 14 families of TEs have been found (Smit and Riggs 1996), including pogo (originally discovered in Drosophila) and Tigger (Robertson 1996).

The *hobo* vector can function in several drosophilids, in *Trichoplusia ni* and *Helicoverpa zea* cells (DeVault et al. 1996), as well as in several tephritids (Handler and Gomez 1996). The stability of *hobo* vectors is open to question because excision from Drosophila could be stimulated by heat shocks that presumably elicited the production of an endogenous *hobo*-like transposase (Atkinson et al. 1993). The excision rate was 8 to 10-fold higher than that seen for the normal host or other Diptera (Atkinson et al. 1993).

CROSS MOBILIZATION AND CONVERSION OF TE VECTORS

Laboratory assays were conducted to compare the ability of *Minos*, *piggyBac*, *mariner* and *Hermes* vectors to 'cross mobilize' each other (Sundararajan et al. 1999). The *hobo* transposase functioned equally well with *Hermes* substrates, but the *Hermes* transposase was able to excise *hobo* only rarely. Cross mobilization is not limited to *hobo* (Sundararajan et al. 1999). Petrov et al. (1995) found that TEs of several types can be mobilized in dysgenic crosses between strains of *D. virilis*. Four structurally diverse elements (Ulysses, Penelope, Paris and Helena) were cross mobilized. These elements are related to the major types of TEs in higher organisms. The simultaneous mobilization was not due to the complementation of some shared defect in the transposition pathways because the mechanisms employed are different and the arthropods examined all showed evidence that the transposition occurred in the manner appropriate to their respective mechanism. Petrov et al. (1995) suggested that the four TEs were mobilized by a sort of "genomic stress", which could have been due to the breakage of double-stranded DNA. Thus, double-stranded breaks from the mobilization of a single TE might induce a cellular response that releases other TEs from repression, mobilizing multiple unrelated elements (Petrov et al. 1995). Disabled TE vectors can be 'converted' to activity. Peronnet et al. (2000) showed that an inactive P element could be converted into an active one through a three-step process. Conversion could make a transgene unstable within the transgenic arthropod's genome and could, in theory, pose a potential risk for horizontal gene transfer.

Conversion and cross mobilization are relatively unstudied phenomena. However, until we know more about them and their frequency, these data suggest that the safest course might be to remove any inserted TE sequences from a transgenic arthropod strain prior to its permanent release into the environment to reduce the likelihood the transgene will move, either within the strain or horizontally between species. Whether horizontal gene transfer would cause harm is open to debate and would certainly depend on the gene in question. Naturally occurring horizontal gene transmission between species has provided some of the variability upon which evolution has acted (Plasterk 1993).

VIRAL VECTORS

A variety of viral vectors have been developed for transforming arthropods and they offer potential risks if transgenic arthropods containing them were released into the environment. So far, no one has requested permission to release arthropods containing these viruses into the environment. However, if such release applications were made, virologists, ecologists, and others who understand the biology, ecology, and genetics of these viruses should participate in evaluating the potential risks such vectors could offer should they, or arthropods containing them, be released into the environment. Risks might include the horizontal movement of the viruses, carrying transgenes, to new hosts. Another concern that needs to be resolved is whether the viruses could recombine with other viruses within their hosts, resulting in new recombinant viruses with unexpected host ranges. Some of these viruses are being used to transform mammalian cells and, thus, their potential host range raises concerns.

Nuclear polyhedrosis viruses (NPV), or baculoviruses, have double-stranded, circular DNA genomes contained within a rod-shaped protein coat. Baculoviruses infect a number of pest insects and several have been used as biological pesticides (Shuler et al. 1994). They also have been exploited as vectors to carry exogenous DNA into insect cells (Miller 1988, Iatrou and Meidinger 1990, Yu et al. 1992). The host range of baculovirus vectors includes human liver cells and baculoviruses have been proposed as vectors for gene therapy in humans (Hofmann et al. 1995). Recombinant baculoviruses recently were found to integrate into Chinese hamster ovary chromosomes in cell cultures, suggesting they could be used to transform mammals in a stable manner (Merrihew et al 2001).

Densonucleosis viruses (Parvoviridae) are linear single-stranded DNA molecules that apparently are restricted to arthropods. Densovirus vectors are being used for gene delivery for laboratory studies and have been suggested for biological control programs (Beaty and Carlson 1997).

Pantropic retroviral vectors have been developed by genetically modifying the Moloney murine leukemia virus so that it contains the G envelope protein from the vesicular stomatitis virus (Burnes 2000). These retroviral vectors have a very wide host range (are pantropic), but are considered to be stable once inserted into the host genome because they lack the genetic information needed to propagate themselves. These vectors are used for human gene therapy and have transformed fish, cows, clams and amoebae, as well as lepidopteran and dipteran cells (Burns 2000). So far, they have only been used to study promoter function and regulation in insect cells because stable transformation of the arthropod germ lines has not been achieved (Burns 2000).

Polydnaviral vectors are derived from multisegmented DNA viruses found in the female reproductive tracts of some hymenopteran parasitoids. Female parasitoids inject the

polydnaviruses when they deposit eggs in a lepidopteran host, and the viruses disrupt the host immune system, making the host more suitable for the developing parasitoid. Polydnavirus DNA can persist within the chromosome of a gypsy moth cell line (McKelvey et al. 1996), and insect cell lines from other lepidopteran and coleopteran species have been transformed by this polydnavirus (Gundersen-Rindal et al. 1999). Thus, polydnaviruses potentially could be used to insert foreign DNA into a variety of arthropods. Whether these viruses could subsequently move horizontally to other species is unknown. Also unknown is whether these viruses could recombine with other viruses in their hosts.

Sindbis virus vectors have been derived from aphaviruses with a single-stranded RNA genome (Beaty and Carlson 1997). These viruses can be grown in mammalian cells and the viruses produced can infect mosquitoes or mosquito cell cultures. Infection is sustained and Sindbis infection was used to express an antisense form of a dengue protein in *Aedes aegypti* adults, making them unable to transmit this human viral disease (Olson et al. 1996, Olson 2000). The Sindbis virus can be fed to mosquitoes, allowing expression of transgenes in the midgut (Olson et al. 2000).

