ENVIRONMENTAL RISK MANAGEMENT AUTHORITY DECISION

Amended under s67A on 22 August 2007 and 30 August 2011

	11 July 2001
Application code	GMD00303
Application type	To develop in containment any genetically modified organism under section 40(1)(b) of the Hazardous Substances and New Organisms (HSNO) Act
Applicant	The Horticulture and Food Research Institute of New Zealand Limited (HortResearch)
Purpose	To develop in containment a Tobacco Yellow Dwarf Virus (TYDV)-based episomal vector as a plant expression vector.
Date received	28 November 2000
Consideration	15 February 2001 - 11 July 2001
Considered by	GMO New Organisms Standing Committee (the Committee) of the Environmental Risk Management Authority

Summary of Decision

The application to develop four strains of genetically modified *Escherichia coli* (Migula 1895); one strain of genetically modified *Agrobacterium tumefaciens* (Smith & Townsend 1907); genetically modified Potato virus X; and genetically modified *Nicotiana tabacum* L. 1753 plants, as detailed in Annex 1 of this decision, is approved, with controls, having been considered in accordance with the relevant provisions of the Hazardous Substances and New Organisms (HSNO) Act 1996, the HSNO regulations, and the HSNO (Methodology) Order 1998:

Legislative Criteria for Application

The application was lodged pursuant to section 40(1)(b) of the HSNO Act. The decision was determined in accordance with section 45, taking into account additional matters to be considered under section 43, and matters relevant to the purpose of the Act, as specified under Part II of the HSNO Act. Unless otherwise stated, references to section numbers in this decision refer to sections of the HSNO Act.

Consideration of the application followed the relevant provisions of the Hazardous Substances and New Organisms (Methodology) Order 1998 (the Methodology). Unless otherwise stated, references to clauses in this decision refer to clauses of the Methodology. The cloning of complete viral genomes is required to be considered by the Authority as it is a Category C(f) experiment, as defined in the Hazardous Substances and New Organisms (Low-Risk Genetic Modification Regulation) 1998. Category C experiments cannot be considered by an Institutional Biological Safety Committee (IBSC) under delegated

authority. The Committee notes that the application also includes a number of Category A experiments and the applicant chose to have the project as a whole considered.

Application Process

The application was formally received and verified on 28 November 2000.

Under section 53(2) of the Act, the Authority has the discretion as to whether or not to notify an application to develop any genetically modified organisms in containment where the Authority considers there is likely to be significant public interest. The project involves the incorporation of more than two thirds of a viral genome, capable of giving rise to infectious particles in plants, so the application is not for a low-risk modification. The working policy of the Authority is that there should be some aspect of the application about which there is good reason to believe that there is a particular public interest that is significantly greater than the general interests of the public in the development of genetically modified organisms. The Genetically Modified Organisms Committee, at a meeting on 12 January 2001, unanimously agreed not to publicly notify the application.

In accordance with clause 5 of the Methodology and section 58 (c) of the HSNO Act, the Department of Conservation (DoC) and the Ministry of Agriculture and Forestry were supplied with verified copies of the application, and invited to comment on it.

Having informed the applicant, ERMA New Zealand sought expert comment on the application from Dr Mark Gibbs, a consulting plant virologist, who is a Visiting Fellow in the Division of Molecular Medicine, John Curtin School of Medical Research, The Australian National University.

Information available for consideration included the application, and the Evaluation and Review Report (E & R report) on the application prepared by ERMA New Zealand; the E & R report included as appendices the expert review by Dr Gibbs, and additional information on containment from the applicant in response to Dr Gibbs' comments. The E & R report noted DoC's response that they had no objection to approval of the application.

The application was considered by the Genetically Modified Organisms Standing Committee of the Authority appointed in accordance with section 19(2)(b) of the HSNO Act. The Committee comprised the following members: Mrs Jill White (Chair), Ms Prue Kapua and Professor Colin Mantell.

On 28 May 2001, the applicant notified ERMA New Zealand that the conditions imposed by the Authority could be met, and set out details of how this will be done.

Purpose of the Application

This application covers the generation of genetically modified plants and plant viruses for the study of their interactions. The research purpose is to test the components of a viral-genetic system that might be used for genetically engineering plants. The experiments will ultimately infect tobacco plants that already carry some TYDV (Tobacco Yellow Dwarf Virus) genes with PVX (Potato Virus X) vectors containing the TYDV replication gene, as well as reporter genes.

In accordance with section 45(1)(a)(i) of the HSNO Act, the Committee determined that this was an appropriate purpose under sections 39(1)(a). *The development of any genetically modified organism.*

The Sequence of Steps in the Consideration

In accordance with clause 24 of the Methodology, the approach adopted by the Committee was to look sequentially at identification, assessment and evaluation of risk, costs and benefits. Interposed with this were consideration of the proposed management regime, and the ability of the organism to escape and form self-sustaining populations. Management techniques were considered in relation to the identified risks (clauses 24 and 12) and those risks identified as significant were assessed (clause 12). Costs and benefits were assessed in accordance with clause 13 of the Methodology.

Risk characteristics were then established, in accordance with clause 33 of the Methodology.

Finally, taking account of the risk characteristics established in accordance with clause 33 of the Methodology, the combined impact of risks, costs and benefits was evaluated in accordance with clause 34.

