ENVIRONMENTAL RISK MANAGEMENT AUTHORITY
NGĀ KAIWHAKATŪPATO WHAKARARU TAIAO

ERMA New Zealand

FORM 4

Application for approval to

FIELD TEST (INCLUDING LARGE SCALE FERMENTATION) IN CONTAINMENT ANY NEW ORGANISM

under Section 40 of the Hazardous Substances and New Organisms Act 1996

Office use only ☐☐☐☐☐☐☐☐

Fees $_____ Date received ___/___ /___ Verified date ___/___ /___ _____Job manager

Application for approval to field test (including large scale fermentation) in containment any new organism under Section 40 of the Hazardous Substances and New Organisms Act 1996

IMPORTANT

Before you fill in this application form please talk to ERMA New Zealand. We can help you scope and prepare your application. The scale of information we need should match the potential significance of the application. For example, applications, which may pose a significant risk to the environment or to human health, need to be supported with more substantial information than applications, which clearly pose a more minor risk.

We need all relevant information early on in the application process. Quality information up front will speed up the process.

Any extra material that does not fit in the application form must be clearly labelled and cross-referenced in the application form. Commercially sensitive information should be collated in a separate document.

All applicants must sign at the end of the form and enclose the correct application fee. The standard notified application fee is $2,500 (excl GST). We are unable to process applications that do not contain the correct fee.

All references to regulations in this form, unless otherwise noted, refer to the Hazardous Substances and New Organisms (New Organisms Forms and Information Requirements) Regulations 1998.

Copies of all our application forms will soon also be available on our website: www.ermanz.govt.nz, and also in electronic form (MS Word format).

If you have any suggestions for improvements to this form, please contact our operations staff at the address below.

You can get more information at any time by telephoning, writing to, or calling in at our Wellington office. One of our staff members will be able to help you.

List of application forms for new organisms:

These are all our application forms related to new organisms. Please check you have the right one.

Form 1 Application for approval under section 34 of the Act to import for release, or release from containment, any new organism – including rapid assessment.

Form 2 application for approval under section (40)(1)(a) of the Act to import into containment any new organism.

Form 3 application for approval under section 40(1)(b) of the Act to develop in containment any genetically modified organism – including rapid assessment.

Form 4 application for approval under section 40(1)(c) to field-test (including large-scale fermentation) in containment any new organism **(this form)**.

Form 5 application for approval under section 47 to use a new organism in an emergency.

Form 6 application for approval under section 62 for grounds for reassessment of a new organism in containment.

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Application for approval to field test (including large scale fermentation) in containment any new organism under Section 40 of the Hazardous Substances and New Organisms Act 1996

Applicant details

1. Name and address in New Zealand of the applicant:

This should be the organisation or person formally responsible for this application.

Name: New Zealand Forest Research Institute Limited

Address: Sala Street, Private Bag 3020, Rotorua

Phone: 07 343 5899

2. The applicant's address for service in New Zealand (if different from above):

Address: N/A

3. Name of the contact person for the application (if different from applicant): This person should have sufficient knowledge to respond to queries and have the authority to make decisions on behalf of the applicant that relate to processing the application.

Name: Dr Christian Walter

Position: Senior Scientist

Phone: s.a.

Fax: 07 347 5444 **Email:** christian.walter@forestresearch.co.nz

4. Summary

Provide a summary of the information contained in this application relating to the identification of the organism.

The information should include summaries of:

- the identity of the organism;
- if it is a genetically modified organism, the source of the donor nucleic acid material and the purpose of the modification;
- what the organism will be used for and why it has been selected.

Provide a summary of the information contained in this application relating to the assessment of the effects of the organism.

The information should include summaries of:

- the risks, costs and benefits and the assessment of these;
- the containment system proposed.

This summary will be used to provide information for those people and agencies (eg, Minister for the Environment, Department of Conservation, Regional Councils, etc.), who shall be notified of the application, and for potential submitters who request information. This information will also be used to prepare the public notice of the application. For these reasons, applicants should ensure that this summary information does not contain any commercially sensitive material.

[No] further information

The New Zealand Forest Research Institute Limited is planning, beginning in 2000 and over the next four years, to field trial transgenic *Pinus radiata* and *Picea abies* (spruce) plants which have already been or will in the next 1-2 years be genetically engineered for resistance to herbicides. Transgenic plants from four transformation events will be planted from the special GMO facility into the trial site, along with non-transformed controls. Transgenic plants will be grown for a maximum of three years (radiata pine) or five years (spruce) respectively, before removal. The material will be assessed regularly using molecular techniques such as PCR or Northern blotting. Data will be obtained on the performance of the plants in the field (growth / height gain / resistance to herbicides), and compared to non-modified control plants.

Forest Research staff will visit the trial once a month to analyse plants and record growth parameters, health and any damage observed. Containment procedures will prevent any spread of genetically engineered material into the environment. Reproductive organs are not expected to develop during the period of this trial. However, should any reproductive organs be detected, they will be destroyed before they will be able to produce pollen or seed. At the end of the trial, autoclaving or incineration will destroy all transgenic material still in the field. Both techniques are suitable to destroy DNA in plant material, and autoclaving will be used for small amounts of material, incineration for greater amounts. The site will be monitored monthly for a period of another two years for the regrowth of any transgenic material. This procedure will be established for safety reasons, and regrowth is not expected for the following reasons:

- Shed plant material of *Picea abies* and *Pinus radiata* has never been observed to regenerate under natural conditions.
- Both species do not have the ability to coppice (regenerate from stumps).
- Seed loss is not an issue in this application. All reproductive structures will be removed before they can develop, let-alone produce viable seed. However, should any seed still be produced and germinate, it will be detected and removed during the post-trial monitoring phase.

Under artificial conditions, plant material from radiata pine and spruce can form roots from young branches when in contact with the ground. This however, requires human interference. Short (around 5 cm) tips of young branches need to be excised and placed in the ground, and watered.

A post-trial monitoring, over a period of two years, will allow the detection of such unexpected occurrence, and destruction of the material.

The total time for this trial is expected to be 11 years (see timeline at the end of this chapter). The planting dates for each set of trees will be as the material is produced, and will be notified to ERMA and MAF.

The genes and promoters included in this application are

i. Genes and promoters, which allow for selection and monitoring of transgenic tissue and plants of *Pinus radiata* and *Picea abies*
ii. Genes and promoters involved in herbicide resistance
iii. Genes and promoters that allow for selection of vectors in *E.coli*

A total of four transformation events will be produced. ***Forest Research*** holds approval to produce the constructs and transformation events in a ***Forest Research*** containment facility (operated under Australia / New Zealand Standard “Safety in Laboratories”, Part3: Microbiology (A/NZS 2243.3:1995)). Both the GMO (Genetically Modified Organisms) greenhouse on ***Forest Research*** campus and the laboratory comply to regulations for PC2 level of containment.

We demonstrate in this proposal that the risk involved with the field trial is either insignificant or low. In the highly unlikely event of gene transfer to another species, the effects would be regarded as similar to those resulting from transfer of the same genes from natural sources to these receptors. Gene transfer to other radiata pine or spruce through pollen dispersal is regarded as unlikely. Further, *Picea abies* is not native to New Zealand and cannot normally establish itself in the natural environment.

The benefits from this field trial are:

i. Research in the area of herbicide resistance will contribute to the understanding of this novel trait in gymnosperms. Herbicide resistance through genetic modification has been tested in a variety of plant species, mainly in an agricultural context. Our trial is the first test world wide on whether the trait will be useful in conifers.

ii. Genes leading to herbicide resistance in conifers may be of high value to the forest industry. Currently the use of herbicides is restricted and those applied are less environmentally friendly than the herbicides we are proposing to use in our experiment. An operational use of herbicide resistant radiata pine, genetically engineered with the genes mentioned in our application, would improve management procedures in forests, and provide a tool for better management and protection of improved genetic material.

iii. Research in this area will maintain our front-end status in conifer genetic engineering. ***Forest Research*** is leading conifer genetic engineering world wide. Herbicide resistant radiata and spruce were first produced in this institute, and to maintain our front-end status we need to demonstrate a capability to trial these plants in field situations.

iv. Research in this area may provide the New Zealand forest industry with an environmentally friendly strategy for weed control (use of environmentally friendly herbicidal formulations). The herbicides in this application are less toxic than those currently in use. This is due to their rapid breakdown to non-toxic products, once they come in contact with soil microorganisms.

v. The results of this trial will help us to ascertain the behavior of transgenes in a field situation. This contributes data to the public debate on genetic engineering.

Currently used herbicide formulations in New Zealand forestry are not ideal from a forest management or environmental point of view.

The risks associated with this trial generally relate to the possibility of the engineered genes entering the environment or with risks associated to the integration of new genes themselves. Risks associated with human interference, sabotage or natural disaster may also be present. All of the genes are derived from natural sequences and homologues are already present in numerous organisms in the environment. This reduces the risks associated with this trial, and we show that we have management procedures in place that reduce any risk to very low levels.

In summary, the results from this field trial are expected to benefit the New Zealand forest industry, the scientific community worldwide, and New Zealand as a whole. In our opinion, the benefits of this study will significantly outweigh any possible risks.

Timeline:

Planting of transgenic plants	2000-2004, when plants become available
Assessment of transgenics	Max 5 years after planting (max up to 2009), according to monitoring plan
Monitoring of trial site after removal of all plants	2 years (max up to 2011)
Report to ERMA and MAF	At completion of trial

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Organism details

5. The identification of the organism:

This should include all information necessary to identify the organism and should include:

- the taxonomic classification and name of the organism;
- the essential characteristics that identify the organism and its behaviour in the environment;
- sufficient information to enable the Authority to uniquely identify the organism in the register as required by section 20(2)(b) of the Act.

(This section may also include the name by which the organism is generally known.)

The information in this section would include, for example, information on the habitat range and climatic sensitivity of the organism. References to the scientific literature supporting this information should be given here if appropriate.

In the separate box below the applicant should provide the name of the organism suitable for inclusion in the Authority's public register.

Information that is commercially sensitive should be clearly identified. If supplied separately, a cross-reference to it should be included.

Taxonomic Name: The parent organism used in this study is *Pinus radiata* D Don, an exotic conifer introduced into New Zealand from native stands in California, and *Picea abies*, an exotic conifer introduced to New Zealand from Northern Europe.

Characteristics

Pinus radiata is an outcrossing monoecious species which relies on wind as a pollen vector. The cones are largely serotinous, although plants can establish from any seed shed from the tree. The population of *Pinus radiata* that is used in commercial forestry originally derived from the New Zealand "land race", which was the product of informal selection (i.e. natural and silvicultural) since the first introduction of the species to New Zealand from its natural habitat in California in the 1860s. The species is used extensively in New Zealand, Chile, Australia, Spain and South Africa for growing for sawn timber and pulpwood. It will grow well under temperate (15-25°C) conditions with moderate precipitation (500-3500 mm pa), spread evenly through the year. It will not grow reliably under freezing conditions, or under conditions of high humidity in spring / summer (Grace 1991). *Pinus radiata* does not coppice.

Picea abies is an exotic conifer introduced from Northern Europe (Norway / Sweden / Denmark). It is a widespread forest tree in the central and northern part of Europe where it plays a vital role in the international trade balance. It is one of the most important conifers in Europe for timber, pulp and paper production and has also several other applications. As a result of its great economic importance, there is an interest to understand its biology in greater detail aiming at future improvement of features such as disease resistance, wood and fiber characteristics and reproductive development.