Paratransgenesis
Genetic engineering of gut symbionts provides a novel form of transgenic arthropod that has been termed 'paratransgenesis' (Durvasala et al. 1997). Genetic manipulation of microbial symbionts that can be cultured outside their host is easier to achieve than transformation of the arthropod itself. Successful examples of paratransgenesis include modification of the extracellular bacterial symbiont of the Chagas disease vector *Rhodnius prolixus* (Beard et al. 1992, 1993, 2000). The symbiont is transmitted to progeny by contamination of egg shells or of food with infected feces and genetically modified symbionts can be transmitted to hosts lacking symbionts (Richards 1993, Beard et al. 2000). Symbionts of tsetse flies, vectors of African sleeping sickness, also have been transformed (Richards 1993, Cheng and Aksoy 1999). Consideration has been given to releases of paratransgenic tsetse flies carrying modified symbionts so the released flies could replace or out-compete native populations but fail to transmit the disease.

One risk issue regarding the genetic engineering of arthropod gut symbionts includes the possibility that the inserted gene could move horizontally between the many types of microorganisms found within the guts of arthropods (and other organisms). Horizontal movement of genes between microorganisms is well documented. For example, *Enterobacter cloacae,* a bacterium found in the guts of insects, and *Erwinia herbicola*, a bacterium that grows on the surface of plants, were found in the guts of silk moth larvae and were able to exchange genetic information at very high rates via plasmids (Watanabe and Sato 1998, Watanabe et al. 1998). The bacteria containing the new genetic information were found in the feces of the insects, suggesting that this method of horizontal gene transfer is a frequent event in nature. Transconjugation between bacteria was shown to occur in the gut of a lepidopteran (Armstrong et al. 1990). If gut symbionts of pest arthropods are transformed with antibiotic resistance genes for pest management programs, these genes could move horizontally to other bacteria within the insect, perhaps leading to antibiotic resistance in pathogens. Antibiotic resistance is a serious medical crisis because some human pathogens are now resistant to almost all available antibiotics and efforts are being made to reduce the rate of development of resistance (Williams et al. 1998, Witte 1998).

WHAT GENES ARE POTENTIALLY RISKY?

Potential risks associated with any transgene are probably relatively low if the inserted gene(s) stay put in the target arthropod. However, there could be risk if the transgene moves horizontally to other populations or species. Quantification of such risks would depend on the specific gene and the nature of the invaded species or population. In theory, the species invaded could suffer genetic damage due to the insertion of the transgene if it inserted into essential coding sequences and the population could be eliminated over time, resulting in reduced biodiversity.

The horizontal movement of pesticide resistance genes from a transgenic arthropod to another species is undesirable, as is the movement of antibiotic resistance genes from transgenic symbionts to nontarget microorganisms in the environment. Plant molecular biologists are removing markers as a method of reducing risk (Yoder and Goldsbrough 1994), and this may be desirable in the future for transgenic arthropods intended for permanent release into the environment. Intrinsic risks associated with certain marker genes in arthropods, such as eye color genes and green fluorescent protein genes, seem low. We have no experience with which to evaluate whether there would be harm associated with the horizontal movement of genes associated with refractoriness to transmission of pathogens or many other traits under study. Creative analyses may be required to assess risks prior to releases of transgenic arthropods containing many of the attributes under consideration.

A proposal was made to develop transgenic mosquitoes that express immunogenic proteins in their saliva as a means for using vector insects to deliver vaccines to human diseases (Stowell et al. 1998). Risk assessments of such genes might ask what harm could occur if too little or too much of the protein were delivered to individuals. A project such as this might also involve reviews by agencies charged with protecting human subjects as well as agencies that are responsible for evaluating ecological risks.

Genes theoretically can be isolated from closely or distantly related organisms for insertion into arthropods by recombinant methods. Plant molecular biologists have decided recently that it is likely to be less risky to isolate a gene from the species being manipulated, alter it, and reinsert it into the germ line than to insert truly novel genes (Moffat 2000).

DEPLOYMENT METHODS AND RISKS

One proposed method of controlling a pest population involves swamping a wild population with some kind of genetic load by releasing a few individuals with a trait that will spread and exert a delayed effect on the fitness or disease-transmission capacity of the wild population (Hastings 1994, Thomas et al. 2000). Genetic loads could be induced in wild populations by the introduction of active TEs or meiotic drive mechanisms or *Wolbachia* (Kidwell and Ribeiro 1992, Kiszewski and Spielman 1998). TEs can cause chromosomal mutations, shut off genes, or cause sterility. If a TE were engineered so that it could not be repressed (inactivated) by its host, then it might increase in a population until it caused so much damage that the population crashed.

The ability of *Wolbachia* to act as a 'drive' mechanism to insert genes into a pest population has not yet been demonstrated. We still know relatively little about the many types of *Wolbachia* that occur in arthropods (Jeyaprakash and Hoy 2000). Efforts to use *Wolbachia* to control mosquito populations by the cytoplasmic compatibility it can induce were first considered by Laven (1951), but the incompatibility induced was incomplete, reducing

effectiveness (Pal 1974). Turelli and Hoffman (1991) report that a *Wolbachia* infection spread rapidly in field populations of *D. simulans* in California and Turelli et al. (1992) concluded that *Wolbachia* ".therefore provides a mechanism for introducing cytoplasmic factors into natural populations...[which] may eventually be useful for introducing deleterious factors into pest insect populations." We do not know if genetically-modified *Wolbachia* would have an increased propensity to move horizontally.

We are still discovering new aspects of the biology of TEs and this lack of knowledge makes it difficult to predict precisely what would happen if arthropods were released that contained active or inactive TEs. As noted by Kidwell and Evgen'ev (1999) "the transposability of mobile elements, their potential for rapid, and sometimes massive, amplification in copy number, their ability to change genomic locations, as well as their propensity for horizontal transfer, makes the generalization of results from model organisms far less reliable. Extrapolation of results from one species to another must therefore be made with caution."