The Identification of the Significant Risks, Costs and Benefits of the Organisms

Significant risks, costs and benefits identified for assessment and evaluation are detailed below, following clauses 9 and 10 of the Methodology, which incorporate sections 5, 6, and 8 of the Act. It was considered that no significant risk existed from the contained development and maintenance of the modified bacteria, viruses and plants.

In accordance with sections 5 and 6 of the HSNO Act and clause 9 of the Methodology Order, the Committee has categorised the potential adverse effects of this application under the headings of environmental, human health, Maori issues and concerns, and economic.

The following are significant risks associated with a breach of containment:

Environmental

- Risks (and the associated costs arising) to native and other valued flora and fauna (in accordance with clauses 9(a), 9(b), 9(c)(i), 9(c)(ii), 10(a), 10(b) and 10(d)), should an escape occur of genetically modified plant viruses, or bacterial cultures or plant material containing the viruses, resulting in adverse impacts on valued plant species. These risks are the effects on plant health of escaped genetically modified PVX viruses or of a new virus that is the result of recombination.

Human Health

- The Committee notes that they considered the risks (and associated costs arising) to human health from the development and use of transgenic bacteria, viruses and plants, including the potential harm to people working with the organisms, and any effects on public health, should the organisms escape, in accordance with the Methodology clauses 9(b)(i) and (ii), 9(c)(iii) and (v) and 10(g). The Committee considered it very unlikely for

there to be any adverse human health effects, and, in view of this, decided that no further consideration of human health effects was required.

Māori issues and Concerns

- The Committee considers adverse effects on Māori culture are very unlikely, based on the fact that no genetic material from humans or native flora and fauna are involved in the research and the research will be carried out in containment. The genetically modified organism(s) are also not approved for field trials or release. The Committee decided therefore, that no further consideration of adverse effects on Māori culture was required.

- The Committee notes the experiments approved in this application may raise issues that would be of interest to local people, including Māori. The Committee advises that providing information to local Māori on the experiments at this stage may facilitate the applicant's information needs should the work eventually lead to a field trial application and/or commercial development.

Economic

- Risks (and associated costs arising) to the economy should an escape of genetically modified plant viruses or bacterial cultures or plant material containing viruses result in adverse impacts on valued plant species, in particular species used agriculture or horticulture (clauses 9 (b)(i) and 9(b)(ii)).

A significant benefit identified was:

- Increase in scientific knowledge about the control and function of TYDV plant virus replicase (in accordance with clauses 9(b)(i) and 9(c)(v).

Inseparable Organisms

The Committee has considered the effects of any inseparable organisms, in accordance with section 45(a)(ii) of the HSNO Act. The Committee noted that the bacteria are well-characterised laboratory strains and very unlikely to contain inseparable organisms, and that the Potato virus X is also a well-characterised isolate that is very unlikely to contain inseparable organisms. The Committee recognises, however, that the tobacco tissue to be modified may contain integrated viral sequences, although these may not produce infective viral particles. Additional controls have been imposed to manage the risks, and the associated costs arising, of viral escape.

Adequacy of the Proposed Containment

The Committee considered the adequacy of containment in accordance with section 45(i)(a)(iii) of the Act, and the magnitude and probability of the risks, costs and benefits at the same time and in an integrated fashion. This is because the former interacts with the latter; this is recognised in clause 12(d) of the Methodology and in section 45(i)(a)(ii) of the Act. For convenience in setting out the decision, the adequacy of containment is discussed first.

Ability to contain the organisms

The Committee considered the ability of the organisms to escape from containment in accordance with section 44(b) of the HSNO Act.
The Committee considered the following:

i. The containment regime proposed.
ii. Inadvertent removal of organisms by personnel within the facility
iii. Sabotage or deliberate removal of organisms from the facility
iv. Development of new viruses through recombination.
v. Escape from containment of viruses.
vi. Escape of MPEs (multi-copy plant episomes)
vii. Escape from containment of plant material containing viruses.

The Committee considered that pathways (i), (ii) and (iii) can be adequately managed by the containment regime and the additional controls imposed by this decision. These controls are discussed further below.

The Committee considered that pathways (iv), (v), (vi) and (vii) provide points of difference from other genetically modified organism applications that the Authority has considered. The pathways that may lead to the escape of the virus from containment and/or the development of a new virus are also managed by the containment regime, including additional controls. The following paragraphs discuss these potential routes of escape and how the additional controls will manage the potential for a breach in containment.

i. The proposed containment facility

The organisms are to be contained in an approved *containment facility* (under the Biosecurity Act 1993) in compliance with the Ministry of Agriculture and Forestry (MAF) Biosecurity Authority/ERMA New Zealand Standards: 154.03.02: *Containment Facilities for Microorganisms* and 155.04.09: *Containment Facilities for New Organisms (including genetically modified organisms) of Plant Species.* In addition, the operation, construction and management of the facility must be in accordance with the relevant provisions of the Australian/New Zealand Standard (AS/NZS) 2243.3:1995 *Safety in Laboratories* Part 3: *Microbiology* at Physical Containment Level 1 (PC1) for the low risk work or Physical Containment Level 2 (PC2) with additional controls for Category C developments.

The Committee considers that PC1 containment is appropriate for the development of the modified *E. coli* bacteria containing no more than 2/3 of the PVX virus because these involve an approved Schedule 2 host and meet the conditions of Category A experiments in the HSNO (Low-Risk Genetic Modification) Regulations. While the HSNO (Low-Risk Genetic Modification) Regulations permit such approved hosts to be held in PC1 containment, the Committee considers that where Category C experiments are involved (i.e. inclusion of more than 2/3 of the PVX virus), then maintenance of *E. coli* containing the viral vector under PC2 conditions is appropriate, since this will more effectively prevent inadvertent escape of the vector.