Vegetative propagation of *Picea abies* by cuttings is possible, but regeneration from tree stumps (coppicing) has never been observed (Mike Menzies, pers. communication).

Life cycle of *Pinus radiata* and *Picea abies*

In its natural habitat radiata pine can get to an age of 100 years, but in plantation forestry in New Zealand it rarely grows for more than 28-30 years before it is harvested. Conventional breeding has significantly improved the growth and form characteristics. Sexual maturity is usually reached by the age of five to eight years, depending on the location and also on the clone. However, viable seed is not produced until more than 2 years after the tree

reaches sexual maturity (ie initiation of formation of reproductive structures). Consequently, pollen shed can be expected soon after sexual maturity is reached, seed shed will be two years later. For a timeline on radiata pine reproductive development see section 8, "Development of reproductive organs on radiata pine".
Picea abies is mainly grown in northern European countries and can reach an age of more than a hundred years. Its sexual maturity is reached at a minimum of 15-20 years, which is long after this trial will be finished and trees destroyed. The reproductive cycle in this species is irrelevant for this application.

Pollen and seed travel:
Radiata pine pollen must successfully penetrate the micropylar chamber of a receptive female strobilus within a few days after being shed, in order to be viable, and must subsequently (1 year later) fertilise receptive ovules (Lill, 1976). Pollen travel is by wind, and most pollen (90% or more) travels less than 300 meter from a point source (Wright, 1952, Wang et al, 1960). However, small amounts of pollen may migrate large distances (Di-Giovanni and Kevan, 1991). Radiata pine seed is normally held in serotinous cones , which open under hot, dry conditions, allowing seed to fall to the ground. Most pine seeds fall within 100m of the parent tree (e.g. Rudis *et al*, 1978). Green cones (containing unripe seeds) are sometimes eaten by possum, and mature seed may be eaten by birds and rodents. Seed may remain viable for up to 10-15 years, under favourable (dry) conditions. Seed will germinate when coming in contact with moist ground, and radiata pine seedlings will develop readily under warm conditions, with moderate light and available moisture.
Picea abies will not produce reproductive organs before age 15 years. Consequently, pollen and seed travel is not regarded as an issue in this application. However, as a safety measure, the plants on the trial site will be regularly (monthly) monitored for the appearance of reproductive organs, and any occurring will be cut off and incinerated or autoclaved. Both are methods to destroy DNA and living material. The trial site will also be monitored for another two years after the trial is completed to ensure that no living transgenic material remains.

The development of reproductive organs on radiata pine: timeline

The following timeline describes the reproductive development process in radiata pine.

Month	Development	Comments
February	Vegetative meristems become influorescent. Reproductive development switched on	Not visible from outside
Around March	Reproductive organs become visible	
July	Some clones will shed pollen	Some pollination takes place
August	Most clones shed pollen Female cones become receptive	Bulk of pollination takes place
September	Some clones shed pollen	Some pollination takes place
August year1 – November year 2	Female cone develops and matures	
November year 2	Fertilisation occurs	
End of March year 3	Embryos in female cone mature	
September year 3	Seed shed	

In summary, the reproductive development of radiata pine from the initiation of reproductive organs to the shed of seed takes more than 2.5 years. Please also see Bollmann and Sweet 1976 a and b.

The reproductive development in *Picea abies* is not described here because it is irrelevant to this application. *Picea abies* will not produce reproductive structures before a minimum age of 15 years. This is long after all plants in this application will have been removed and destroyed.

Source of plant material:

Forest Research, on behalf of the NZ Radiata Pine Breeding Cooperative, manages a breeding population of *Pinus radiata* aimed at genetic improvement of the species through recurrent selection. The most highly improved progenies are multiplied for forest plantations, employing techniques of controlled pollination and vegetative multiplication (vegetative propagation) (Carson *et al.*, 1992; Menzies, 1994). In this study, embryogenic cell lines of *Pinus radiata* were genetically modified (Walter *et al.*, 1998a, b). These lines are derived from open pollinated (OP) immature zygotic embryos or from immature zygotic embryos (Smith, 1996) that have resulted from controlled pollination using superior, progeny tested genotypes. The *Picea abies* material used in this study was obtained as sterile tissue culture from Norway (Norwegian Forest Research Institute). The disease-free status of the material was monitored by the suppliers (Phytosanitary certificate supplied with tissue) and MAF authorities in New Zealand. Tissue transformation and plant regeneration takes place in the laboratory and the GMO greenhouse using techniques described in Walter et al, 1999.

List of transformation events:

Pinus radiata (*bar*)
Pinus radiata (*ALS*)
Picea abies (*bar*)
Picea abies (*ALS*)

The term in brackets indicates the gene of interest introduced into the respective species. Bar stands for a resistance gene against the herbicide “Buster” and ALS stands for a family of resistance genes against sulfonylurea herbicides (for example: “Escot”).
Each transformation event will result in a number of transgenic lines, and from each line, plants can be regenerated. Some plants will be retained in the containment laboratory for further analysis, while others will be transferred to the GMO greenhouse for propagation before planting in the trial.

[No] further information

[No] commercially sensitive information

Name of the organism that may be used for the Authority’s public register:

Pinus radiata D.Don (radiata pine)

Picea abies Kaarst (spruce)

6. If the organism is a genetically modified organism, information on the details of the genetic modifications:

This information shall include full details of the genetic constructs and modifications and the source and characteristics of the foreign nucleic acid.

This information should clearly identify the source of the donor genetic material and the characteristics. The desired characteristic (eg, herbicide resistance) and any other significant characteristics that may be expressed by the donor genetic material in the organism should be described.

Information on the stability and homogeneity of the construct should be given, if known. If this information is not known then this should be explicitly stated. References to the scientific literature supporting this information should be given here if appropriate.

Information that is commercially sensitive should be clearly identified. If supplied separately a cross-reference to it should be included.

[No] further information [No] commercially sensitive information

Plasmid vectors used for transformation of *Pinus radiata* and *Picea abies*, to introduce herbicide resistance:

Vectors for transformation of embryogenic tissue contain a selection gene (*npt*II), a *uid*A reporter gene, an antibiotic selection gene for maintaining these vectors in *Escherichia coli* bacteria (the *bla* gene for ampicillin resistance), and a further gene potentially introducing herbicide resistance in regenerated transgenic plants. These include the *bar* gene for resistance against the herbicide "Buster", and variations of the *ALS* gene for resistance against sulfonylurea herbicides. All vectors are based on the bacterial cloning vector pUC (Vieira and Messing, 1982). Promoters include a bacterial promoter for expression of the *bla* gene, the Cauliflower Mosaic Virus (CaMV) promoter for expression of *npt*II in plant tissue, the maize ubiquitin promoter for expression of genes such as the *uid*A gene, the ubiquitin promoter from *Pinus radiata* and the FMV promoter from figwort mosaic virus.

Construction of vectors:
Vector cloning is according to standard molecular cloning techniques (Sambrook *et al.*, 1989). See Appendix for examples of vectors. Further vectors will be cloned, and this will be done using the same principal techniques and genes and promoters as described in Tables 1 and 2. However restriction sites may be different. The vector backbone however, will be the same (pUC) in all constructs. It contains the bla gene (Ap-r) driven by the lac promoter (see tables 1 and 2, and below (Expected characteristics of genes introduced / Expected characteristics of promoters introduced)). Plant transformation will be performed using techniques described by Walter 1998a, b, and Walter *et al.,* 1999.
All cloning and transformation experiments are performed in laboratories that comply to the Australia / New Zealand Standard "Safety in Laboratories", Part3: Microbiology (A/NZS 2243.3:1995).

Gene transfer to *Pinus radiata* and *Picea abies*:
Biolistic transformation of embryogenic tissue is performed according to a protocol described by Walter *et al.* (1998a, b, and 1999). Briefly, the DNA to be transferred is coated onto gold particles and bombarded into embryogenic tissue using the DuPont biolistic particle apparatus (PDS 1000He). Tissue with the selection gene *(npt*II) is propagated on selective media supplemented with the antibiotic geneticin. Non-transgenic tissue dies on these selective media. Selected tissue is used for somatic embryogenesis and regeneration of transgenic plants. Molecular analysis is performed at the tissue and plant stage. Plants are being and will be grown in the ***Forest Research*** GMO greenhouse until released for field trials. The GMO greenhouse complies to standards set out in the Australia / New Zealand Standard "Safety in Laboratories", Part3: Microbiology (A/NZS 2243.3:1995).

Integration of new genes:
Molecular analysis is used to confirm the presence and integration of novel genes into transgenic material. PCR techniques, Southern and Northern blotting, ELISA, histochemical and fluorometric GUS staining are examples of techniques being used to confirm the status and expression characteristics of the integrated DNA. Protocols are described in Walter *et al.* (1998a and b, and 1999).

Stability of new genes:
Foreign genes in transgenic plants are subject to a range of mechanisms that may affect the stability of their expression. Silencing mechanisms are well known and described in angiosperm plants (Matzke & Matzke, 1995), but information for conifers is scarce. Indications from transformation experiments (Walter *et al.,* 1998a and pers comm.) confirm the continued expression of introduced genes in 2-year-old transgenic radiata pine. Recent studies with *Pinus radiata* and *Picea abies* plants resistant to the herbicide Buster indicate the stability of this construct in both species: Transgenic plants are resistant against herbicidal spray tests (Grace, pers. comm.). However, the result of such silencing would have a negative impact in terms of the commercial application of a gene, but is not expected to have a negative effect on the environment or on human health.

The project

This field trial is an extension of research performed at ***Forest Research*** in containment laboratories. *P. abies* and *P. radiata* plants resistant to the herbicide formulation Buster have been produced using the introduction of the bar gene. The plants have been spray tested with the herbicide, and were confirmed transgenic and resistant. A publication describing the research and the results was submitted to an international scientific journal recently. The proposed field trial will analyse the behaviour of those plants in a field situation, particularly in terms of growth performance and herbicide resistance, compared to controls. Another gene that makes plants resistant against sulfonylurea herbicides, will be included in further genetic engineering experiments, and field trialing.

The data collected in this trial will contribute to the public debate on the use of genetic engineering technologies in forestry.

Expected characteristics of genes introduced (also see Table 1):

Genes used in this application are used to select for and/or monitor tissue and plants for their transgenic nature, and some are expected to introduce herbicide resistance to *Pinus radiata* and *Picea abies*.

In detail:

(a) The *npt*II gene (Beck *et al.*, 1982) for selection of transgenic tissue on the antibiotic geneticin:
The *npt*II gene (Genbank accession number: U00004 and L19385) was originally isolated from the gram-negative bacterium *Escherichia coli*, and the sequence found on the bacterial transposon Tn5. The gene product, an aminoglycoside 3'-phosphotransferase, confers resistance to aminoglycoside antibiotics such as kanamycin, geneticin and neomycin. Aminoglycoside antibiotics are inactivated by the transfer of a phosphate group to the antibiotic (Sambrook, 1989).
This gene is widely used in bacterial genetics (Sambrook, 1989) and plant transformation experiments (Klee 1985; An *et al.*, 1985; Ma *et al.*, 1992; Datla *et al.*, 1992; Jones *et al.*, 1992). It is present on the bacterial transposon Tn5, and can spread naturally across gram-negative bacteria through conjugation and transposition. Consequently, *npt*II can be regarded as abundant in gram negative bacteria (Bukhari *et al.*, 1977; Berg and Berg, 1983; Pühler and Timmis, 1984, Clark and Warren, 1979). For a study on the biosafety of this gene see Nap et al., 1992.