The relatively recent horizontal transfer of *P* elements into *D. melanogaster* provides some of the best data for horizontal movement in arthropods in 'recent' time (Houck et al. 1991). Jordan et al. (1999) showed that a long terminal repeat retrotransposon moved from the D. melanogaster group to *D. willistoni*, perhaps within the last 100 to 200 years. How often such movements occur remains unclear because relatively little effort has been made to document them.

Horizontal transfer of DNA could be mediated by insect viruses, but the frequency and impact of such movements is unknown. The *piggyBac* element was discovered embedded within the genome of a baculovirus (Fraser 2000). If horizontal transmission of transgenes by viruses were to occur in the field, there is no guarantee that genes inserted into an insect species using a viral or TE vector would remain within that species.

Horizontal gene transfer might even occur when DNA is eaten. While most consumed DNA is degraded, that is not true for all (Schubbert et al. 1998). For example, bacteriophage DNA fed to mice can persist in fragmented form in the gut, penetrate the intestinal wall and reach the nuclei of leukocytes, spleen and liver cells (Schubbert et al. 1998). Fetal and newborn progeny of mice fed such DNA during pregnancy had the phage DNA in various organs. Furthermore, the foreign DNA was located in the nuclei and associated with the chromosomes, although the DNA had not integrated into them (Schubbert et al. 1998). Such an association of DNA with the chromosomes could affect normal gene function.

Whether horizontal gene transfer will cause harm would certainly depend on the gene(s) transferred and its location. The most serious harm might occur if the TE or viral vector inserted into germ line tissues so it could be transmitted to succeeding generations. However, harm might occur even if the TE only damaged somatic tissues; for example, the movement of *mariner* in the soma reduced the lifespan of Drosophila simulans males (Nikitin and Woodruff 1995). The movement of retroelements into human breast, colon and testicular tissues can induce cancer or Duchene's muscular dystrophy (Capy et al. 1996).

RISK ASSESSMENT PROCEDURES FOR TRANSGENIC ARTHROPODS

For the near future, releases of transgenic arthropods in the USA will be evaluated by regulatory agencies on a case-by-case basis. Initial permits for releases will be for short term releases in controlled situations so that unexpected outcomes might be mitigated (Young et

al. 2000, USDA 2001). Permission for long term and large scale releases may only occur after several years of evaluating small scale releases. Thus, risk assessment of transgenic arthropods, as it has with transgenic crops and microorganisms, will add a significant cost in both time and resources to pest management projects. It took about ten years for the first transgenic crop to become commercially available and could take as long for transgenic arthropods to be released permanently into the environment.

Transgenic arthropods should be contained with appropriate facilities and effective procedures until permits have been obtained from regulatory authorities to allow their release into the environment (Hoy et al. 1997, OECD 1998). Such containment is necessary if all releases of transgenic arthropods are purposeful and have occurred after thorough scientific review. Unfortunately, the US Department of Agriculture provides no specific guidelines regarding containment facilities or procedures for transgenic arthropods. The guidelines and facilities used to contain arthropod natural enemies imported in classical biological control programs could be used to contain transgenic arthropods and these protocols and facilities could be adopted voluntarily by the scientific community to allay concerns by the public about accidental escape of transgenic arthropods.

Short term releases
Risk assessments associated with releasing arthropods that have been manipulated with recombinant DNA techniques will likely include, as a minimum, the questions or principles outlined in Table 2 (Hoy 1990; 1992a, b; 1993; Tiedje et al. 1989, USDA 1991). Concerns can be summarized as questions about: (1) whether the transgene is stable within the transformed population, (2) whether the host or prey range of the transformed population has been altered, and (3) whether the population's potential to persist in the environment (geographic distribution and climatic tolerances) has been altered. (4) Another concern is whether the transgenic population can affect ecosystem functions. For the foreseeable future, releases will be evaluated by regulatory agencies on a case-by-case basis. The question of how far and how quickly the transgenic arthropod strain can disperse from the experimental release site is even more important with transgenic arthropods than it is with transgenic plants. Less is known about dispersal behaviour of many arthropods than might be desirable and it is currently necessary to retrieve transgenic individuals from the release site at the end of the experiments.

Permanent releases
Currently, there are no guidelines in the USA for evaluating the risks associated with permanent releases of transgenic arthropods into the environment (Hoy 2000). How should those guidelines be developed? Risk assessment involves scientific, political and social components, as well as genetic, ecological, conservation and economic issues. It is my opinion that the guidelines for evaluating risks associated with permanent releases of transgenic arthropods should be developed by a diverse group of scientists, regulatory officials, and policy specialists. While such releases are likely to be evaluated on a case-by-case basis, the procedures and process should be based on the best current scientific information. Permanent releases need to be evaluated thoroughly and from diverse viewpoints because it could be very difficult to mitigate harm if the released transgenic arthropod produced unintended consequences. Unlike transgenic plants, which are (except for their pollen) relatively slow dispersers when planted in an agricultural field, arthropods are able to disperse over large distances relatively rapidly and only a very few have been successfully eradicated with great expense.

Questions that might be asked include: Will the transgenic population migrate or be moved accidentally through human trade and commerce to new environments to establish where it is not wanted? Experience suggests that the probability that an organism will become established in a new environment is small (Williamson 1992). However, historical examples of biological invasions of pests or of classical biological control agents demonstrate a lack of predictability and the importance of scale, specificity, and the speed of evolution (Ehler 1990). Williamson (1992) also speculated that the greater the genetic novelty, the greater the possibility of surprising results.

Discussions of risk should include questions about survival, reproduction, and dispersal of transgenic populations and their effects on other species in the community (Table 2). Questions also should be asked about the origin of the inserted DNA, its stability, and its possible effect on other species should the gene be transferred.