The genetic modification of *A. tumefasciens* with the PVX vector and the infection of plant tissue require PC2 level containment since these experiments involve the use of pathogenic viral vectors, and so do not meet the requirements of an approved Schedule 2 host in the HSNO (Low-Risk Genetic Modification) Regulations.

The Committee accepts the external reviewer's advice that a greater level of security and monitoring is required than that for genetic modifications of plants that do not involve viral vectors. The higher level of containment will be important when the transgenic plants are grown, as this is when any adverse interactions might take place. To achieve this, the Committee has imposed additional controls on top of standard PC2 containment conditions.

To reduce the chances of inadvertent infection by other plant viruses, the genetically modified plants shall be grown in a plant house where there is limited access, the genetically modified plants shall be kept physically separate from other plants, and no other experiments involving plant viruses are to be conducted in the same plant house.

The Committee also imposes an additional control to monitor for the generation of any novel viruses. The Committee considers that such experiments will be essential to any future consideration of risks associated with this work, such as an application to field trial the viral vector.

The MAF/ERMA and AS/NZ standards, supplemented by the additional controls, are considered by the Committee to sufficiently address the requirements for the maintenance in containment of the organisms to be created in these experiments, i.e. microorganisms, plant cultures and whole plants.

ii. Inadvertent removal of organisms by personnel within the facility

The Committee notes that the applicants have experience in maintaining plants and microorganisms in containment. Access to the facilities is limited to authorised staff (as required by AS/NZS 2243.3 and the MAF/ERMA Containment Standards 154.03.02 and 154.04.09) who are highly skilled. The Committee considers it is very unlikely that inadvertent removal of organisms from containment would occur, subject to containment controls and the integrity of the containment regime being maintained.

iii. Sabotage or deliberate or accidental removal of organisms from the facility

The Committee considers that the possibility exists for the containment facility to be damaged, either by accident through act of nature, or by human interference. The result could be new organisms escaping from the facilities. The HortResearch containment manuals describe security procedures and contingency plans in the event of deliberate or accidental damage. Since the plants will not be grown to flowering stage, there is limited risk of any damage allowing the dispersal of seed or pollen. Removal of the plants themselves from the containment facility is the most likely route for genetically modified plant material to escape from containment should damage occur. Given the restrictions on access to the facility and on removing such material (as detailed in the containment manual) the Committee considers that it is unlikely that containment would be breached through uncontrolled removal of plant material.

iv. Development of new viruses through recombination

The Committee considered that there is the potential for several types of recombination involving the viral vector, namely recombination between:

- vector sequences
- the self-replicating viral vectors and other viruses
- the integrated vector and other viral sequences.

There is, however, uncertainty in determining the likelihood and consequences of such recombination events. The Committee considers that, in accordance with clause 30, it must take into account the need for caution in managing these potential adverse effects. Consequently additional controls, described below, are included to reduce the chance of recombination, as well as to prevent the escape of viruses. These additional controls are:

i. keeping the genetically modified plants physically separate from other plants, to reduce the chance of becoming infected with other viruses
ii. not conducting experiments on other viruses within the same plant house
iii. fumigating the plant house, to prevent insects or other invertebrates infecting the genetically modified plants with other viruses.

The Committee considers that these additional controls, as well as the small and contained scale of the experiments, are sufficient to manage the risks of new viruses generated by recombination.

The Committee notes that, based on advice from the external virologist, new viruses do not appear to commonly result from natural plant viral infections. Consequently, the Committee considers that despite uncertainty, the likelihood of a new virus forming in containment is probably low.

v. Escape from containment of viruses

The Committee notes that for the PVX or a new recombinant virus (if formed) to escape from containment, it would need to be released from the plant and infect another host. This may potentially occur via pollen or seed, an insect (or other transmission) vector, or direct mechanical transmission by contact between plants.

The Committees considers that additional procedures and plant house containment, at a level higher than required for plant house containment level 2, would make it very unlikely that any plant viruses would escape from containment in these experiments. The Committee is satisfied that the following additional controls will achieve this:

i. training of personnel by an experienced virologist so that those with day to day contact with the plants will be familiar with signs of unusual viral infections, and methods of viral spread.
ii. keeping infected plants physically separated from other plants to prevent direct contact and virus transmission
iii. excluding insects from the plant houses by prior fumigation and sealing any gaps in the plant house, to prevent insect-mediated viral transmission
iv. sterilisation of equipment used on the genetically modified plants before they are used on other plants, to prevent mechanical dissemination of viruses

v. sterilisation of soil to be used on the genetically modified plants, to kill any invertebrates that may potentially transmit a virus
vi. sterilisation of soil used on genetically modified plants before disposal, to prevent subsequent transmission of plant viruses
vii. prevention of plant reproduction, to preclude dissemination of viable plant material that may potentially contain viruses
viii. inspection of plants for viral infection before samples are taken, to prevent inadvertent spread of new viruses
ix. transport of plant samples to laboratories in sealed containers, to prevent dissemination of viruses.

vi. Escape of MPEs (multi-copy plant episomes)

These experiments will also produce plants that contain self-replicating, but non-infectious, viral elements, called multi-copy plant episomes ("MPEs"). The Committee is satisfied that the additional controls for preventing the escape of viruses and plants will make it very unlikely that MPEs could escape from containment. If they did escape, the Committee considers that the magnitude of an effect would be minimal since they lack the features of viruses that enable them to infect cells.

vii. Escape from containment of plant material containing viruses

Pollen and seeds do not generally transmit PVX, but pollen and seeds of genetically modified plants will have the viral replication unit (amplicon) integrated into their genome. Consequently, if the genetically modified plants escape from containment, or pollen or seed escape from the plant house, there is the potential for escape of the virus as well as the plant.