(b) The *bla* gene for selection of transgenic *Escherichia coli* bacteria containing plasmid vectors (Vieira and Messing, 1982; Blattner *et al.*, 1997):
The *bla* gene (Genbank accession number: AE000487 and U00096) was isolated from the gram negative bacterium *E. coli* and is present on a wide range of cloning vectors used in bacterial and eucaryotic genetics. It codes for the production of a beta-lactamase, an enzyme that cleaves the beta-lactam ring of beta lactam antibiotics (such as penicillin and ampicillin), rendering them inactive. The *bla* gene is part of the *E. coli* K12 genome. Beta-lactamases are also found in other microorganisms, such as *Klebsiella ozeanae*, where the sequence is located on a plasmid (Huletsky *et al.*, 1990, Genbank accession number: M95179 and M35595).

(c) The *uid*A reporter gene for confirmation and continuous monitoring of the transgenic nature of tissue and plants (Jefferson *et al.*, 1986; Schlaman *et al.*, 1994):
The *uid*A gene (Genbank accession number: S69414) was isolated from the gram-negative bacterium *E. coli*, and is located on the bacterial chromosome. *Uid*A codes for the production of the enzyme beta-glucuronidase, which is an acid hydrolase catalysing the cleavage of a variety of beta-glucuronides. The gene is very useful in monitoring transient and stable gene expression in genetically engineered plant tissue and organs (see Gallagher, 1992 for a summary). When cells are incubated with a specific colorless staining substrate, they will

turn blue when the enzyme is active, confirming a successful transformation event. For a study on the biosafety of this gene see Gillissen et al., 1998.

(d) The *bar* gene for resistance against the herbicide glufosinate-ammonium (Buster) (DeBlock 1987).
The *bar* gene (Genbank accession number: X17220) was isolated from of the bacterium *Streptomyces*. It encodes a phosphinothricin acetyltransferase (PAT) and is widely applied in genetic engineering. The respective enzyme can use phosphinothricin (the active ingredient of the herbicide formulation Buster) as a substrate and will acetylate it to acetyl-phosphinothricin. This product is non-herbicidal to plant cells and is also degraded rapidly by the plant metabolism. The end products of this degradation process are water, phosphate and carbon dioxide.

(e) The ALS gene (*csr*1-1, sometimes termed *aro*-A or HRA) for resistance against sulfonylurea herbicides (Brasileiro 1992). ALS stands for a family of very similar genes.
This gene (Haughn 1986; Li *et al.*, 1992) was isolated from a mutant *Arabidopsis thaliana* plant found in nature, and which was resistant against sulfonylurea herbicides. The gene codes for an acetolactate-synthase, which is changed by naturally occurring mutation processes to have a lower affinity to sulfonylurea herbicides. Consequently, the herbicide is less toxic and the plant carrying this enzyme can survive herbicide applications. Introduction of this gene or improved sequences for higher resistance levels, have resulted in the production of sulfonylurea-herbicide resistant plants (Botterman and Leemans, 1988; Hartnett *et al.,* 1990; Haughn *et al.,* 1988). The resistance mechanism is always based on a modified enzyme, which does not bind to the sulfonylurea class of herbicides, as described above.

Expected characteristics of introduced promoters (Table 2):

(a) The Cauliflower Mosaic Virus (CaMV) promoter (35S):
This promoter was isolated from the Cauliflower Mosaic Virus (Franck *et al.*, 1980) which infects cauliflower and a range of other plants. It has been used widely in plant transformation experiments and controls a variety of foreign genes in transformed monocotyledonous and dicotyledonous plant species. 35S has been successfully used to express foreign genes in gymnosperms (examples see Rey *et al.*, 1996, Charest *et al.*, 1996, Drake *et al.*, 1997, Humara *et al.*, 1998, Walter *et al.*, 1998 a and b). Experiments in *Pinus* and *Pseudotsuga* species have confirmed the function of this promoter, but have also indicated that the expression level is significantly lower compared to other promoters such as the maize ubiquitin promoter (Walter *et al.*, in preparation).
For a discussion of risk associated with the use of the 35S promoter, please see section 14.

(b) The maize ubiquitin promoter (Christensen 1989):
This promoter was isolated from maize. Expression of genes under the control of this promoter is up to ten-fold higher compared to the 35S promoter (Christensen *et al.*, 1992). Experiments with gymnosperms have indicated that this promoter expresses at a significantly higher level than CaMV 35S (Walter *et al.*, in preparation).

(c) The *Agrobacterium tumefaciens* nos (Nopalin Synthase) promoter:
This promoter is part of the *Agrobacterium tumefaciens* genome and has been isolated from the Ti-plasmid (Depicker *et al.*, 1982). In *Agrobacterium*, it expresses the nopalin synthase gene, which codes for a gene involved in the production of nopalin. When infecting susceptible plants, *Agrobacterium* transfers this gene with its promoter into host plant cells, where it is expressed and where nopalin is produced. The promoter has been used in various plant transformation experiments (for examples see An *et al.*, 1985, Klee *et al.*, 1985, Jones *et*

al., 1992, Datla *et al.*, 1992, Ma *et al.*, 1992). The nos promoter is active in *Pinus radiata* embryogenic tissue (Walter, unpublished).

(d) The lac promoter:
This bacterial promoter is part of the *E. coli* lactose (lac) operon (Genbank accession numbers J01636, J01637, K01483, and K01793). It controls constitutive expression of genes in gram negative bacteria and is part of a wide range of bacterial cloning vectors (for example the pUC derived vectors). It is used to express the bacterial resistance gene *bla* for resistance against beta-lactam antibiotics (Blattner *et al.*, 1997). In this proposal, the lac promoter together with the *bla* gene is used to select for the presence of pUC derived plant transformation vectors in *E coli*.

(e) The FMV promoter from the Figworth Mosaic Virus (Genbank accession numbers XO6166 and X16673):
This promoter was isolated from a virus that is very similar to the Cauliflower Mosaic Virus. The promoter was tested in transient expression studies and was found to induce expression of genes located downstream of the promoter approximately two fold higher than the CaMV 35S promoter (Walter, pers. comm.). In this proposal, FMV, and an enhanced version of FMV (eFMV, Maiti *et al.,* 1997; Cooke, R. 1990; Sanger *et al.,* 1990)will be used to control the expression of herbicide resistance genes such as bar and ALS.

(f) The promoter of the *Pinus radiata* polyubiquitin gene (Moyle *et al.*, Genbank submission in preparation):
This promoter was isolated from *Pinus radiata* based on the cDNA sequence of polyubiquitin genes from other species. This class of genes is usually expressed constitutively at a high level, which makes the promoter ideal for expressing genes at high levels, in all organs, and during all stages of the development of the tree.

In summary, all genes and promoters to be used in this trial are derived from natural resources - none are the result of artificial DNA synthesis. The genes and promoters are abundant in nature, and some of them are found in bacteria inhabiting the human gut (i.e. *npt*II, *uid*A, *lac and bla*). The same is the case for promoters used in this application. They are derived from abundant microorganisms or plants. The CaMV 35S promoter is derived from a plant virus that infects a range of plants that are part of the human diet (See discussion of effects, section 14).

7. The reason why an application is necessary for the organism:

Refer to the definitions set out in Section 2 of the Act, to the prohibited organisms in the Second Schedule of the Act, and for genetically modified organisms, to the exemptions in the HSNO (Organisms Not Genetically Modified) Regulations 1998.

Pinus radiata and *Picea abies* are not prohibited organisms under the Act. The application is necessary because the field trial described in this proposal includes genetically modified *Pinus radiata* and *Picea abies* plants. Genes have been inserted by *in vitro* techniques.

8. The nature and method of the field trials and the experimental procedures to be used:

Information on the location and duration of the field trial and associated facilities. Details of the target ecosystem, full trial design and experimental plan, capability of the test organism to disperse and survive, specific potential effects being tested, and methods of disposal of the organism from the test site.

Responsibilities:

Forest Research will be responsible for all aspects of the trial. No part of the work will be subcontracted to a third party. ***Forest Research*** will, through its IBSC (Internal Biosafety Committee) appoint a principal investigator to the trial. This person will have the following responsibilities:

- Carry out and supervise planting and analysis
- Establish and supervise the monitoring regime
- Supervision and training of relevant staff
- Reporting to IBSC, ERMA and MAF

The principal investigator will have the power to delegate individual tasks to appropriate staff members.

Location and duration of the field trial, and monitoring:

The planting for this field trial will be conducted over the next four years, in Rotorua, New Zealand. A map of the trial site is attached in the Appendix, and is confidential to ERMA to reduce the likelihood of sabotage. The trial will be supervised over the full period (minimum of monthly visits to the trial site) . During each visit, the following items will be observed for each tree, and recorded in a log book.

- Growth normal? Any abnormalities observed?
- Does the plant show any signs of damage by insects or pathogens?
- Development of reproductive structures? (These will be removed immediately and destroyed)

During the trial, herbicide sprays will be applied to confirm the continued expression of herbicide resistance genes, and to quantify the resistance level of individual plants.

The close environment of the trial site includes plantations of several different *Pinus radiata* genotypes, as well as other conifer and eucalyptus species. Naturally regenerating trees are removed if they appear. Further information is submitted to ERMA in confidence.

This trial will be planted over a period of four years, and the plants kept for a period of up to five years.

In total, and including the 2-year monitoring phase after the trial is completed, the trial will operate for a maximum of 11 years.

Non-transgenic radiata pine will be planted in parts of the trial area that is not used at the time. This will help to make the site appear “non-suspicious” to possible saboteurs.

Details of monitoring are described in this section, below.

Removal of reproductive structures:

The development of reproductive structures will be detected during monthly visits and individual inspection of every tree. They will be removed long before pollen or seed can develop, and destroyed by autoclaving or incineration. Both methods are suitable to destroy DNA and living matter.

Height of trees in field trial:

Pinus radiata is expected to grow to a maximum height of 3m (3 year trial), and spruce to a height of around 2m during the trial period of five years.

Associated facilities:

The ***Forest Research*** campus contains all molecular biology, plant propagation, GMO greenhouses, and nursery and maintenance facilities necessary to perform and supervise the trial, as well as to perform all analysis and, if necessary, removal procedures. The laboratory where transgenic plants were produced, complies to the Australian / New Zealand standard for Safety in laboratories (AS/NZS 2243.3:1995). The GMO facility (glasshouse for genetically modified organisms) is operated under PC2 containment procedures, under the same standard.

Details of the target ecosystem:

The area for the proposed trial is not considered to be vulnerable to any major disturbances such as extensive flooding or fires. Disturbances resulting from volcanic or seismic activity may be expected in this area. However, from the perspective of this trial, additional contingency plans to cope with such conditions are not necessary, since such a disaster would effectively destroy the plantation. However the following plans are set up to manage the plantation in the event of:

1. The premature cessation of the project.
   All material in the field will be removed and incinerated. The area will be monitored for two years after the cessation of the project, and any regrowth will be destroyed by incineration. ERMA and MAF will receive a final report after the two year time period.
2. The abandonment of the campus due to transient volcanic activity or other natural disaster:
   Once staff are allowed back onto the campus, the plantation will immediately be assessed and action taken to prevent any spread of material. Material that has to be removed will be incinerated.
3. The trial is managed under the responsibility of New Zealand Forest Research Institute Limited.
   The institute will, at all times, provide a manager (the principal investigator) to supervise the trial.
4. Deliberate sabotage of the trial.
   We are not planing to fence the trial area in a way that would prevent access to saboteurs. Such a structure would rather attract possible saboteurs. Further, a structure, that can prevent access under all circumstances, would be impossible to construct, and beyond any economics. Strategies to reduce the likelihood of sabotage are described above.