Table 2 Some risk issues relevant to short term releases of transgenic insects into the environment
(Modified from Tiedje et al. 1989; USDA 1991; and from a discussion held at a conference on "Risks of Releasing Transgenic Arthropod Natural Enemies", held November 13–16, 1993 in Gainesville, Florida.)
Attributes of the unmodified organism
What is the origin of the transgenic organism (indigenous or nonindigenous) in the accessible environment?
What other ecological relationships does it have?
How easy is it to monitor and control it?
How does it survive during periods of environmental stress?
What is the potential for gene exchange with other populations?
Is the insect involved in basic ecosystem processes?
Attributes of the genetic alteration
What is the intent of the genetic alteration?
What is the nature and function of the genetic alteration?
How well characterized is the genetic modification?
How stable is the genetic alteration?
Phenotype of modified organism compared to unmodified organism
What is the host/prey range?
How fit and effective is the transgenic strain?
What is the expression level of the trait?
Has the alteration changed the organism's susceptibility to control by natural or artificial means?
What are the environmental limits to growth or reproduction (habitat, microhabitat)?
How similar is the transgenic strain being tested to populations previously evaluated in field tests?
Attributes of the accessible environment
Describe the accessible environment, whether there are alternate hosts or prey, wild relatives within dispersal capability of the organisms, and the relationship of the site to the potential geographic range of the transgenic strain.
Are there endangered/threatened species present that could be affected?
Are there agents that could move the transgenic strain present in the release environment?
Do the test conditions provide a realistic simulation to nature?
How effective are the monitoring and mitigation plans?

SOME METHODS TO REDUCE POTENTIAL RISKS?

The ability to introduce cloned genes into the germline at a predictable chromosomal site reduces the likelihood of "position effects" on gene expression and reduces concerns about horizontal gene movement through conversion or cross mobilization. Genes introduced by TE and viral vectors insert more or less randomly into chromosomes, making it difficult to predict how well the

130

transgene will be expressed. One method for accomplishing precise insertion is based on the FLP-FRT system found in *Saccharomyces cerevisiae*. This system was modified to insert DNA into a specific site in a Drosophila chromosome (Konsolaki et al. 1992, Simpson 1993, Golic et al. 1997) and, if applied to other arthropods, could eliminate concerns about unstable transformation or position effects. The FLP-FRT system is best suited for species undergoing intensive and long term genetic analysis and manipulation but would reduce risks associated with releases of transgenic insects containing TE vectors that could move with the aid of endogenous elements or through conversion. A few experiments have delivered linear or circular plasmid DNA into the genome of arthropods without using a specific vector (Walker 1989, Presnail and Hoy 1992, 1996). This approach has the advantage of reducing potential risks of instability or horizontal transfer of inserted genes via TE or viral vectors.

There is a risk that a transgenic arthropod population released into the field could fail to function as expected due to a phenomenon called 'gene silencing'. Transgenic plants and mammals are known to inactivate transgenes that overexpress proteins or are otherwise abnormal (Dorer and Henikoff 1994, 1997, Wolffe 1997, Henikoff 1998). This phenomenon is thought to be due to systems that have evolved to prevent high levels of expression of TEs or viruses that cause genetic damage to their hosts. In fungi and plants, gene silencing is associated with several mechanisms, including methylation of DNA, or posttranscriptional and transcriptional processes. Multiple mechanisms of transgene silencing occur in *D. melanogaster* (Dorer and Henikoff 1994, 1997, Pal-Bhadra et al. 1999, Jensen et al. 1999). Thus, methods may have to be developed to eliminate transgene silencing in other arthropods or this phenomenon could reduce the effectiveness of pest management programs. The use of insulators and boundary elements may limit gene silencing (Bell et al. 2001) as could elements such as histone deacetylase RPD3 (De Rubertis et al. 1996).

RELATIVE RISKS

Risk equals the potential for damage and the likelihood of its occurrence. Risks may be different for pest and beneficial arthropods and may depend on whether the arthropod is expected to persist in the environment or is unable to reproduce and thus cannot persist. Risks also will vary with the transgene(s) inserted, the insertion method, and the stability of the transgene's expression. It is possible to suggest potential types of harm but more difficult to quantify the likelihood of their occurrence without additional research.

An example of a low risk transgenic arthropod could be the domesticated silk moth (*B. mori*), which is unable to survive on its own in the wild. Most transgenic *B. mori* seem unlikely to have a negative effect on the environment because they should not be able to persist. Another example of a low risk transgenic pest or beneficial arthropods includes populations that are sterile and unable to permanently establish. Transgenic pest or beneficial arthropods that are unable to persist because the environment is unsuitable during a portion of the year are also likely to pose a low risk. Honey bees, *Apis mellifera*, are semi-domesticated and could escape human management to survive in the wild; thus, transgenic honey bees could pose a greater risk than the domesticated silk moth.

Transgenic pest arthropod populations containing *Wolbachia* or active TEs as 'drivers' should require different risk assessments than populations lacking active TEs or *Wolbachia* because we know very little about the mechanism(s) and frequency of horizontal transfer of these agents.

RESEARCH NEEDS

Evaluating risks associated with releasing transgenic arthropods will likely include, as a minimum, the questions or principles outlined in Tables 1 and 2, but other issues may become important as we learn more. Evaluations of transgene stability in the population and host or prey range can be answered by a variety of laboratory experiments (Li and Hoy 1996, McDermott and Hoy 1997, Presnail et al. 1997). Questions about horizontal gene transfer and unintended effects on nontarget species in the environment are more difficult to analyze in the laboratory and additional research is needed.

Models
Can we use models to predict the outcome of releases of transgenic arthropods in pest management programs? Many types of population and genetic models could, in theory, be used to predict what will happen when genetically-modified arthropods are released into the environment. We do not know, however, which types of model are most likely to be predictive of the actual outcome of field releases because few have been validated.

The difficulties in predicting field results from a mathematical model are exemplified by three models developed to predict the success or failure of a biological control program involving applications of fungi for control of grasshoppers and locusts (Wood and Thomas 1999). All three models fit the empirical data, but one predicted sustained control would occur after a single pathogen application. The other two models predicted that repeated pathogen applications would be necessary. The results demonstrated that two assumptions, often made by ecologists and modelers, are suspect: (1) quantitatively similar models need not give qualitatively similar predictions (contrary to expectations); (2) sensitivity analyses of model predictions are not always sufficient to ensure their accuracy (Wood and Thomas 1999).