The plants are to be grown within a plant house that is a MAF-registered containment facility, and the plants will not be grown to a stage where they produce flowers.

Taking into account adherence to the controls imposed, the Committee considers that it is very unlikely that viruses or genetically modified plant material will escape from containment.

Ability of the organisms to establish undesirable self-sustaining populations and ease of eradication

In accordance with sections 43 and 37 of the HSNO Act, the Committee considered the ability of the organisms to establish undesirable self-sustaining populations, should they escape from containment, and the ease with which such populations could be eradicated. In evaluating these matters, the Committee took into account the nature of the organisms.

The Committee notes that the laboratory strains of bacteria to be used in the research are unlikely to persist in the environment due to specific mutations and requirements for supplementary nutrients. The *E. coli* strains are derivatives of *E. coli* strain K12, which is known to be unable to establish a self-sustaining population outside of laboratory culture. The *Agrobacterium tumefaciens* strain is a disarmed laboratory strain that does not produce plant tumors. *A. tumefaciens* and the various *E. coli* strains have been used in many laboratories for many years with no adverse effects. Furthermore, *E. coli* and *A. tumefaciens* occur naturally in the environment, so that the laboratory strains are likely to be at a selective disadvantage. Although there is some uncertainty (clause 30) over whether the various *E. coli* strains and the laboratory strain of *A. tumefaciens* to be used in the research would be able to establish self-sustaining populations, the Committee considers it is very unlikely that bacterial cultures will escape from containment, or infect other plants, given the controls it has imposed.

In the event that viruses or genetically modified plants escape from containment, the magnitude of an adverse effect would depend upon the viability and pathogenicity of the virus, and whether the plants contain or produce viable viral particles and come into contact with plants susceptible to the virus. The Committee considered that there is uncertainty over whether viable genetically modified viruses would establish self-sustaining populations. Having identified, in terms of clause 30, a need for caution in the face these uncertainties, the Committee considered it prudent to assume that if viruses were to escape, that they could form self-sustaining populations.

PVX is usually transmitted by direct contact between plants so that the magnitude of an effect in this case may be minimal if no susceptible wild plants are infected. If, however, plants were infected, then the virus could establish a self-sustaining population. The Committee considered that in the event of a virus establishing outside of containment it might be very difficult to eradicate. Additional controls have, therefore, been imposed to minimise the likelihood of viruses or genetically modified plants escaping from containment.

Tobacco plants are not commercially grown near the HortResearch campus, so that plants that escaped from containment are likely to be detected and destroyed. However, tobacco is able to hybridise with related *Nicotiana* species, and these may be cultivated in gardens or be growing wild, so that there is the potential for cross-pollination if tobacco pollen escaped. The Committee notes, however, that tobacco is generally self-fertilising, and plants will not be permitted to flower, so that it is very unlikely that viable genetic material from modified tobacco plants will escape and establish a self-sustaining population. The Committee considers the additional control requiring the area surrounding the plant house to be kept clear of any *Nicotiana* plants during the experiments further reduces the likelihood that the experimental plants could cross-pollinate *Nicotiana* species.

Assessment of the significant risks and costs of the organism

The risks and costs assessed were those identified as potentially significant, having regard for those matters set out in clauses 9 and 10 of the Methodology. Risks were considered in terms of the requirements of clause 12 of the Methodology, including especially the assessment of consequences and probabilities, the impact of uncertainty and the impact of risk management. Costs were considered in terms of clause 13 of the Methodology.

The evidence available was largely scientific in nature and was considered in terms of clause 25 of the Methodology. This evidence comprised principally that provided by the applicant, additional evidence set out in the E & R report prepared by ERMA New Zealand, and in the expert review by Dr Gibbs

Risks to the environment

If genetically modified plant viruses, or bacterial cultures or plant material containing viruses, should an escape from containment, there is a risk of adverse effects to the health of native and other valued plant species. This risk is that escaped genetically modified PVX viruses, or of a new virus that is the result of recombination, could be pathogenic, causing plant disease (clause 12(a)).

The Committee notes there is uncertainty about the potential adverse effects of the escape of bacterial cultures containing the viruses, and the need for a cautious approach to the managing these risks (clause 30). There is uncertainty as to whether the strains of *E. coli* and *A. tumefaciens* hosting the genetically modified viruses developed by the research, would survive and form self-sustaining populations (clause 12(b)), should they escape from containment. In terms of clauses 12(b), 12(c) and 12(e), the Committee notes that the laboratory strains of bacteria to be used in the research are unlikely to persist in the environment due to specific mutations and requirements for supplementary nutrients. The *E. coli* strains are derivatives of *E. coli* strain K12, which is known to be unable to establish a self-sustaining population outside of laboratory culture. The *Agrobacterium tumefaciens* strain is a disarmed laboratory strain that does not produce plant tumors. *A. tumefaciens* and the various *E. coli* strains have been used in many laboratories for many years with no adverse effects. Furthermore, *E. coli* and *A. tumefaciens* occur naturally in the environment, so that the laboratory strains are likely to be at a selective disadvantage. The Committee considers it is very unlikely that bacterial cultures will escape from containment, or infect other plants, given the controls it has imposed (in accordance with clause 12(d)).