   If in an act of sabotage some or all trial material is destroyed, we will consider partial or total replanting based on additional material in the GMO greenhouse. Prior to that, any destroyed plant material will be collected and destroyed by incineration.

Full trial and experimental plan:

We propose to plant up to thirty transgenic *Pinus radiata* and *Picea abies* plants per line of each "transformation event", together with non-transgenic controls. The trial area for this application is capable to hold up to 330 trees. Planting is planned to commence in 2000 and finish in 2004. The trial will continue over a maximum of 5 years per transformation event. The trial has 4 transformation events, listed under section 5. Each event will produce a number of lines. This number can be as low as 1 or as high as several hundred. It depends on the individual transformation experiment and is not predictable. We estimate an average of 5 lines per event. The total number of plants is defined by the size of the trial area, and we will not extend this area.
As an example, the following scenario will illustrate this:

4 transformation events plus 1 non-transformed control

5 lines per event,
Planting of an average of 9.4 plants per event
Total number of plants: 5*5*13.2=330 plants.
This is within the capacity of trial site.

ERMA and MAF will be informed of exact numbers of plants per transformation event before the planting commences.
Growth and development of the plants will continuously (monthly intervals) be monitored and logged by ***Forest Research*** staff under the close supervision of the Principal Investigator. The Principal Investigator will be a scientist closely related to the project, and appointed by the IBSC. Molecular analysis to monitor the continued expression of transgenes will be performed as needed using techniques such as PCR, Northern and Southern blotting, gus staining and ELISA. The general morphology and, in particular, the apparent physiological age of the transformed plants will be compared with seedling controls. This evaluation will be carried out during monitoring visits, and plant height will be recorded. Reproductive development is not expected but will be assessed and any reproductive structures removed (see also: Section 15, containment system). Plants will two to three times during the trial be spray tested with operational herbicide formulations to confirm continued resistance against the respective herbicide. Upon termination of the experiment, all plants will be cut at ground level, and incinerated. Coppicing of stumps or regeneration from roots has not been observed in *Pinus radiata* over the 50-plus years of tree breeding research at ***Forest Research*** (M Carson, pers. comm.), and is not expected for *Picea abies*. However, the area will be continuously monitored (monthly) for two years after completion of the trial, for any re-emergence of transgenic material. Research indicated that seed of spruce and pine would either germinate shortly after it has reached the ground, or be destroyed by fungal attack.
In our trial, the conditions would favour germination of any shed seed within a year and resulting plantlets would be easily detectable. We have chosen a period of 2 years post monitoring to make sure that no growth of seedlings from shed seed goes unnoticed.

Transport of transgenic material to and from the trial site (GMO house, laboratory, and incineration facility) will be in sealed plastic containers and only by authorised personnel.

Time line for this proposal:

Planting of transgenic plants	2000-2004, when plants become available
Assessment of transgenics	Max 5 years after planting (max up to 2009), according to monitoring plan
Monitoring of trial site after removal of all plants	2 years (max up to 2011)
Report to ERMA and MAF	At completion of trial

Plants for this trial will not become available at the same time and planting is expected to spread across years one to four. All plants will be removed from the trial prior to them becoming sexually mature. Any developing reproductive structures will be removed from the plants, once they are visible. From the time they become visible, to maturity will be several months during which they can be detected and removed. See also above, section 5

"Organism details" for a timeline of radiata pine reproductive development. For spruce, no development of reproductive structures is expected during the length of this trial.

Monitoring plan for each transformation event:
Each transformation event will become available for planting in the trial at a different time over the next 4 years. A monitoring plan will be set up by the principal investigator for each transformation event, following the rules specified here:

- Plants added to the trial will be monitored once a week for the first two months, to ensure establishment of the plant in soil
- Thereafter, plants will be monitored on a monthly basis
- Starting in January each year, plants will be monitored on a weekly basis, for development of reproductive structures. It is not expected that any will develop and we include this item for safety reasons.
- In June each year, monitoring will be reduced to monthly. The production of reproductive structures is regarded long finished by this time.

Details on monitoring:
During monitoring visits to the trial, every tree will be assessed for the following items, and the results logged in the trial logbook:

- Growth normal? Any abnormalities observed?
- Does the tree show any signs of damage by insects or pathogens?
- Any other interference?
- Development of reproductive structures?
- Plant material removed?
- Was removed plant material destroyed or used for further work?
- Actions taken

ERMA and MAF will be notified of planting dates, and will receive a copy of the monitoring plan for each transformation event.

For a site map see appendix 4 (Information confidential to ERMA).

Standards and internal procedure:
The facilities used for the experiments comply to Australian / New Zealand standards for safety in laboratories (Part3: Microbiology, AS/NSS 2243.3:1995). With regard to the GMO glasshouse, the relevant MAF standard for genetically modified plants is not yet finalised, and the glasshouse will be registered against controls imposed by the Authority. The glasshouse complies with PC2 requirements of the above mentioned standard (AS/NSS 2243.3:1995).

Site map:
For a site map see appendix 4 (CONFIDENTIAL to ERMA)

Other matters:
To the best of our knowledge, there are no other matters to be discussed at this stage.

See comments 12,13,14,15

9. The purposes of the field testing and large scale fermentation for which approval is sought:

Reference should be made to the purposes specified in section 39(1) of the Act and the information should also provide sufficient details on the purpose of the application to enable the Authority to provide the information required in the register (under section 20(2)(c) of the Act).

The information in this section should be as expansive as possible. While the applicant may have only one potential use in mind, an approval would enable other uses as well. To enable the Authority to have access to all relevant information all the potential uses of the organism should be provided. The information on how well the organism performs these uses is necessary to enable the Authority to determine the performance characteristics of the organism.

In the separate box below the applicant should provide a statement describing the purpose for making the application suitable for inclusion in the Authority's public register.

Information that is commercially sensitive should be clearly identified. If it is supplied separately a cross-reference to it should be included.

The purpose of field testing the organism that approval is sought for, is:

- To grow radiata pine that has genes for herbicide resistance integrated, in a field environment
- To obtain scientific data on the expression characteristics of novel genes in conifers
- To obtain scientific data on the growth characteristics of genetically modified radiata pine
- To obtain data on the continued herbicide resistance of transgenic radiata pine plants under field condition
- To provide the public with data relevant to decision making on the safety of use of genetically modified conifers in New Zealand
- To ascertain whether genetic engineering of conifers changes any visible or otherwise assessable growth or development pathways in the plant, over a time period extending that possible in a GMO greenhouse, and under field conditions

[No] further information [No] commercially sensitive information

Provide in this box a statement describing the purpose for making the application. This statement may be included in the Authority's public register (please use a maximum of 255 characters):

Application for approval under section 40 (HSNO Act 1996) to field test, in Rotorua, New Zealand over a period of 10 years, *Pinus radiata* and *Picea abies* plants genetically engineered in herbicide resistance. The total duration of this project is 11 years.

10. Information on any likely inseparable organisms:

Information should be provided on any organism, which is unable to be separated from any new organism at the time of making the application. Examples may include foot and mouth and scrapie causing organisms in animals and viruses in plants.

[No] further information

All transgenic material used in this application has been regenerated from sterile embryogenic or organogenic tissue cultures which are not known to contain any microorganisms or any pests or diseases. Transgenic plants are grown in sterile containers in the light rig before planting in soil in the GMO greenhouse. Before planting, they are checked for the presence of any disease or associated insects. However, the presence of these is regarded as unlikely since the GMO greenhouse is continuously maintained for the absence of disease and insects.

Assessment of Effects

The information to be provided in these sections should cover the assessment of effects (both adverse and positive) of the organism. Where appropriate these sections may be combined in section 14 below.

Effects should be clearly assessed where relevant, including details as to how the risks will be controlled by the proposed containment system. **Where these adverse effects are identified, in the first instance by the applicant, as being minor then these do not require in-depth assessment.**

11. Information on all the possible adverse effects of the organism on the environment:

This should include information on the effects of the organism on ecosystems, public health, and Maori culture and taonga. It should also include information relevant to the matters in sections 4, 5, 6, 7, 8, and 37 of the Act and any regulations made under section 41 of the Act. The assessment should identify and assess risks, costs and benefits.

The information should give particular regard to:

Environmental and ecosystem effects (section 6(a) and (b) of the Act)

- assessment of the known and possible adverse effects throughout the life cycle of the organism on the sustainability of native and valued introduced flora and fauna and on the intrinsic value of ecosystems. *[Include an assessment of the ability of the organism to establish an undesirable self-sustaining population and the ease with which the organism could be eradicated if it was established.]*

Public health effects (section 6(c) of the Act)

- assessment of the known and possible adverse effects throughout the life cycle of the organism on public health. *[Assessment should take account of aspects of public health and safety including, where appropriate, effects from occupational exposure and effects from environmental exposure to the organism.]*

Relationship of Maori with taonga (section 6(d) of the Act)

- assessment of the known and possible adverse effects throughout the life cycle of the organism on the relationship of Maori and their culture and traditions with their ancestral lands, water, sites, wahi tapu, valued flora and fauna, and other taonga. [Include details of consultation (if any) carried out.]

The ability of the organism to escape from containment.

Information on all the possible adverse effects of the organism on the environment: see section 14

12. In the identification and assessment of risks, costs and benefits and other impacts which may occur as a result of the escape of the organism include those matters set out below.

The information should comprise of the risks identified and include:

- the nature of the adverse effects of the organism.
- the probability of occurrence and the magnitude of each adverse effect.
- the risk assessed as a combination of the magnitude of the adverse effect and the probability of its occurrence.
- the options and proposals for managing the risks identified.

- the uncertainty bounds on the information contained in the assessment, expressed quantitatively where possible but otherwise through narrative statements.

The identification and assessment of costs and benefits required in each application must include.

- the nature of the costs and benefits associated with the proposed new organism and whether they are monetary or non-monetary;
- the magnitude or expected value of the costs and benefits and the uncertainty bounds on the expected value.

Relevant costs and benefits will be those which pertain to the New Zealand economy, society and environment and which would not arise if the application was not approved (ie the opportunity cost to New Zealand). They shall include the long term as well as short term, and consequential as well as direct costs and benefits.

The information on risks, costs and benefits shall include the distributional effects over time, space and groups in the community. It shall also include the uncertainty intervals associated with these estimates.

Identification and assessment of risks, costs and benefits and other impacts which may occur as a result of the release of the organism include those matters set out below: See section 14

– **Information on the positive effects of the organism:**

Information on the positive effects of the organism: see section 14

14. Assessment of effects

If the assessment of effects is combined into this section, applicants should clearly indicate how the information requirements in sections 11, 12 and 13 of this form are addressed.

[No] further information

[No] commercially sensitive information

The assessment of effects is combined into this section 14.

POTENTIAL RISKS

The probability of occurrence and the magnitude of adverse effects

In this application, the assessment of risk is based on the use of qualitative descriptors. ERMA New Zealand have developed a set of descriptors to quantify the likelihood and magnitude of an effect. These descriptors are specified in Tables 3 and 4.