Some current population models may lack key ingredients, such as partial reproductive isolation. For example, Caprio and Hoy (1995) developed a stochastic simulation model that varied the degree of mating bias between pesticide-resistant and susceptible strains of a pesticide-resistant parasitoid, its genetic structure (haplo-diploid), degree of dominance of the resistance allele, and degree to which mating biases extended to the hybrid progeny. The results obtained were counterintuitive and illustrated that models can offer insights into the complexities of population genetics and dynamics that might otherwise be overlooked. The common assumption made in models is that all genotypes of a species mate at random, but this assumption may mask important interactions. The efficacy of transgenic arthropod release programs could be jeopardized if mating biases exist between released and wild populations.

Empirical data generally are lacking to compare the relative usefulness of different model types in predicting insect population dynamics. Theoretical ecologists usually assume homogeneous and continuous populations. Metapopulation models, by contrast, assume that populations exist in patches varying in area, degree of isolation, and quality. Metapopulation biology increasingly is being recognized as relevant to our understanding of population ecology, genetics, and evolution (Hanski 1998). Recent data, and a variety of metapopulation models, indicate that spatial structure affects populations as much as birth and death rates, competition, and predation (Caprio and Hoy 1994).

CONCLUSIONS

Transgenic methods probably should be used to modify pest and beneficial arthropods only if conventional genetic tactics are ineffective because transgenic methods are more expensive

and difficult. Furthermore, recombinant DNA techniques generate concerns about risk, which adds a significant cost in both time and resources to such projects. However, there are circumstances in which a transgenic arthropod could provide an effective and appropriate solution to a pest management program. The issue is not if transgenic arthropods should be released, but when, how, and after what type of evaluation?

The debate over evaluation methods and interpretations should include a variety of viewpoints. Ewel et al. (1999) reported the conclusions of a workshop on deliberate introductions of species into new environments. The participants did not discriminate between the potential risks of genetically modified organisms and unmodified organisms. Many of the comments appear relevant to releases of transgenic arthropods. For example, Ewel et al. (1999) concluded that the

> *Benefits and costs of introductions are unevenly distributed among ecosystems, within and across regions, among sectors of society, and across generations. Although an introduction may meet a desired objective in one area, at one time, or for some sectors of society, unwanted and unplanned effects may also occur. Introduced organisms can, therefore, simultaneously have both beneficial and costly effects. Furthermore, the relative magnitudes of costs and benefits vary both in space and over time.*

Ewel et al. (1999) listed research questions under four headings: Guarding against risks without sacrificing benefits; Alternatives to introductions; Purposeful introductions; and Reducing negative impacts. They recommended a single framework for evaluating all types of introductions, a need for retrospective analyses of introductions, a holistic view of the invasion process, and fewer, more effective introductions. Ewel et al. (1999) concluded

> *At the extremes, these views range from a handful of advocates of no introductions, or of such rigorous pre-introduction proof of benignness that all introductions are effectively prohibited, to an equally small group that advocates a freewheeling global eco-mix of species. Most proponents of purposeful introductions understand the risks (but believe that technology can deal with them), and most conservation biologists recognize the potential benefits to be derived from carefully controlled introductions. Clearly, there is a need to bring all parties together on common ground that can lead to objective, science-based decisions by policymakers.*

I wish to echo this recommendation to bring together a broad array of people with differing scientific backgrounds in order to assess the potential risks of introducing transgenic arthropods into the environment. Enhanced funding and effort should be devoted to fundamental research on risk assessment methods for transgenic arthropods.

It is important that the use of transgenic arthropods not be "sold" as "the only" solution to difficult problems. The potential value of developing transgenic arthropods for pest management programs has been justified by the social, public health, and economic costs associated with serious problems such as malaria (Curtis and Townson 1998). For example, malaria is an increasingly important health problem with at least 500 million people infected and approximately 3 million deaths annually. Will deployment of transgenic mosquitoes unable to vector malaria contribute to a solution? Miller (1989) reviewed malaria control strategies and pointed out that there is unlikely to be" ... a magic bullet that will eliminate malaria". He noted that "Even DDT could not be called such a weapon, at least in retrospect." The strategy of relying on a single tactic in pest management, whether it be the use of

pesticides or of transgenic technology, is likely to fail. For example, the complexity of genetic structure in *Anopheles gambiae* populations in West Africa (Lanzaro et al. 1998), which may be reflective of the complex genetic architecture of other arthropod populations, suggests that release programs involving a single transgenic strain are unlikely to be successful.

It is urgent that we learn how to evaluate the potential ecological and genetic risks of releasing transgenic arthropods into the environment. The possibility of damaging the long term sustainability of ecosystems (critical to the world's food supply for the estimated 8 to 11 billion people that are projected to inhabit the earth in 2050) is a serious concern. Such evaluations must occur despite the fact that some critics argue that we can't yet predict ecological trends 30 to 40 years in the future based on experiments conducted over a few years. For example, Rasmussen et al. (1998) pointed out, "Conclusions based on 10 to 20 years of data can be very different than those based on 50-plus years of data. Current technology is continually expanding our capability to measure and monitor chemical or biological components that were not possible to measure two or three decades ago."

Because we face uncertainties in risk evaluations of transgenic arthropods, it seems prudent to exhibit great care in our initial releases. We are just now beginning to understand how genomes evolve and how genetic diversity is developed and maintained. Only a few years ago, we believed that genomes were relatively stable and we had little knowledge of the potential role played by TEs, retroelements, introns, noncoding DNA, and horizontal gene transfer in remodeling the genomes of organisms. Much remains to be learned about genome organization and evolution, but it is becoming clear that foreign DNA sequences inserted into the genome can sometimes serve as a source of variation that is selected on and capable of surviving over a long evolutionary period (Britten 1997, Jeltsch and Pingoud 1996, Miller et al. 1997).