In considering the nature of the adverse effects (clause 12(a)), the Committee notes, that there is uncertainty over whether the presence of the TYDV replicase genes will affect PVX pathogenicity and viability (clause 12(b) and 12(c)). There is also uncertainty over whether the presence of MPEs will lead to adverse effects (clauses 12(a), 12(b) and 12(c)). The Committee notes that information on these issues can only be obtained by experiments of the type proposed here, but that in view of present uncertainties a cautious approach was required to managing these risks (clause 30). While data provided by the applicant indicated that the genetically modified virus is less pathogenic, the Committee notes that adverse effects on plant health can still result. The magnitude of any adverse effects, should the modified PVX virus escape, will depend upon the plant species infected and the viability and pathogenicity of the virus (clauses 12(b), 12(c) and 12(e)).

The Committee considered two main scenarios. The first was escape of the PVX vector. In the event that recombination leads to the loss of the foreign genetic material in the viruses then viruses similar to wild-type PVX may result. In terms of clause 12(b) and 12(c), while PVX is likely to be already present in the New Zealand environment, the Committee recognises that different viral strains may result in different pathogenicity, and it is unknown whether the PVX strain to be used is present in the New Zealand environment (clause 12(e)). Consequently, there may be differences in pathogenicity if the PVX vector escapes and infects plants outside of containment.

The second scenario involves the escape of a new virus that is the result of recombination. There is uncertainty over the likelihood and consequence (clauses 12(b), 12(c) and 12(e)) of new viruses being formed by recombination. In the worst case, where a range of plant species are infected with a very virulent virus, the consequences could be widespread, irreversible and lead to decline or loss of native species (clause (12a)). At the other extreme, a recombinant virus may not be viable.

Should the genetically modified PVX virus, or a new virus resulting from recombination, escape, it could infect some native or other valued plant species and cause plant disease (clause 12(a)). However, there is uncertainty over host range, since not all plants have been tested with PVX, and the nature of any recombinant viruses that may be generated cannot be predicted (clause 12(e)).

In consideration of these issues, the Committee recognises that it is essential that the likelihood of escape be reduced to a very low level by use of careful experimental procedures and diligent application of the controls (clause 12(d)).

The Committee acknowledges the uncertainty in predicting the likelihood and magnitude (clauses 12(c) and 12(e)) of effects related to the development of new viruses by recombination. However, based on advice from the experienced plant virologist involved in the assessment of this application, the Committee considered that the uncertainties and risks would be minimised by the inclusion of the set of additional controls imposed by this decision (clause 12(d)).

Economic risks

The Committee considered the economic risks of the development of these genetically modified organisms in containment, in accordance with the Methodology clauses 9(b)(i) and (ii), and 9(c)(iii) and (v).

The Committee notes that, based on advice from the external virologist, new viruses do not appear to commonly result from natural plant viral infections. In terms of clauses 12(a), (b) and (c), the Committee notes however, that Dr Gibbs, in his expert review, stated that "*There is a very small but significant chance that the experiments described on the application will create a novel plant pathogen that could cause a new plant disease and so have adverse effects on crops or wild populations*". The controls (clause 12 (d)) imposed by the Committee are directed at preventing escape from containment of organisms developed by the research described in the application. Because of these controls, the Committee considers it very unlikely that any adverse economic effects would result from approval of the application (clause (12a)).

Costs

A "cost" is defined in clause 2 of the Methodology Order as "the value of a particular adverse effect expressed in monetary or non-monetary terms".

The adverse effects on both the environment and the economy will, if they eventuate, have associated monetary and non-monetary costs, which are potentially major (clause 13(a)). In the unlikely event of escape of new pathogenic microorganisms or plants developed as a result of the research, there is uncertainty (clauses 12(e), 25(1), and 29) in terms of clause 13(b), about the magnitude of costs, which would depend on the species involved, and their future uses and values. This uncertainty, which has not been quantified, has been taken in to account by the Committee in the controls it has imposed, in accordance with clause 30. The costs are unlikely to be restricted over time, space or to particular groups in the community (clause 13(c)).

Assessment of benefits (beneficial effects)

A "benefit" is defined in clause 2 of the Methodology Order as "the value of a particular positive effect expressed in monetary or non-monetary terms". Benefits that may arise from any of the matters set out in clauses 9 and 10 of the Methodology were considered in terms of clause 13.

In terms of clause 13(a), the Committee considers that the primary benefits to New Zealand associated with this application are the monetary and non-monetary benefits accruing from an increase in scientific knowledge about the control and function of TYDV plant virus replicase. Potential indirect non-monetary benefits to the applicant and New Zealand are those associated with maintaining New Zealand's standing in international science. A successful outcome to the research might increase the applicant's ability to attract research funding.

The applicant considers that the research described in the application will have long-term benefits to the New Zealand economy through providing a more detailed understanding of the TYDV replicase function, which will lead to future developments of efficient and precise vectors for plant gene expression (clause 13(a)). The Committee recognises that the research could lead eventually to development of new plant expression systems in economically important crops. However, given the speculative nature of the postulated economic benefits (clause 13(b)), the Committee has not evaluated them further.

These benefits are unlikely to be restricted over time, space, or to particular groups in the community (clause 13(c)).