Descriptor	Description
Very unlikely	Not impossible, but only occurring in exceptional circumstances
Unlikely	Could occur, but is not expected to occur under normal conditions.
Likely	Will probably occur at some time.
Very likely (almost certain)	Is expected to occur

Table 3: The likelihood of a risk to occur. Adapted from ERMA 1999

Descriptor	Description
Minimal	Insignificant (repairable or reversible) environmental impact, no observable cultural effects, other effects slight (reversible) or very small
Minor	Reversible environmental impact, limited adverse cultural effects (affecting small area or localised community), other effects small and limited in scope.
Moderate	Some slight effect on native species, adverse cultural effects to wider area but not considered serious, other effects medium or mid range.
Major	Irreversible environmental effects but no species loss, adverse cultural effects widespread but remedial action available, other effects large.
Massive	Extensive irreversible environmental effects, including species loss, adverse health effects, severe adverse cultural effects over whole country with no possible remedial action, other effects very large and widespread.

Table 4: The magnitude of effects. Adapted from ERMA 1999

Following this assessment technique, the risk is a combination of likelihood and the magnitude of an effect. To calculate risk, table 5 is used.

	Magnitude of effects				
Likelihood	Minimal	Minor	Moderate	Major	Massive
Very unlikely	Insignificant	Insignificant	Low	Medium	Medium
Unlikely	Insignificant	Low	Low	Medium	High
Likely	Low	Low	Medium	Medium	High
Very likely (almost certain)	Medium	Medium	High	High	High

Table 5: Calculations of risk levels. Adapted from ERMA 1999

The risks described in the following assessment, are assessed following this guide. At the end of the risk assessment section, table 6 summarises the level of risk for each item of risk in this application.
This risk identification is based on

- Brainstorming session
- Experience from earlier trial
- Experience from trials in other countries
- Scientific evidence.

Effects on public health and safety:

No adverse effects on public health and safety are expected from the trialing or accidental release of transgenic *Pinus radiata* or *Picea abies* plants (Carson *et al.*, 1996 and 1997). *Pinus radiata* is not normally part of the human diet, nor is it part of the diet of any organism used for human consumption. We have no scientific evidence indicating that the introduction of the genes discussed in this application, will alter the allergenicity of pollen. Radiata pollen is unlikely to be absorbed by respiration (due to its size and hydrophobic character). Further, our trial design and management procedures will make the release of pollen unlikely.
Picea abies will not produce pollen during this trial due to the fact that its sexual maturity is not before the age of 15 years.
The formation of reproductive organs will not occur in this trial and consequently the transfer of any of the genes or promoters to other organisms and subsequent threats to public safety and health are not expected.

Effects on the environment, genetic diversity or significant deterioration of natural habitats:

Pinus radiata and *Picea abies* do not form part of the natural ecosystem in New Zealand, although *Pinus radiata* is often used to stabilise land areas affected by wind and water erosion. *Picea abies* does not have any significant impact on the New Zealand landscape or ecosystem.

With respect to inherent genetic diversity, the release of genetic material from genetically engineered radiata, if it occurs, can be considered similar to the effects of release of novel *Pinus radiata* genotypes obtained through conventional breeding, which is commonly practiced in plantation forestry (Carson, 1997). The commercial use of genetically engineered material could be viewed as a minor enrichment of the genetic diversity of the species, at least in terms of use of such diversity for financial benefit. It is not expected that transgenic *Pinus radiata* plants will have any different effect on biological processes and natural habitats compared to non-transgenic plants. *Picea abies* is not used in commercial forestry in New Zealand. However, in this field trial we are not proposing the release of transgenic material into the environment beyond the trial site. The proposed containment strategy includes mechanisms to prevent movement of transgenes into the environment.
In the event of accidental release of transgenic material, there is no scientific evidence indicating significant risk to the environment. For a discussion and examples see Nap et al, 1992 and Gilissen et al, 1998.

Effects on Maori culture and taonga:

We do not expect, from a scientific point of view, any effect of the proposed field trial on Maori traditional resources. We do not intend to release any genetically engineered material, and the procedures in place for this trial ensure containment of transgenic material.
Non-transgenic *Pinus radiata* have been grown in plantations in New Zealand for more than 130 years, and the addition of the genes mentioned in this proposal is not expected to change any aspect of Maori traditional resources. Picea abies is exotic to New Zealand and has no known significance with regard to Maori culture and taonga.
All genes and promoters mentioned in this application are isolated from natural sources which are present in New Zealand and elsewhere in the world. The genes can be regarded as already being abundant in the natural environment of New Zealand.
We have entered into a Maori consultation process to obtain the Maori view on this issue, and to ascertain whether our trial has any potential risk on key outcomes for Maori. This process and its result to date are detailed in the attached document “Maori consultation”. However, we view consultation as an ongoing process and we will involve relevant Maori over the full period of the trial.
It is noted that the Authority has, in the decision relating to a field trial application by Carter Holt Harvey (GMF98011), adopted the view that for a containment application, information on the risk to the relationship of Maori and their culture and traditions with taonga, is required primarily in respect of local Maori. We have therefore concentrated our consultation efforts on Te Arawa. However, Ngati Tuwharetoa and Ngati Raukawa were invited to submit their views as well.

Affinities of the transgenic *Pinus radiata* and *Picea abies* to other organisms:

From a scientific viewpoint it can be regarded as highly unlikely that these transgenic plants will cause disease, be parasitic, or act as a source for human or animal pathogens. The transgenic plants are no different from non-

transgenic plants, other than having novel genes introduced, and which are derivatives of genes found in the natural environment in New Zealand. We expect these plants to behave identically to non-transformed plants with respect to their disease potential.

Pinus radiata in New Zealand is associated with a range of diseases and pests. This includes fungal diseases of needles and shoots such as *Dothistroma pini*, *Cyclaneusma minus*, and *Diplodia pini*. *Armillaria limonea* root disease is also common in newly established *Pinus radiata* plantations in areas that have previously been covered by native forests. *Sirex noctilio* is an established insect pest responsible for occasional economic damage, while Asian gypsy moth is one of numerous theoretical threats to New Zealand radiata pine plantations that have not yet been introduced. *Picea abies* are rarely planted in New Zealand.
Mycorrhizal fungi are found in a beneficial association with radiata pine. They can come into close contact with root cells, due to their ability to penetrate roots. Theoretically, this association might allow transfer of genes from radiata pine root cells to cells of mycorrhizal fungi, and subsequent spread of the genes through the microbial population. We are not aware of any scientific evidence of such transfer occurring in nature and the authority has, in their decision on application GMF98011 (Carter Holt Harvey radiata pine), adopted the view that "the probability of the transfer of genetic material via this route is very low".
Introduced possums or rabbits may eat fresh plant material from transgenic radiata pine or *Picea abies*, but there is no scientific evidence that this would have any negative effect on the herbivores or other organisms in the habitat. Even if gene transfer occurred in the gut of those animals and the genes became integrated into individual cells of the animals or any member of the gut flora, the effects are not regarded as being different from those expected when the animals eat other plants or associated microorganisms which have the same genes integrated in their genome. Gene transfer from transgenic radiata pine or spruce to pathogens, insects or herbivores and establishment of those genes, is regarded as highly unlikely.
In their decision on application GMF98011, the Authority considered the events necessary to happen for a transfer of genetic material from transgenic plants to gut bacteria to occur. The Authority concluded that "the probability of this sequence of effects occurring is low and therefore considers the cumulative probability of the transfer of genes derived from these genetically modified radiata pine trees to be low".

Ability of transgenic *Pinus radiata* and *Picea abies* to form undesirable self-sustaining populations, either by themselves or following hybridisation with other organisms:

Pinus radiata will regenerate naturally through shed seed in many parts of New Zealand, although it has been much easier to control any such spread than for say, *Pinus contorta* (which has caused relatively minor problems in parts of the Central North Island, and Otago). Such "wildling" trees are generally only considered undesirable if they spread into areas reserved to indigenous plant species, and even then they can be (and are) controlled using conventional physical and chemical management methods. *Picea abies* is uncommon in New Zealand, and will not regenerate reliably from seed. It produces little seed, and spread of "wildlings" has not been reported.

Unintended pollen or seed production:

The management procedures set out for this trial will make it highly unlikely that pollen containing transgenes are released. Unintended pollen release could result from
Failure to detect developing male cone over a prolonged period of time, and during more than 10 monitoring visits.

- Inefficient monitoring

- Natural disaster making the area inaccessible during pollen formation, and leaving the plants intact

However, any such release would have a very low likelihood of successfully pollinating female cones on adjacent radiata pine. Small amounts of pollen from trial trees would need to compete with the very large loads of pollen naturally present. Any resultant regenerating seedlings would be removed before they, in turn, flowered. The probability of trial pollen reaching mature pines would be very low and levels would be highly diluted by ambient pollen from nontransgenic trees closer by. Any subsequent spread of an aberrant genotype would take years to accomplish, and could be easily controlled at any stage.
The transformation events detailed in this application, are not expected to change the composition of the cell wall or outer area of pollen grains. Therefore, a change in allergenicity of any accidentically release pollen, is not expected.

The production of seed on transgenic radiata pine cannot be 100% excluded, but the probability for this occurring is very low. Our management procedures to avoid seed production/escape are as follows:

- Plants will not be grown long enough to allow the formation of reproductive structures to occurr.
- Male and female organs that are unexpectedly initiated will be removed before maturity and pollen shed.
- Should any seed be produced and germinate on the ground, this will be detected during the monitoring visits. The seedling will be removed and incinerated.
- *Picea abies* will be removed from the trial area 10 years before any sexual maturity is likely.
- Monthly inspections over a period of two years after the trial will ensure that any developing seedlings are detected and destroyed.

Hybridisation with other species:
Pinus radiata will hybridise in nature with *Pinus attenuata*, but has not been shown to cross readily with any other pine species (and records of successful artificial hybridisation are quite rare (Critchfield, 1967). *Pinus attenuata* is of no commercial or other importance in New Zealand, and is known to be highly susceptible to the needle-blight fungus *Dothistroma pini*. The *P. radiata* x *P. attenuata* hybrid has been trialed in cold regions of New Zealand by *Forest Research* staff, and is likely to have only limited utility for plantation use. Any subsequent gene transfer between transgenic *P. radiata* and this hybrid would raise no new issues than those already present for non-transgenic plants. Exotic pines, including radiata pine, have never been shown to hybridise with other coniferous species, and New Zealand has no indigenous pine species. We are confident that there is very little risk of gene transfer from the proposed trial, and that risks of undesirable impacts of any unexpected release would be negligible. *Picea abies* is so uncommon in New Zealand that there is little risk of successful crossing either within or across related species. As with radiata pines, *Picea abies* have no near relatives in the indigenous flora, and there is no likelihood of successful outcrossing.

Regeneration of roots from young branches under artificial conditions:
This is a very hypothetical issue and our comments are based on rare observations of this happening. Expertise at ***Forest Research*** indicates that the only way that this can happen is if the small (<5cm) tips of new young branches are excised, and somehow end up with their bases in the soil. It needs human interference to achieve this and we would not expect this to happen naturally. However, even if branches rooted, the resulting tree would be detected and removed.

Effect of genes, should seed or other plant material escape from the trial.
The herbicide resistance genes expressed in transgenic plants have an effect only when a herbicide is applied to the plant. In this case, the plant will be protected by the action of the resistance gene and will survive the treatment. The gene will not change any other characteristic of the plants, such as their reproductive development, pollen, or growth and form. When no herbicide is applied, there is no scientific evidence indicating that the plants will have a competitive advantage over non-transgenic plants.