Assessing the risks associated with releasing transgenic arthropods into the environment raises a number of complex ecological questions that cannot be answered readily with our current level of knowledge. This is not unusual; scientific information relevant to policy is lacking for many ecological issues. A conference on "Science, Policy and the Environment" was conducted by the US National Academy of Sciences in December 2000 and several conclusions appear relevant to assessing ecological risks associated with transgenic arthropods (National Council for Science and the Environment 2000):

(1) The breadth, depth, and diversity of the scientific specialties involved in successful environmental decisionmaking make **interdisciplinary** and **multidisciplinary** approaches essential.

(2) Serious voids in scientific knowledge make resolution of current environmental problems and prevention of any future problems extremely difficult. Significant **investment** in environmental science and engineering is needed.

(3) There is a crucial need for periodic **knowledge assessments** that can provide scientists and policymakers with reliable and timely "tate of the science" reports on the environment as a whole as well as on particular topics. Such assessments will require coordinated, multi-agency environmental tracking, monitoring, and inventory programs.

(4) Sound environmental decisionmaking is dependent on an effective **interface between scientists and policymakers** based on reliable and timely "translation" of information and views between the two communities.

(5) The integration of environmental knowledge, assessments, research, information, communication, and education is vital if our society is to achieve a requisite level of sustainability.

One field release of a transgenic arthropod has occurred (Hoy 2000), and it is logical to assume that releases of sterile transgenic arthropods (such as sterile Mediterranean fruit flies that contain a transgenic marker) could occur relatively soon because the sterile flies will be unable to establish permanently in the environment. The ultimate deployment of a transgenic arthropod in a pest management program will be an awesome challenge, requiring risk assessments, as well as detailed knowledge of the population genetics, biology, and behaviour of the target species under field conditions, and coordinated efforts between molecular and population geneticists, ecologists, regulatory agencies, and pest management specialists. Last, but not least, effective education and communication is necessary to assure the public that appropriate risk assessments have been completed satisfactorily.

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144

COMMERCIAL IMPLICATIONS AND IPR RELATED TO THE USE OF TRANSGENIC ARTHROPODS

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Abstract

We have recently created a company (Insecta Ltd) for the express purpose of fully exploiting both the traditional Sterile Insect Technique (SIT) and the potential benefits of using transgenic arthropods to control agricultural and medical pest insects or, more generally, animal and human diseases. This short paper briefly considers some of the general issues surrounding the commercial exploitation of transgenic arthropods both through the improvement of the SIT and for other, more innovative, approaches to disease and pest control.

INTRODUCTION

Throughout the world many important pests and diseases remain significant problems, while controlling them is becoming increasingly expensive and difficult as resistance develops, environmental and food residue limits are tightened, and insecticides are withdrawn for safety reasons. Traditional control using broad-spectrum insecticides has adverse effects on the environment, and even the new generation of chemicals is not without their side effects. The mass-scale production and release of sterile insects represents a cost-effective, very environmentally benign solution to many of these pests and diseases.

InSecta Ltd is a company that we established in 2000 with the objective of producing and releasing insects on a mass scale and on a fully commercial basis to control or eliminate:

- Pests such as medfly, olive fly and date moth
- Diseases such as sleeping sickness, malaria and dengue fever

The company encompasses both traditional SIT and new innovative ideas for disease and pest control. Insecta will concentrate initially on traditional SIT, with its focus in the first years on medfly and tsetse. It will provide a full range of services, from strain development and colony establishment, through sterile fly supply and aerial release, to project management and field evaluation. The use of mass insect technologies requires: (a) the development of viable insect strains, (b) efficient, quality-controlled production and distribution systems, and (c) professionally designed and managed field-release programmes. InSecta has a world class technical team with expertise in all of these key disciplines, access to state of the art insect strains, as well as access to the necessary production and distribution technologies to fulfill all these requirements. Funding has now been obtained to build a modular, medfly rearing facility at Zvolen in Slovakia with a capacity of 60–80 million pupae per week and plans are fully developed for construction of the facility. The facility has been located in Slovakia in order to service the needs of the accessible medfly SIT market in the Mediterranean basin. This market is estimated to be in the region of 4 billion flies per week. Zvolen has also been chosen because it is outside the normal range for medfly, yet close to the IAEA facility at Seibersdorf and good airports to allow rapid transportation of pupae to the markets around the Mediterranean.

The initial R&D programme for the company is focused on the key factors that will improve the commercial viability of the medfly strains and the rearing process so as to enhance production efficiency and make it as cost efficient as possible. Essentially, the focus will be on improving production and distribution logistics so as to improve end-user value added. In parallel, research will be undertaken focusing on bringing one other (yet to be defined) agricultural pest species to the state where we can bring it to market for SIT. We will also begin to explore the issues around mass rearing of a mosquito species with the intention of defining whether SIT can be used to reduce mosquito vector populations in selected situations. In addition, the expectation is that the early assessment of releasing transgenic insects will require that the insects are sterile before release. This approach will allow an environmental impact assessment to be carried out without any possibility of the transgene persisting in the environment.

In the longer term, Insecta is aiming to explore the use of transgenic systems to improve our ability to control agricultural and medical pest insect populations. One possibility is to use transgenic systems to improve the SIT through novel strain creation to improve rearing efficiency or effectiveness in the field following release. We also have an interest in using transgenic approaches to create and deploy refractory insects as part of SIT programmes and, perhaps, more generally for the future control of vector borne diseases. Finally, we have created transgenic strains of mosquito that contain model vaccine genes that are expressed in the salivary gland of the insect. Here, the concept involves using transgenic haematophagous insects as a means to deliver vaccines to target animal populations when they take a blood meal. Some of these approaches are discussed briefly below in the context of their potential application on a commercial basis. The actual detailed technology to undertake these manipulations is discussed by others in this meeting and will not be covered in this paper.