Establishment of the approach to risk in the light of risk characteristics

The Committee considered the characteristics of risks posed by the application in terms of clause 33 of the Methodology. In the case of the present application, the relevance of clause 33 is much reduced because the application is "in containment" and it has already been concluded that the containment provisions and other controls will reduce most biological and physical risks to a low level. Given the controls that the Committee has imposed, escape of the organisms is very unlikely. The Committee notes that, in the very unlikely event that organisms did escape,

- exposure to the risks would be involuntary (clause 33(a));
- it is likely that the risks will persist over time (clause 33(b)) ;
- it is likely that the risks would be subject to uncontrollable spread and extend beyond the immediate location of the incidence (clause 33(c));
- escape of plant material containing viruses would be potentially reversible through application of herbicides to the escaped plants, once they are located, but the escape of viruses is likely to be irreversible (clause 33(d));
- the general public has a poor understanding and little direct experience of the potential adverse effects of newly developed plant viruses (clause 33(e)).

Because the risks that would result from the escape of the genetically modified PVX virus, or a new virus resulting from recombination, are likely to persist over time and be subject to uncontrollable spread, the Committee has taken a cautious approach to these risks (clause 30), which is reflected in the controls it has imposed.

Overall Evaluation of Risks, Costs and Benefits

The overall evaluation of risks, costs and benefits set out below was carried out having regard to Clauses 22 and 34 of the Methodology and in accordance with the tests in clause 27 of the Methodology and section 45 of the Act.

The Committee concludes that the risks to native and valued flora and fauna, human health and the economy can be considered together (clause 34).

The Committee considers that the escape from containment of viruses used in or developed by this application, although very unlikely, could have effects of a magnitude that are potentially major, and therefore the overall risk was not negligible. The decision therefore, is made in accordance with clause 27 of the Methodology.

It is evident that there are no common units of measurement for combining groups of risks, costs and benefits, so clause 34(a) cannot apply. Instead, as set out in clause 34(b), the Committee identified the dominant risks.

These risks (and the costs arising) are:

1. Risks to native and other valued flora and fauna from escape of the genetically modified PVX virus, or a new virus resulting from recombination, leading to exposure to new plant disease; and
2. Economic risks to commercial crops or wild populations of plants from exposure to new plant viral disease.

The benefit that contributed most to the overall balance, in the Committee's judgement, was that of an increase in scientific knowledge, coupled with the economic and other potential benefits that may be generated by the new knowledge.

The Committee, having considered the characteristics of the risks (clause 33), concludes that, given the controls it has imposed on the application, the benefits of the application outweigh the risks (and costs arising) associated with the application

Decision

1. Pursuant to section 45(1)(a)(i) of the Act, the Committee is satisfied that this application is for one of the purposes specified in section 39(1) of the Act, being section 39(1)(a): The development of any genetically modified organism.

2. Having considered all the possible effects of the organisms detailed in Annex 1 in accordance with sections 45(1)(a)(ii) and (iii) of the Act and pursuant to clause 27 of the Methodology, and based on consideration and analysis of the information provided and taking into account the application of risk management controls specified in this decision, the view of the Committee is that the risks (or costs) of adverse effects associated with the development in containment of the organisms are outweighed by the benefits of conducting the research.

3. The Committee is satisfied that the proposed containment regime together with the additional controls imposed will adequately contain the organism as required by section 45(1)(a)(iii) of the Act

4. In accordance with clause 36(2)(b) of the Methodology, the Committee records that, in reaching this conclusion, it has applied the balancing tests in section 45 of the Act and clause 27 of the Methodology and has relied in particular on the following criteria in the Act:

    - Section 5(a). The safeguarding of the life-supporting capacity of air, water, soil, and ecosystems.
    - Section 5(b). The maintenance and enhancement of the capacity of people and communities to provide for their own economic, social, and cultural well-being and for the reasonably foreseeable needs of future generations.
    - Section 6(a). The sustainability of all native and valued introduced flora and fauna.

5. The Committee has also applied the following criteria in the Methodology:

    - clause 9 - equivalent of sections 5, 6 and 8;
    - clause 10 - equivalent of sections 36 and 37;
    - clause 12 – evaluation of assessment of risks;
    - clause 13 – evaluation of assessment of costs and benefits;
    - clause 21 – the decision accords with the requirements of the Act and regulations;
    - clause 22 – the evaluation of risks, costs and benefits – relevant considerations;
    - clause 24 – the use of recognised risk identification, assessment, evaluation and management techniques;
    - clause 25 – the evaluation of risks;
    - clause 27 - risks and costs are outweighed by benefits;
    - clause 33 – risk characteristics; and
    - clause 34 – the aggregation and comparison of risks, costs and benefits.

6. The application for development in containment of the organisms detailed in Annex 1 to this decision is thus **approved**, with controls, as follows:

Containment Controls

In order to provide for the matters detailed in Part I of the Third Schedule to the Act, Containment Controls for Development and Field Testing of Genetically Modified Organisms, the approved organisms are subject to the following controls:

1. To limit the likelihood of any accidental release of any organism or any viable genetic material[1]:

1.1 The containment facilities shall be approved by Ministry of Agriculture and Forestry (MAF) in accordance with the MAF Biosecurity Authority/ERMA New Zealand Standards 154.03.02[2] or 154.04.09[2], and the controls of the Authority.