Horizontal gene transfer
Our contained field trial does not include the production of sexual organs, or the shed of reproductive units. However, other avenues of horizontal gene transfer from the trial cannot be completely excluded.
Indications are that horizontal gene transfer (other than that involving reproductive organs) is indeed taking place in the natural environment, and this includes transfer between non-related organisms. For instance, it is well known that DNA can move around between bacterial species, and also between higher organisms and microorganisms. The latter however is not conclusively demonstrated for natural conditions (discussed in Nielsen et al, 1998). Gene transfer from microorganisms to higher organisms is also possible in the natural environment, and is actually used in plant genetic engineering (*Agrobacterium* transformation). However, to our knowledge, the transfer of a specific gene from plant cells via microorganisms to another plant species, has not yet been demonstrated to occur in nature. This does not imply that such transfer did not exist, and we agree with authors such as Syvanen (1998) that horizontal gene transfer is a process happening in the natural environment, actually providing a tool for evolution.
The issue of horizontal gene transfer is extensively covered in the following references:
Clark et al, 1979 ; Jain et al, 1999, Syvanen,M. and Kado C.I. 1998.
In the decision on application GMF98011, the Authority have confirmed their position which is “that while the scientific evidence available is inconclusive, horizontal gene transfer from transgenic plants to soil microorganisms is unlikely” (to occur).

Effects through integration of new genes
We do not expect the introduced genes and promoters as such to pose any disease potential. All of them are well characterised and there is no scientific evidence for the occurrence of such potential in conifer genetic engineering. However, gene integration may indeed disrupt metabolic or other pathways and silence genes essential for development. Due to the huge size of the radiata pine genome, the integration of our constructs into essential genes is probably a rare occurrence , but cannot be excluded. In many cases of integration into expressed genes, the plant will either not be viable, or show a change in its characteristics which will phenotypically be detectable. Those plants will not be used for the trial. The integration of genetic elements not resulting in visible changes is also possible.
In general, the integration of DNA sequences into expressed genes followed by disruption / silencing of the gene is a well known process in the genetics of most organisms analysed so far. Transposons, as an example, are genetic elements present in many (if not all) organisms. These elements have the ability to move within the genome and integrate more or less randomly within the genome. This leads to integrative mutations, some of which are fatal, others lead to changes in the organisms characteristics that go unnoticed. Others however, will result in an advantage, speeding up the evolutionary process and it has been hypothesised that integrative elements such as transposons are in fact evolutionary tools used by nature.
We maintain the view that the genetic modifications carried out in our experiments, do not represent a higher risk than the movement (and integration!) of genes already occurring during natural processes. We believe that our elements have in fact a lower likelihood of causing integrative changes since once integrated, they are not known to transfer within the genome.

For more information on transposons see the following references: Berg and Berg (1983), Bukhari et al, 1977, Jones et al (1992).

Trial GMF 99005 is designed to collect data on herbicide resistance in conifer trees. The research is purely scientific and we are not intending to use any modifications in the trial in commercial plantations. However, we discuss in our application the possible outcomes of our trial, which may have an impact on forestry management and tree quality. We wish to stress that by collecting data and making comments on possible outcomes, we are not intending to make recommendations on how and whether the technology used in our trial, and the outcomes of the trial, should be used in commercial forestry in New Zealand or elsewhere. We aim to provide data to the public, scientists and the forest industry which can be used to make fact based decisions on the use of this technology, thereby contributing scientific evidence to the genetic engineering debate.

Effects related to the production of novel proteins through integration of novel genes:
In earlier sections (particularly section 6) we have detailed the effects expected as a result of expression of novel genes. It is part of the aim of this trial to evaluate whether or not the introduced genes have the expected effect. The selection and reporter genes used (section 6 a-d) are well characterised in hundreds of genetic engineering experiments in radiata pine, and we do not expect any different characteristics expressed in plants of this trial. The other genes used are mainly isolated from other plant sources and were characterised in those environments. Form a scientific viewpoint, and based on experience in plant genetic engineering, we are convinced that these genes will behave similar in radiata pine. The final proof however, is the experiment itself, and a conclusive answer regarding the predictability of gene expression for intended field release proposals can only be arrived at by conducting the experiment in a contained trial.

Other effects of the introduced genes, than those expected, could be:

- Lethal effects on plant development
- Lethal effects on reproductive development
- Delay of reproductive development

Early reproductive development (in terms of months or years earlier)

All of these effects can safely be controlled by our management procedures. Lethal effects would only result in premature cessation of the project, rather than a negative effect on the environment. Delay of reproductive development would not have a negative effect on the environment, and early development would be detected through our management procedure. Corrective action would be taken in time, before reproductive structures can mature.

Effects related to the Cauliflower Mosaic Virus 35S (CaMV-35S) promoter

The Cauliflower Mosaic Virus 35 promoter is a short genetic element derived from CaMV, a widespread virus that infects a range of plants of the family of cabbages, including broccoli and cauliflower. CaMV is present in agricultural areas world wide, and it is estimated that around 10% of all cabbages are infected with this virus. . *Pinus radiata* and *Picea* abies are not a host for CaMV, and there is no known virus infecting these species. The 35S promoter is a small DNA sequence from the CaMV genome, responsible to control the expression of a structural protein. The promoter is constitutively expressed in most plant species tested so far, and it is used to control the expression of various novel genes in transgenic plant species. Recently, various authors have questioned the biosafety of this promoter, particularly in the context of human food production. Concerns are that the presence of the promoter might induce the formation of novel pathogenic viruses, or that the promoter itself might have undesired effects in the human gut.

From our point of view, no scientific evidence of harmful outcomes of use of the 35S promoter was ever presented, despite numerous speculations on possible risks associated to this element (for an example see Cummins, 1998). The promoter and the virus are both abundant in nature, and humans frequently are consuming plants that carry the complete virus. However, a negative effect of such consumption has never been recorded, and any possible negative effects would have had thousands if not more years to develop and manifest themselves. We are not aware of any such ever happening, and we conclude that 35S is perfectly safe in our trial. The trial is in containment, and any plant matter produced will be destroyed after the trial is finished. The plants in our trial are not for human consumption, and they are not a normal part of the human food chain. Further, from a scientific point of view, the 35S promoter and the CaMV cannot be regarded harmful.

We attach a reference by Morel and Tepfer (2000) who address this issue in more detail, and make reference to the appropriate literature. The article is going to be published in the French journal Biofutur, and we have obtained an English translation from the authors.

In their decision in relation to application GMF98011, the Authority have made a number of comments relating to the use of 35S:

1. Evidence available suggests that *Pinus radiata* is not susceptible to viruses, and in particular is not susceptible to infection by CaMV. Presence in the modified radiata pine of a promoter sequence derived from the CaMV in itself cannot cause a CaMV infection.
2. Therefore the probability of the development of either CaMV in genetically modified radiata pine or any other novel viral infection, as a result of the use of the 35S promoter region from CaMV is small.
3. Further, in a previous decision of the Authority (GMF98005 and GMF98006, both relating to the field testing of genetically modified maize), the Authority noted that " to date there is no evidence that the CaMV promoter, which has been widely used for many years, has any risk associated with its use".

Deliberate sabotage

Sabotage of our trial is possible in case the location of the trial becomes known to possible sboteurs. It is expected though, that such group would conduct sabotage for reasons of feeling threat from transgenic plants. They would most certainly make sure that any transgenic material removed is destroyed. We have mentioned two techniques to destroy DNA and plant material in our application, incineration and autoclaving.

Other unlikely impacts:

A range of theoretically possible but highly unlikely impacts of transgenic plants on the environment have been suggested and include:

- Weediness: Transgenic plants may have a significant advantage over existing, non-transgenic populations and will overgrow these.
- Transfer to weeds: Transgenic plants may, through an as yet unknown mechanism, transfer the introduced genes into existing weeds and increase their weed potential.
- Antibiotic resistance: The antibiotic resistance genes used to select for transgenic material may be transferred into microorganisms associated with the transgenics, and increase the resistance of those microorganisms against antibiotics.

It would be impossible to argue that any of these cannot happen, however unlike one may feel this was. The important task however is to ascertain whether the technique of genetic engineering, and the resulting product have

the potential to introduce any risk greater than what has already been introduced through conventional technologies, such as conventional breeding or risks already present in the natural environment.

Genetic engineering introduces single or few well-characterised genes into a given line, whereas conventional breeding results in a mix of several thousand genes which may or may not have already been combined in this way by evolution. Rearrangement and deletion of genes commonly happen during this process. Traditional breeding in agricultural and forestry backgrounds has resulted in the recombination of various traits (genes) and has introduced novel traits into preferred cultivars. The effect of transgenic plants should not be greater than that from plants obtained through conventional breeding.

Further, the development of both conventional and transgenic crops routinely includes intensive testing and screening of candidate varieties, and usually only a small proportion of such varieties (i.e. less than 1%) are subsequently developed as commercial crops. We would expect any aberrant types to be identified and rejected during this screening phase.

If gene transfer to weeds occurred, a possible negative effect, such as an increase of the weediness of such plants, cannot be totally excluded. However, nature has had several million years to test the effect of this hypothetical gene transfer since genes used in this proposal have been available in nature (including New Zealand) for that period. There is no known example of the development of a “superweed” through such gene transfer.

The same argument must be applied when concerns about antibiotic resistance in microorganisms are discussed. The genes for antibiotic resistance used in this study have been isolated from natural sources, and can be considered as abundant in nature. Selection for the presence of these genes is applied at the early stages after transformation, in sterile tissue culture. Once the plants are in the greenhouse and in the field, the respective antibiotics will no longer be applied to trees, or on surrounding land. A selection of organisms having acquired the resistance genes therefore is unlikely, if not excluded.

Long term unanticipated environmental and health effects

In the past, submissions the Authority has received submissions in relation to applications GMF98005, GMF98006 and GMF98011, expressing concerns with regard to unanticipated long term adverse effects on either the environment or human health. The Authority has taken this into consideration, and has stated that:

“concerns regarding scientific uncertainty, and potential long term adverse impacts on future generations are more relevant to release applications than to an application for a small-scale contained field test. There may be some scientific uncertainty regarding the potential consequences of the genetic modifications proposed in the present applications, but this will not result in adverse consequences for the environment, human health, or future generations while the field test is undertaken in containment. The caution required of the Authority relates to the adequacy of the containment conditions and management regime. In this regard the Committee considers the risks to be negligible for current and future generations alike”

We are convinced that the same applies to our application.

Eradication of transgenic plants if they established an undesirable self-sustaining population.

In the highly unlikely event that transgenic *Pinus radiata* or *Picea abies* established an undesirable self -sustaining population, a range of control measures are available to completely eradicate this population. These include physical removal and incineration, and eradication through a range of commercially available herbicides such as glyphosate or sulfonylurea herbicides (ie herbicides other than those the specific plants are resistant to).

The trial site will be continuously and regularly monitored over the period of the trial and two years beyond. Identification of the material will have occurred well before maturation of reproductive organs and further spread, and all undesired plant material will be eradicated once identified. Molecular techniques may be used to identify

undesired material, if necessary.
In the event of fire, the trial population together with any undesirable self sustaining population, will be eradicated.

Summary:
Risks associated with this trial relate to the direct effects on the transgenic organism, and the possibility of engineered material escaping into the environment outside the trial site. Since the genes used in this application are present in various organisms in the environment, the additional risk posed by the genes used in this trial, for the environment and human health, appear to be negligible from a scientific viewpoint. However, we have established risk management procedures to address the possible escape of engineered material.

Table 6 determines the risk associated with this trial, using a technique described at the beginning of this section (see tables 3-5).