USING AND DEPLOYING TRANSGENIC APPROACHES TO IMPROVE THE SIT ON A COMMERCIAL BASIS

We have identified a number of key priority areas for research in terms of deploying transgenic approaches to eventual improvement of the SIT. Some of the research will be done 'in house' whilst much will be done in collaboration with other groups or exploited through licensing agreements. Insecta is not focusing on the development of the tools for transgenic manipulation of insects, rather how they may be exploited for improvement of the SIT.

Creating male-only strains for use in the SIT
The efficiency of the SIT approach is significantly enhanced by rearing, sterilising and releasing only males, rather than both males and females (Whitten, 1969; Hendrichs et al 1995). The conventional approach to creating sexing strains involves the use of Mendelian genetics. However, this is extremely laborious and time consuming, and requires an extensive knowledge of the genetics of the target organism before it can be attempted. The process of bringing the appropriate mix of genes together in a single strain may have implications for the eventual fitness and stability of the strain both in mass rearing and following release. A transgenic approach has the potential to radically speed up the process, and is more easily applicable to a range of insects without having to create an extensive genetic map of the organism. There is also the potential of manipulating a range of gene systems that may impact on different aspects of the insect physiology so as to improve the SIT. Also, the approach would seem to lend itself to manipulating the genome of insects recently colonised from the target control area. This may be beneficial in terms of the future fitness and efficiency of the

SIT strain, although the transgenic approach will also involve significant selection from single founder flies which may negate some of the benefits of this approach.

A number of approaches for creating male only strains are possible and these have been discussed previously by Robinson and Franz (2000). Possibilities include the introduction of a system of conditional lethality where a female-specific lethal gene can be induced at the appropriate (preferably early) stage in the life cycle. Various configurations are possible using sex specific promoters and a number of different genes and/or splicing alternatives. The use of transgenic approaches to rapidly create new sexing strains for a variety of different species that are capable of mass rearing is a major priority for Insecta. Important considerations as to the eventual viability of the systems that are developed (in addition to the regulatory issues surrounding their eventual use in the field) include:

- The cost, toxicity and environmental issues of chemicals that may be required as the selective agent.

- The sexing system must be very effective such that, under ideal circumstances, no males are killed and all females are killed following induction, and no females are affected under non-induced conditions.

- The pressure on keeping costs to a minimum and efficiency of production high means that elimination of females should occur very early in the rearing cycle.

- Ideally, the selection system could be rapidly integrated into current rearing programmes, or that their introduction is so cost effective as to warrant their use.

The efficient creation of new sexing strains through transgenic means for a range of SIT target species remains our highest, long term research priority.

Creating transgenic strains which may be sterilised (or effectively sterilised) without the use of radiation
Currently, SIT uses radiation prior to release to disrupt sperm DNA to the extent that the released males are sterile. An alternative system that does not require the use of radiation with its inherent safety and cost issues could be highly beneficial. The 'release of insects carrying a dominant lethal' (RIDL) approach described by Thomas et al (2000) represents one of a family of methods that may provide just such a system. Essentially, transgenics are used to create a strain of the target insect that contains a conditional, dominant, sex-specific lethal where the permissive condition can be created in the rearing facility but which would never be encountered in the normal environment. Other approaches are possible and a number are being explored. However, in all cases it would be essential to ensure the stability and reliability of the system and to determine the longer-term fate of the transgenes of such a system in the environment before contemplating replacing radiation sterilisation.

Using transgenic approaches to improve the cost effectiveness of the rearing process
In principle, transgenic approaches could be highly effective to 'tweak' the genetic makeup of SIT strains so as to improve rearing efficiency. For example: the strains could be made more resilient to the rearing or the sterilisation process; they could be altered so that cheaper alternative food sources could be utilised; the ability to store or stockpile strains would be beneficial; new strains could be developed which were resistant to infection; and new strains could be created which were more easily deployed to a variety of different locations. Finally, it would be beneficial if larger, more robust insects could be reared in a shorter time. These

manipulations would seem to be possible in the future, and would have significant advantages in terms of reducing the unit cost for production and hence to the buyer — all critical commercial concerns. However, at this stage they remain lower priorities in comparison to the creation of efficient sexing strains for a larger range of target species for the SIT.

Using transgenic approaches to improve the effectiveness of sit insects following release
Again, transgenic approaches have the potential to significantly improve the effectiveness of sterile males once released into the environment. The focus would be to enhance end-user value added. Possibilities include:

- The insects could be made more resilient and hence longer lived in the environment through, perhaps, enhancing the insect immune system (Dimopoulos et al, 1997);

- It would be beneficial if the ability of the males to compete for females in the wild could be enhanced perhaps through modifying the insect pheromones or certain behavioural/mating characteristics (Meller and Davis, 1996)

- Modifying the behaviour of the insect in such as to suppress the detrimental effects it may have on the agricultural product – for example suppressing fruit stinging in medfly.

- In addition, a simple application of the transgenic approach would be to tag the released males in such a way that they could be easily discriminated from wild flies on a very large scale and at low cost. The use of Green Fluorescent Protein GFP as a marker (Catteruccia et al, 2000) is being explored as one potential tagging system. The ability to trap and classify reared, released flies from wild flies is critical in terms of defining the effectiveness of any SIT programme and Insecta would expect to incorporate such a tag into any transgenic strains which it creates.

USING TRANSGENIC TECHNIQUES TO CREATE REFRACTORY STRAINS

As indicated above, Insecta has an interest in using transgenic approaches to create and deploy refractory insects as part of SIT programmes. The main focus is to ensure that any sterile released males are not capable of transmitting disease-causing organisms. In many cases, where only the female transmits the disease, this may not be an issue. However, where the male is also capable of disease transmission, then it is essential that some means of drastically reducing or eliminating their ability to act as vectors needs to be found. A transgenic approach would certainly lend itself to assisting in creating strains with the appropriate refractory genes incorporated into them. Much effort is currently underway to identify the molecular basis of refractory mechanisms in a wide range of vector pathogen systems (eg Zheng et al, 1997). When appropriate, these genes systems could be incorporated into new SIT strains to create refractory insects. Clearly, much of the current effort in the molecular analysis of refractory mechanisms is directed towards controlling disease transmission but not using the SIT. Here the concept is to spread the beneficial refractory gene through target vector populations using either transposable elements or other genetic drive mechanisms following the release of fertile transgenic insects (Crampton et al, 1990). The release of fertile, as opposed to sterile, transgenics into the environment poses much more significant regulatory issues. Clearly, Insecta is developing major capacity in mass rearing and release and we are focusing our attention entirely on the use of mass release of sterile insects at this stage.