DNA manipulations and cloning using *Escherichia coli*

1.2 The construction and operation of the containment facility shall be in accordance with the:

a) MAF Biosecurity Authority/ERMA New Zealand Standard 154.03.02[2]: *Containment Facilities for Microorganisms*

b) Australian/New Zealand Standard AS/NZS 2243.3:1995[2] *Safety in Laboratories*: Part 3: (*Microbiology*), Physical Containment Level PC1.

DNA manipulations and cloning using *Agrobacterium tumefaciens, and Nicotiana tabacum* transformations and maintenance of plant tissue in plant growth rooms (infection of plant tissue with a pathogenic viral vector)

1.3 The construction and operation of the containment facility shall be in accordance with the:

a) MAF Biosecurity Authority/ERMA New Zealand Standard 154.03.02[2]: *Containment Facilities for Microorganisms*

b) Australian/New Zealand Standard AS/NZS 2243.3:1995[2] *Safety in Laboratories*: Part 3: (*Microbiology*), Physical Containment Level PC2.

Additional controls to PC2 laboratory:

1.4 On establishment of root or shoot growth in tissue culture the transformed plants shall be transferred in sterile plastic sealed containers to PC2 plant house containment (with the additional controls as described below).

1.5 All tissue culture products shall be autoclaved before being disposed of.

Maintenance of whole plants in plant house (greenhouse)

1.6 The construction and operation of the containment facility shall be in accordance with the:

[1] Viable Material is biological material that can be resuscitated to grow into tissues or organisms. It can be defined to mean biological material capable of growth even though resuscitation procedures may be required, e.g. when organisms or parts thereof are sublethally damaged by being frozen, dried, heated, or affected by chemical.

[2] Any reference to this standard in these controls refers to any subsequent version approved or endorsed by ERMA New Zealand

a) MAF Biosecurity Authority/ERMA New Zealand Standard 155.04.09[2]: Containment Facilities for New Organisms (including genetically modified organisms) of Plant Species

b) Australian/New Zealand Standard AS/NZS 2243.3:1995[2] *Safety in Laboratories*: Part 3: (*Microbiology*), Plant House Level PC2.

Additional controls to PC2 plant house[3]:

1.7 The genetically modified plants shall not be in the same plant house as other plants.

1.8 No other experiments with plant viruses shall be carried out in the plant house used for this experiment.

1.9 Plants containing different viral vectors shall be kept in different plant houses.

1.10 The plant house shall be made insect free by fumigation prior to introduction of the plants, and by sealing any gaps in the plant house.

1.11 Plants shall be grown in sterilised soil, and the soil re-sterilised prior to disposal or re-use.

1.12 A washbasin shall be located within the anteroom or in the plant house close to the entry. Where a laboratory is directly connected to the plant house, the basin may be in the laboratory.

1.13 Personnel shall decontaminate their hands by washing with soap and warm water in the wash-basin provided on entering and leaving the plant house. When entering, personnel shall put on overshoes, covering clothes (e.g. lab coat) and a hat in the anteroom. These garments shall be removed on leaving the plant house and kept in the anteroom (or laboratory) between uses and shall only be used in this specific plant house. Garments shall be laundered at least every two weeks, with overshoes and disposable hats autoclaved and disposed of after use. Clothing shall be stored and laundered in a manner that prevents contact with clothing used in other plant houses.

1.14 Contact of general plant house equipment, such as hoses, with plants containing viruses shall be avoided to prevent transmission of viruses to other plants. Equipment that comes into contact with plants that contain the viruses shall be sterilised or disinfected before being used in other plant houses or on other plants.

1.15 The plants shall be monitored at least weekly to check for the development of any flowers.

1.16 Prior to the development of any flower structures the plants shall be destroyed by autoclave or incineration.

1.17 Samples taken for DNA and protein analysis shall be transported in sealed containers to a PC2 laboratory.

2. To exclude unauthorised people from the facility

2.1 The identification of entrances, numbers of and access to entrances, and security requirements for the entrances and the facility shall be in compliance with the requirements of the standards listed in controls 1.2, 1.3 and 1.6.

[3] For the purpose of these additional controls to the PC2 planthouse, the term planthouse may refer to an entire planthouse facility, and may also refer to separate rooms within such a facility, providing that these rooms are each fully enclosed so as to effectively contain the organisms, that laboratory coats and any other protective equipment stays in that room (except when securely removed for washing), and that personnel wash their hands after each period of work in any room.

3. To exclude other organisms from the facility and to control undesirable and unwanted organisms within the facility

3.1 The exclusion of other organisms from the facility and the control of undesirable and unwanted organisms within the facility shall be in compliance with the standards listed in controls 1.2, 1.3 and 1.6, and the additional controls 1.7 to 1.14.

Additional control:

3.2 No insects that are used in the biological control of plant house insect pests shall be introduced into the plant house facility used for the containment of genetically modified tobacco plants.

4. To prevent unintended release of the organism by experimenters working with the organism

4.1 The prevention of unintended release of the organism by experimenters working with the organism shall be in compliance with the standards listed in controls 1.2, 1.3 and 1.6, and the additional controls 1.11 to 1.15.

To control the effects of any accidental release or escape of an organism

5.1 Control of the effects of any accidental release or escape of an organism shall be in compliance with the standards listed in controls 1.2, 1.3 and 1.6.

5.2 If a breach of containment occurs, the facility operator must ensure that the MAF Inspector responsible for supervision of the facility has received notification of the breach within 24 hours.