Risk	**Likelihood to occur**	**Magnitude of effect**	**Risk level**
Effects on public health and safety	Very unlikely	Minimal	Insignificant
Effects on the environment, genetic diversity or significant deterioration of natural habitats	Very unlikely	Minimal	Insignificant
Effects on Maori culture and taonga	Very unlikely	Minimal	Insignificant
Affinities of the transgenic *Pinus radiata* to other organisms	Unlikely	Minimal	Insignificant
Ability of transgenic *Pinus radiata* and *Picea abies* to form undesirable self-sustaining populations, either by itself or following hybridisation with other organisms	Unlikely	Minor	Low
Unintended pollen or seed production	Unlikely	Minor	Low
Hybridisation with other species	Unlikely	Minor	Low
Regeneration of roots from young branches under artificial conditions	Very unlikely	Minimal	Insignificant
Effect of genes, should seed or other plant material escape from the trial	Very unlikely	Minimal	Insignificant
Horizontal gene transfer	Unlikely	Minimal	Insignificant
Effects through integration of new genes	Unlikely	Minimal	Insignificant

Effects related to the production of novel proteins through integration of novel genes	Unlikely	Minor	Low
Effects related to the Cauliflower Mosaic Virus 35S (CaMV-35S) promoter	Very unlikely	Minimal	Insignificant
Deliberate sabotage	Unlikely	Minor	Low
Other unlikely impacts:	Very unlikely	Minor	Insignificant
Long term unanticipated environmental and health effects	Very unlikely	Minimal	Insignificant

Table 6: Calculation of risk level, based on descriptors (see also tables 1-3)

Potential benefits:

The benefits expected from this field trial are also discussed in sections 4 and 6. Further, scientific and commercial benefits, and benefits for the New Zealand GMO discussion, from this test are discussed in more detail here:

Importance of field trials of transgenic radiata pine and spruce:
Transgenic *Pinus radiata* and *Picea abies* can be, and have been, grown in containment greenhouses for a limited period of time. In our experience, plants will grow sufficiently well for the first year after germination in containment, but due to limitations of container size and size of the greenhouse, they perform less well compared to field planted trees by two years after establishment. Genetic engineering technologies for conifer trees have become routine, and several novel genes have been introduced, producing a large number of transgenic trees, thus limiting space in the GMO greenhouse. Field trials will allow us to grow trees further, and to obtain data on their performance in a field situation. Field trials are also necessary to confirm traits observed *in vitro*, and to make estimates of the performance of transgenic trees over the full range of the plantation forestry environment.

Importance of genetic engineering for herbicide resistance:
Genetic engineering for herbicide resistance as a research tool is used for a number of reasons:

(i) to understand herbicide action and resistance in gymnosperms and compare it to the data obtained in similar experiments in angiospermous plants;

(ii) to assess the feasibility of a new marker (herbicide resistance) in conifer genetic engineering (this has not yet been achieved world-wide); and

(iii) to demonstrate the applicability of a concept which has proven successful in angiosperms, to gymnosperms. Herbicides are currently used in New Zealand forestry, but they are mainly applied before plantation. The herbicides related to this application are regarded as environmentally friendly as a result of their rapid breakdown in soil (Götz *et al.,* 1983).

The field trial proposed will enable us to collect data on the function of several genes and promoters involved in angiosperm and gymnosperm herbicide resistance projects. The results will improve our understanding of herbicide action and resistance in gymnosperms, allow us to make comparisons to angiosperms, and provide a basis for genetic modification in major forest tree species worldwide. The benefits of this should be regarded as being extremely high both in terms of knowledge in a very intensively researched area, and in terms of value gain for the forest industry worldwide.

Benefits to the forest industry:

The New Zealand forest industry has identified herbicide resistance in radiata pine as a valuable trait for commercial forestry. Plantations using herbicide resistant trees can be established economically on heavily weed-infested sites. Also, plantation forestry depends on the ability to successfully establish new plantations after a rotation has been completed, and which have superior characteristics compared to the plantation before. A major problem is the spread of wildlings resulting from seed production in the older non engineered population. These wildlings can easily be controlled at the beginning of a new plantation, if the new plants are herbicide resistant.

Forest Research will benefit from this trial by maintaining its competitive advantage in the area of conifer genetic engineering world wide.

Contribution to public debate:

The New Zealand public is currently involved in a detailed debate on the use of genetic engineering technologies for plants, animals and human medicine in this country. Very often the debate lacks appropriate scientific information and all parties would benefit from the availability of data from contained field tests of transgenic plant material. This data can serve as a basis for informed decision making on the biosafety of genetically engineered organisms, and our trial is in part designed to provide this data. We will obtain information on the behaviour of transgenic plants in a field situation, their growth and performance, and their continued (or not) expression of introduced genes.

Cost associated with this trial:

We are not expecting any adverse effect of this trial on the environment or human health. Consequently, the direct cost associated with this proposal is regarded as negligible. However, if the application was not approved, the cost for the New Zealand forest sector and the scientific community would be significant. Research projects would come to a premature end and the knowledge and expertise gained in this area would be lost. ***Forest Research*** would lose its competitive advantage in this area and would no longer be regarded as being at the forefront of international science, in these and related subject areas.

Containment System

15. Information on the proposed containment system:

Provide information on how it is proposed that the organism be adequately contained including how the proposed containment system conforms to the requirements of the Parts I and II of the Third Schedule of the Act as appropriate.

This may include reference to, and outlines of, appropriate standards and codes of practice.

[No] further information

To limit the likelihood of any accidental release of any organism or any viable genetic material:
The DNA vectors for this proposal are cloned in the ***Forest Research*** molecular biology laboratory. It complies to the Australian/ New Zealand Standard for Safety in Laboratories (Part3: Microbiology) (AS/NZS 2243.3:1995, PC2 level of containment).
DNA constructs, consisting of vector sequences and also the gene sequences described in this proposal, are contained within the laboratory and only under sterile conditions or within nonpathogenic *Escherichia coli* bacterial species. For disposal of DNA, and all material that comes into contact with DNA sequences, autoclaving is used to completely destroy the DNA before it is disposed of in the waste containers.

Transformation of *Pinus radiata* and *Picea abies* involves embryogenic tissue, which is grown in sterile culture in petri dishes. After transformation and selection, the tissue is propagated in sealed containers within the laboratory. Following plantlet regeneration, small plantlets are grown in sterile containers in the light rig in the laboratory. For disposal, tissue and all material having come into contact with transgenic tissue and plants is autoclaved. When transgenic trees grow bigger, they are transferred to the GMO glasshouse situated on the ***Forest Research*** campus. This facility is operated under the Australian/ New Zealand Standard for Safety in Laboratories (Part3: Microbiology) (AS/NZS 2243.3:1995, PC2 level of containment).
The containment ensures that non-transgenic plants are not released into the environment, and that any organisms with a potential to transfer parts of transgenic plants into the environment, are excluded from the house.

Material taken from transgenic trees will be transferred back to the laboratories, in sealed containers, for further analysis. After analysis, incineration or autoclaving will destroy all tissue. Some material will be stored as reference material in the freezer at minus 80°C. When transfer of tissue or plant material, to collaborating institutions in New Zealand or overseas becomes necessary, the current guidelines set by MAF for such transfer will be adhered to (MAF Reg. Standard 144.03.02).

When the trial is finished, all remaining aerial parts of the trees will be removed and incinerated/autoclaved. ***Forest Research*** has the necessary equipment and expertise to handle trees of large size, and in large numbers. Regrowth from stumps or root systems has never been observed in radiata pine or spruce, but the trial site will be monitored up to two years after the trial is finished, to detect and destroy any growing transgenic material.

To exclude unauthorised people from the facility:
Access to the laboratories, the GMO greenhouse and the trial is by approval through the operator of the containment facilities only. A log book for GMO house and trial will record all access and the book will be available for inspection by MAF / ERMA. The trial area not be signposted, in case of attempts of deliberate sabotage. Further, the trial site will be kept confidential to ERMA, to reduce the likelyhood of sabotage. Also, all non-used land

at a time will be planted with non-transgenic radiata pine plantlets, to maintain the site and to distract possible saboteurs.

To prevent unintended release of organisms by experimenters working with the organism:
Only trained and properly instructed personnel will have access to transgenic plant material. Handling of plants will ensure that no unintended release of genetically engineered material will happen.

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

Although highly unlikely, the formation of viable seed of trial plants is theoretically possible and such seed may germinate during or after completion of the trial. Seedlings emerging within the trial area will become obvious during the trial, or during the post-trial monitoring phase. They will be removed immediately and incinerated. The trial plants will be checked for new growth, particularly in the form of reproductive buds. These can be detected at an early stage (usually by March each year), long before the reproductive organ is actually formed (usually in the period July-September each year). Reproductive organs will be removed from the trees and incinerated. The time-line under section 5 details the formation of reproductive organs and the reproductive cycle of radiata pine. In spruce, the formation of reproductive structures is not expected before the age of 17-20 years (minimum observed:15 years).

International and related matters

16. Information on all occasions where the organism has been considered by the government of any prescribed State or country or by any prescribed organisation and the results of such consideration: Where no countries or organisations are prescribed by regulations made under section 140(1)9k) of the Act, this section can be omitted.

If the applicant is aware that the organism has previously been considered by, for example, any OECD or APEC country, information on the nature of that consideration, including the result, should be provided if known.

[No] further information

Genetic engineering of *Pinus radiata* and *Picea abies* including the regeneration of transgenic plants has only been achieved at ***Forest Research*** in New Zealand. An application for a field trial of *Pinus radiata* with novel genes was made to the Ministry of Environment in 1997, and a field trial was approved and planted in early 1998.

On a worldwide scale, many hundreds of applications for field trialing and also commercial release of transgenic plants have been approved (James and Krattiger, 1996). This includes trees such as transgenic poplar, eucalyptus species, and larch. Transgenic trees have integrated selection genes such as *npt*II, reporter genes such as *uid*A and genes of commercial importance such as herbicide resistance genes, genes involved in lignin biosynthesis, herbicide resistance, and genes influencing reproductive development (James and Krattiger, 1996).

17. Information on New Zealand's international obligations that may be relevant to the application:

Where the applicant is aware that New Zealand's international obligations may be relevant to the application, indicate the nature of the obligation and the effect this may have on the application.

If the applicant is aware of obligations such as the WTO Agreements, the Convention on International Trade in Endangered Species (CITES), Trans Tasman Mutual Recognition Agreement and the like that may be relevant to the application, then information on these obligations should be provided, if known.

[No] further information

The applicant is not aware of any international obligations relevant to this application that may either deny or enforce the approval or rejection of the application. The transgenic *Pinus radiata* and *Picea abies* lines developed in this study were all developed at *Forest Research* and all vectors used to produce these plants were developed in New Zealand. Those vectors and plants that are still in development, will be produced in our laboratories over the next two years.

Previous considerations

18. If the application relates to an organism that has been previously considered by the Advisory Committee on Novel Genetic Techniques or the Minister for the Environment on the recommendation of the Interim Assessment Group, details of the consideration and its results:

[No] further information

An application to field trial transgenic *Pinus radiata* plants was made to the Ministry of Environment in 1997. The trial was approved and planted in April 1998. The plants have a *npt*II antibiotic resistance gene and a *uid*A reporter gene integrated into their genome. The total planting consists of 30 transgenic *Pinus radiata* plants along with nontransgenic control plants. All transgenic plants are growing normal compared to the controls, and no adverse effects have been detected. Reproductive structures have not formed yet on these plants.
A field trial with similar material was applied for by Carter Holt Harvey in June 1999 (GMF98011) and approved by the Authority, with controls in December 1999. We have no information on planting dates, or any results from this trial.