USING TRANSGENIC TECHNIQUES TO CREATE HAEMATOPHAGOUS INSECTS WITH VACCINATING POTENTIAL

Insecta is undertaking one other area of research, although it should be stressed that this represents a much longer-term objective of the company. The work explores the concept of using such haematophagous insects as a vehicle to deliver antigens directly to animal hosts at the time a blood meal is taken (Crampton et al, 1999). Antigen would be delivered intradermally, a common route in vaccination (Levin and Clarke, 1993). Such an approach would lend itself to being deployed through mass rearing and release of sterile transgenic insects such as mosquitoes. As a model system, we have already created transgenic strains of the mosquito *Aedes aegypti* that contain the gene coding for the ookinete antigen Pbs21 from the rodent malaria parasite *Plasmodium berghei* (Tirawanchai and Sinden, 1990). Pbs21 is a model for a class of so called transmission blocking vaccines. This gene is expressed in the insect's salivary gland and the vaccine antigen secreted as a component of the saliva. Each time the female takes a blood meal, small amounts of the antigen is delivered via the saliva which would be sufficient to induce, sustain or boost an immune response in the bitten animal. Vaccination does not confer protection on the host, but when the insect ingests antibodies to the antigen, they effectively block invasion by the parasite and hence onward transmission to another host. Insecta is interested in developing this concept because of the potential it may have for vaccinating large numbers of agricultural animals against a range of diseases, such as Rinderpest, Paratuberculosis and Bluetonge, in a cost effective way. There may also be opportunities to deploy this approach to vaccinate vertebrate reservoirs of zoonoses (i.e. diseases which on occasion 'spill over' from animal reservoirs to infect the human population) but which are difficult or expensive to control through other means.

IPR ISSUES ASSOCIATED WITH THE COMMERCIAL EXPLOITATION OF TRANSGENIC ARTHROPODS

Many of the applications of transgenic technology discussed briefly above will involve the manipulation and introduction of multiple elements, including DNA vectors, promoters, and genes for introduction. The creation of transgenics may therefore involve bundling a number of patented or protected elements together to create the desired construct for manipulation. Finally, in a very limited number of cases, the actual process or concept being use as part of novel strain creation may have been protected or patented. Thus, to deploy a commercially useful transgenic strain may require quite a complex set of negotiations relating to licensing and royalty payments with a number of organizations and individuals. Depending on the nature of the protection, and the attitude of the patent holder, this could have significant implications in relation to the costs associated with the creation of new strains for commercial use. This issue is not unique to the insect field – much has been done, for example, in plant biotechnology. At present, we do not foresee that IPR issues are likely to be either insurmountable or significantly impede the use and deployment of transgenic arthropods through commercial means.

CAGED POPULATION STUDIES TO ANALYSE THE FITNESS PARAMETERS AFFECTING TRANSGENIC ARTHROPODS

In all of the situations described above, there is a need to consider very carefully all of the risks and environmental consequences before any release of either fertile or sterilised insects could be considered. Fundamental to any risk assessment is the need for a full understanding of the life history characteristics of the transgenic individuals and populations in relation to wild type insects. The genetic modification of any organism will always be accompanied by

feedback consequences as a result of fundamentally altered gene expression, caused by the insertion of foreign genes or deletion of specific genetic components of the target organism's genome. However, the impact of altered gene expression will be manifest in the fitness of both the individual and the population.

Insecta is particularly interested to explore the issues around fitness parameters which are likely to affect sterile transgenics in relation to their interaction with wild flies and hence their effectiveness in the SIT. As indicated above, we have created a number of transgenic strains of *Aedes aegypti* that contain the Pbs21 gene. In these model systems specifically, any effect of the modification on longevity and the ability to effectively compete for females in the field will critically affect the success of these transgenics in any SIT programme and hence their commercial viability.

We are now studying the biology of these transgenic strains in comparison with non-transformed mosquitoes in caged population studies. The main aim of the work is to define the Darwinian fitness of the 'loaded' transgenic mosquitoes and to determine the fate of the transgene in mixed, caged populations. We wish to understand these factors in the context of beginning to define the environmental impact and risks associated with the release of transgenic mosquitoes as part of a disease control programme. In addition, using a range of transgenic strains, we will be able to assess the impact of transgene size and nature on the fitness and life history characteristics of these mosquitoes.

We will use a combination of detailed individual level and population level analyses to explore the fitness consequences of introducing the transgene into the mosquito. At the individual level we will determine the life history characteristics of the loaded and unloaded transgenic strains in comparison with their wild type counterparts. In addition, we will directly compare the population dynamics of wild type and transgenic strains in pure and mixed populations. These data are essential if we wish to accurately describe and predict the outcome of releasing such an organism into the environment. We are then going to undertake a similar assessment for releasing **sterilized** transgenic insects into caged populations of fertile, wild type mosquitoes. In this instance, we would assess the impact of the sterile, transgenic individuals on the wild type population. We would also want to generate data that provides reassurance that these sterile, transgenic individuals were relatively persistent in a mixed population, and that the transgene showed no signs of being passed to fertile individuals by whatever means.

CONCLUSIONS

In summary, Insecta is committed to the commercial exploitation of mass rearing of insects for the control of agricultural and medical pest insects in an ethical and environmentally friendly manner. In the short term, the focus will be to deploy and expand the use of standard SIT approaches to medfly and a broader range of target insects. Research is also underway to explore the potential of incorporating transgenic approaches to improve the efficiency and cost effectiveness of the SIT and for other purposes such as disease control. However, Insecta wishes to ensure that it is fully engaged and compliant with the relevant national and international regulatory developments and procedures that will needed to be in place before any release of transgenic insects can be contemplated.

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