5.3 In the event of any breach of containment the contingency plan for the attempted retrieval or destruction of any viable material of the organisms that have escaped shall be implemented immediately. The contingency plan shall be included in the containment manual in accordance with MAF Biosecurity Authority/ERMA New Zealand Standards 154.03.02[2] and 154.04.09[2]

5.4 Prior to any experiments commencing all *Nicotiana* plants shall be removed from the vicinity (at least 5 metres) outside of the plant house(s) in which the experiments are being carried out, and kept clear of these species during the experiments to create an exclusion zone. The monitoring of this zone for growth of *Nicotiana* species shall be carried out at least once every 2 weeks.

6. Inspection and monitoring requirements for containment facilities

6.1 The inspection and monitoring requirements for containment facilities shall be in compliance with the standards listed in controls 1.2, 1.3 and 1.6.

6.2 The containment manuals shall be updated, as necessary, to address the implementation of the controls imposed by this approval, in accordance with MAF Biosecurity Authority/ERMA New Zealand Standard 154.03.02[2]: Containment Facilities for Micro-organisms.

Additional controls:

6.3 An experienced plant virologist shall at all times maintain oversight of the experiments and assess the plants for viral symptoms. Any symptoms that differ from those expected for the PVX vector and the fate of those plants shall be recorded.

6.4 The applicant shall carry out and record the results of DNA hybridisation experiments or other appropriate assessments to determine if novel viruses are generated. Where novel viruses are detected that do not meet the organism description or purpose of the application MAF and ERMA New Zealand should be notified immediately and all research involving the organism must cease. The organism can be held in storage for up to one year while a new approval is sought. If a new approval is not obtained within a year, the organism must be destroyed.

7. Qualifications required of the persons responsible for implementing those controls

7.1 The training of personnel working in the facility shall be in compliance with the standards listed in controls 1.2, 1.3 and 1.6

Additional controls:

7.2 An experienced virologist shall train all staff working with the organisms to ensure they are familiar with the principles of containment for plant viruses and the containment procedures of the facility.

7.4 The person(s) responsible for the particular research area and/or the person(s) responsible for the operation of the containment facilities shall ensure that all personnel who work with the Category C organisms to sign-off that they have received training from the experienced virologist on the principles of containment of viruses and of the containment procedures required by these controls.

_____ Date: 11 July 2001

Mrs Jill White, Chair
Genetically Modified Organisms
Standing Committee of the Authority

Amendment: November 2006
Changes to controls:

- Addition of footnotes to the containment facility references and the Australian/New Zealand containment facility references to "future proof" the decision
- Standardise the wording of the breach of containment control
- Removal of the control regarding inspection of facilities by the Authority, its agent or enforcement officers

_____ Date: 22 August 2007

Dr Kieran Elborough
Chair, GMO Standing Committee

Amendment: August 2011

Deletion of control 7.2 -The identity of the experienced virologist overseeing the research (and any subsequent changes) shall be recorded in the containment manual and notified to ERMA New Zealand and MAF.

Rewording of control 6.3 and 6.4 regarding testing for novel plant viruses and what to do in the event that something novel that falls outside the scope of the approval is detected.

_____ 30 August 2011

Richard Woods Date

Chair, Decision Making Committee,
Environmental Protection Authority

ANNEX 1:

Organisms approved by this application:

Escherichia coli (Migula 1895) Casterllani & Chambers strains DH5 alpha, DH10B, JM101, and JM109 modified by non-conjugative plasmid cloning vectors that may contain a ColE1 origin of replication, antibiotic resistance marker genes, the lacZ reporter gene, no more than 2/3 of the potato virus X genome, and the replicase genes from tobacco yellow dwarf virus (TYDV) (Category A development, so long as infectious pathogenic particles are not produced).
Escherichia coli (Migula 1895) Casterllani & Chambers strains DH5 alpha, DH10B, JM101, and JM109 modified by non-conjugative plasmid cloning vectors that may contain a ColE1 origin of replication, antibiotic resistance marker genes, the lacZ reporter gene, more than 2/3 of the potato virus X genome, and the replicase genes from tobacco yellow dwarf virus (TYDV) (Category C development).
Escherichia coli (Migula 1895) Casterllani & Chambers strains DH5 alpha, DH10B, JM101, and JM109 modified by pART27 or the pCAMBIA series transformation vectors containing the potato virus X vector P2C2S that is under the control of the cauliflower mosaic virus 35S promoter. In addition, these vectors may also contain the TYDV replicase genes, the *uidA* (β-Glucuronidase; "GUS") reporter gene, and the green fluorescent protein ("GFP") reporter gene (Category C development).
Agrobacterium tumefaciens (Smith & Townsend 1907) Conn strain LBA4404 modified by pART27 or the pCAMBIA series transformation vectors containing the potato virus X vector P2C2S that is under the control of the cauliflower mosaic virus 35S promoter. In addition, these vectors may also contain the TYDV replicase genes, the GUS and/or GFP reporter genes (Category C development).
Potato virus X (vector P2C2S) that may contain the TYDV replicase genes, the GUS and/or GFP reporter genes (Category C development).
Nicotiana tabacum L.1753 plants modified by pART27 or the pCAMBIA series transformation vectors containing the potato virus X vector P2C2S that is under the control of the cauliflower mosaic virus 35S promoter. In addition, these vectors may also contain the TYDV replicase genes, as well as the GUS and/or GFP reporter genes. The tobacco plants to be infected have previously had inserted into them a T-DNA vector containing the TYDV large and small intergenic regions and the replicase gene, as well as a GUS reporter gene (Category C development).