Other relevant legislation

19. Information on other legislation relevant to the organism and its use throughout its life cycle.

If the organism is also subject to other legislation (eg. an Import Health Standard under the Biosecurity Act 1993, or resource consent under the Resource Management Act 1991), details should be provided.

[No] further information

The development work to produce the plants covered in this application will be performed in the molecular biology laboratory at ***Forest Research*** in Rotorua. ***Forest Research*** holds the necessary approvals for development work. Some of the material to be field trialed is still to be developed, and the technologies are similar to those used for material already produced. In addition to the HSNO Act, legislation in the Biosecurity Act, 1993, and the Resource

Management Act, 1991, will sometimes have provisions relevant to the growing and management of radiata pine plantations.

Glossary

20. A glossary of scientific and technical terms used in the application.

This may be appended to the application on a separate form if desired.

[No] further information

Please see appendix.

Other relevant information

21. Provide here any other information required by the Act or regulations not included under any other section of this form.

[No] further information

[No] commercially sensitive information

The field trial described in this application is open to inspection by ERMA and MAF at any time. Material removed from trees (such as needles, stem tissue) will be made available to collaborating institutes for analysis, following national and international guidelines and legislation for the handling and movement of such material.

Summary of Application Contents

(Please check the application is complete and identify attachments)

[Yes] Fees paid

[Yes] Assessment of effects included

[Yes] Confidential information supplied

[Yes] Signed and dated

[Yes] Appendices attached and cross-referenced (list below)

Appendix 1: Glossary

Appendix 2: Tables

Appendix 3: Vector maps

Appendix 4: Trial site map (CONFIDENTIAL to ERMA)

Appendix 5: References

Appendix 6: Report: Maori Consultation

Signature of applicant or person authorised on behalf of applicant _____ **Date:**

APPENDIX:

1.Glossary

β-glucuronidase	An enzyme which is present in a number of organisms and which catalyses a specific biochemical reaction.
ACNGT	Advisory Committee on Novel Genetic Techniques
Agrobacterium	A soil bacterium causing the plant disease crown gall by transferring specific genes to the plant.
Allergenic	A substance, usually a protein, which causes a harmful antigen-antibody reaction in the body.
Angiospermous	Belonging to the angiosperm plant group. Producing flowers and reproducing by seeds enclosed within a carpel. Opposite to gymnospermous
Anatomical	Relating to anatomy (the science of the body)
Antibiotic	A substance, which has an inhibiting, or killing effect on organisms.
*aph*IV gene	Aminoglycoside - Phosphotransferase IV gene. A gene which confers resistance against the antibiotic hygromycin B.
Arabidopsis	A plant belonging to the Brassicaceae
Arboretum	A place where rare trees are grown for study and display.
bar gene	A gene which codes for herbicide resistance
Biolistics	A technique where foreign DNA is shot into cells using a highly accelerated metal particle as a vehicle.
Biosynthesis	The production of organic molecules by living organisms.
bla gene	A gene which confers resistance against beta-lactam antibiotics.

Breeding	Combination of two genotypes resulting in an offspring with a mixture of characteristics.
Breeding population	All trees actively being used for tree improvement.
CaMV	Cauliflower Mosaic Virus. A virus infecting cauliflower.
Chromosome	The structure that contains the DNA in eucaryotes, usually complexed with histones.
Clonal material	The entire stock of plants obtained by budding, grafting, cuttings or other means of vegetative multiplication from one original parent.
Cloning	Production of a group of identical units from a common ancestor.
Cone	Here: A plant organ involved in reproductive development
Coniferous	Belonging to the Coniferae - the chief class of Gymnospermae with about 400 species, all woody, mostly large evergreen trees forming forests.
Containment	Restriction to a defined area.
Conventional	Following tradition.
Coppicing	Growth from roots
DNA	A polymer of nucleotides connected via a phosphatedeoxyribose sugar backbone; the genetic material of the cell.
ELISA	Enzyme linked immunosorbent assay
Embryogenesis	The term used to describe the process of embryo formation from the zygote.
Endogenous	Growing or originating from within
Eradication	Complete destruction
Escherichia coli	A bacterium commonly found in mammalian guts
Eucalyptus	Tree genus native to Australasia
Exotic	Imported breeds
Expression	Here: Transferring genetic information into a phenotype

Fluorometric	Absorbing light of a specific wavelength and emitting light of a longer wavelength

Fungal	Adj. Fungus. (Group of organisms including moulds, yeasts, mushrooms and toadstools)
Gene	A unit of heredity; a segment of DNA specifying a particular protein or polypeptide chain.
Genetic improvement	Improvement of specific traits of an organisms by genetic techniques (breeding)
Genetic modification	Modification of an organism by gene technology
Genome	The complete set of genes present in an organism.
Genotype	The genetic constitution of an individual.
GMO	Genetically modified organism
gus-gene	A gene from *Escherichia coli* coding for the enzyme β-glucuronidase.
Gut	Intestine
Gymnospermous	Belonging to the plant group of gymnosperms (which have seeds unprotected by an ovary)
Herbivores	Animals feeding on plants
Histochemical	The identification and distribution of the chemical constituents of tissues by means of stains, indicators, and microscopy.
Hypersensitivity	An immune reaction. Usually harmful to the animal, caused either by antigen-antibody reaction or cellular-immune processes.
Immature	Undeveloped, not mature.
Indigenous	Native to a country; not imported.
Inter alia	Among other things.
in-vitro	In artificial conditions. Opp: in vivo
Land-race	Population that has developed (usually in an exotic location) from introductions from the native range of species

Larch	Conifer tree species
Light rig	Shelves used for plant tissue culture and illuminated by incandescent light.
Lignin	A compound found in plant material, mainly in wood.
Microorganism	An organism with one or a small number of cells, and which can only be visualised by microscopy.
Microprojectile bombardment	Use of high velocity gold or tungsten particles for delivering DNA into living cells. See also: Biolistics.
Molecular analysis	An analysis technique relating to molecules rather than organs.
Monoecious	Having separate staminate and pistillate flowers on the same individual plant.
Morphology	The study of the form of things.
Nopalin Synthase gene	A gene from *Agrobacterium tumefaciens*, encoding for the production of the chemical compound napoleon.
Neomycin-phosphotransferase	An enzyme which phosphorylates aminoglycoside antibiotics and thereby reduces their toxicity.
Northern blotting	A technology to confirm the expression of genes in organisms.
npt II gene	Neomycin phosphotransferase gene conferring resistance to the antibiotics geneticin, kanamycin, neomycin and related substances.
Octopin synthase	An enzyme encoded by *Agrobacterium tumefaciens* DNA and catalysing the production of octopin.
Organogenic	The formation and development of organs.
Outcrossing	Mating with anything beside oneself.
Parasitic	Relating to an organism living in or on another organism.
pat gene	A gene that confers resistance against a herbicide
Pathogen	Any disease – producing microorganism.
PCR	Polymerase Chain Reaction – technique to produce many identical copies of a particular DNA fragment.

Physiological age	State of physiological maturity, which may differ in organisms of similar chronological age
Plasmid	An extra-chromosomal genetic element not essential for growth.
Pollen	Male gamete fertilising the female ovule in plants.
Pollination	The transfer of pollen from an anther to a stigma, preliminary to fertilisation.
Poplar	A tree of the genus Populus.
Precocious	Earlier development than usual.
Progenies	Offspring
Promoter	A genetic element controlling the expression characteristics of a gene.
Provenances	Regions; group of trees growing in the same place
Pulp	Wood reduced to a mass of soft spongy tissue by mechanical or chemical means.
Recurrent	Happening repeatedly
Regeneration	Renewal or replacement of an organ or structure.
Repository	Place where things are stored.
Reproductive development	Process by which gametes are produced
Seismic	Relating to an earthquake
Selection	Things from which a choice may be made.
Self-sustaining	Support itself for a long period
Serotinous	Cone that opens only after a fire or in very hot conditions
Silvicultural	Cultivation of forest trees in forest plantations involving pruning and thinning.
Somatic embryogenesis	Production of embryos from somatic cells, i.e. cells of the body.
Southern blotting	A Molecular Analysis technique used to prove the presence of a

	DNA sequence in a genome.
Spruce	A conifer plant belonging to the Piceaceae
Sterile	Unfruitful, unproductive, free of living matter.
Traits	Characteristics.
Transformation	Transfer of genetic information via free DNA.
Transgenic	Used to describe genetically modified plants or animals containing foreign genes inserted by recombinant - DNA means.
uidA A gene	See *gus* gene.
Vector	A genetic element able to incorporate DNA and cause it to be replicated in another cell.
Vegetative	Concerned with growth and development, and distinct from sexual reproduction.

2. Tables

Table 1 : Genes included in this application.

Gene	Reference	Source	Expected function/characteristics
*npt*II	Beck *et al.*,1982.	*Escherichia coli*	Resistance against aminoglycoside antibiotics, such as kanamycin and geneticin.
bla	Blattner *et al.*, 1997.	*Escherichia coli*	Resistance against β-lactam antibiotics.
*uid*A	Jefferson *et al.*, 1986.	*Escherichia coli*	Production of the enzyme β-glucuronidase,
bar	DeBlock *et al*., 1987	*Streptomyces hygroscopicus*	Production of the enzyme phosphinothricin-acetyl-transferase (PAT)
ALS (csr1-1) or HRA	Brasileiro 1992	*Arabidopsis thaliana and various other sources*	Production of a modified acetolactate-synthase (ALS)

Table 2: Promoters included in this application

Promoter	Reference	Source	Characteristics
CaMV 35S	Franck *et al.* 1980	Cauliflower mosaic virus	Constitutive promoter for expression of genes in mainly dicotyledonous plant species
Maize ubiquitin	Christensen *et al.*, 1989.	*Zea Maize*	Constitutive promoter for expression of genes in mainly monocotyledonous plant species
nos	Depicker *et al.,* 1982.	*Agrobacterium tumefaciens*	Constitutive promoter for expression in plant tissue
lac	Jefferson *et al.*, 1986.	*Escherichia coli*	Constitutive bacterial promoter used to express genes in *Escherichia coli*
FMV and eFMV	Sanger et al., 1990	Figwort Mosaic Virus	Constitutive promoter used to express genes in plant tissue
Pinus radiata polyubi	Moyle *et al.*, in prep.	*Pinus radiata*	*Pinus radiata* promoter of a polyubiquitin gene. Expected to constitutively express genes in *Pinus radiata*.

3. Vector maps

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4. Trial site map

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The area around the trial site:

Further information relating to the close environment of the trial site:
The close environment of the trial site includes plantations of several different *Pinus radiata* genotypes, as well as other conifer and eucalyptus species, since a major part of this 20 hectare area is maintained as an arboretum and clonal archive. Naturally regenerating radiata pine is removed if it appears in the archive. Immediately adjacent to the nursery, at its eastern side, is the Whakarewarewa forest reserve, a 3500 hectare area of mainly exotic forest containing a wide range of largely coniferous tree species. Whakarewarewa forest contains a substantial component of radiata pine (which is subject to artificial regeneration) managed as commercial stands although these are located at least 800m from the trial site.

The area outside the trial site further includes a range of native and exotic non-plantation species, for example Douglas Fir, Poplar, various Eucalyptus species, various Cypressus species. It also includes some residential area. This area is not under ***Forest Research*** management and we do not have a detailed map of existing trees available to us. Since ***Forest Research*** does not own this land, we cannot make predictions on what species will be there over the period of the trial. While we know that currently there are no plantations of radiata pine, we are unable to guarantee the absence of this species in this area during the length of the trial. The land that will be used for planting transgenic trees, is owned by ***Forest Research***.

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