ENVIRONMENTAL RISK MANAGEMENT AUTHORITY
NGĀ KAIWHAKATŪPATO WHAKARARU TAIAO

FORM NO2N

Application for approval to

IMPORT INTO CONTAINMENT ANY NEW ORGANISM THAT IS NOT GENETICALLY MODIFIED

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

Application Title: Import into Containment of Antarctic micro-organisms

Applicant Organisation: Victoria University of Wellington

ERMA Office use only

Application Code: ☐☐☐☐☐☐☐☐

Formally received:____/____/____

ERMA NZ Contact: _____

Initial Fee Paid: $

Application Status:

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Cnr Waring Taylor & Customhouse Quay
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ENVIRONMENTAL RISK MANAGEMENT AUTHORITY
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Application for approval to import into containment any new organism that is not genetically modified, under Section 40 of the Hazardous Substances and New Organisms Act 1996

IMPORTANT

1. An associated User Guide is available for this form. You should read the User Guide before completing this form. If you need further guidance in completing this form please contact ERMA New Zealand.
2. This application form covers importation into containment of any new organism that is not genetically modified, under section 40 of the Act.
3. If you are making an application to import into containment a **genetically modified organism** you should complete **Form NO2G,** instead of this form (Form NO2N).
4. This form, together with form NO2G, replaces all previous versions of Form 2. Older versions should not now be used. You should periodically check with ERMA New Zealand or on the ERMA New Zealand web site for new versions of this form.
5. You can talk to an Applications Advisor at ERMA New Zealand who can help you scope and prepare your application. We need all relevant information early on in the application process. Quality information up front will speed up the process and help reduce costs.
6. This application form may be used to seek approvals for importing more than one new (non-genetically modified) organism into containment where the organisms are of a similar nature.
7. Any extra material that does not fit in the application form must be clearly labelled, cross-referenced, and included as appendices to the application form.
8. Commercially sensitive information must be collated in a separate appendix. You need to justify why you consider the material commercially sensitive, and make sure it is clearly labelled as such.
9. Applicants must sign the form and enclose the correct application fee (plus GST). The initial application fee can be found in our published Schedule of Fees and Charges. Please check with ERMA New Zealand staff or the ERMA New Zealand website for the latest schedule of fees. We are unable to process applications that do not contain the correct initial application fee.
10. Unless otherwise indicated, all sections of this form must be completed for the application to be progressed.
11. Please provide an electronic version of the completed application form, as well as sending a signed hard copy.

You can get more information by contacting us. One of our staff members will be able to help you.

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Application for approval to import into containment any new organism that is not genetically modified, under Section 40 of the Hazardous Substances and New Organisms Act 1996

Section One – Applicant Details

1.1 Name and postal address in New Zealand of the organisation or individual making the application:

Name > Victoria University of Wellington

Postal Address > School of Biological Sciences,
Victoria University of Wellington,
PO Box 600,
Wellington.

Physical Address > School of Biological Sciences,
Victoria University of Wellington,
Room KK705, Kirk Building,
Kelburn Parade,
Wellington.

Phone > (04) 463 6083

Fax > (04) 463 5331

E-mail > ken.ryan@vuw.ac.nz

1.2 If application is made by an organisation, provide name and contact details of a key contact person at that organisation

This person should have sufficient knowledge to respond to queries and have the authority to make decisions that relate to processing of the application.

Name > Dr Ken Ryan

Position > Senior Research Fellow

Address > School of Biological Sciences,
Victoria University of Wellington,
PO Box 600,
Wellington

Phone > (04) 463 6083
Fax > (04) 463 5331
E-mail > ken.ryan@vuw.ac.nz

Application for approval to import into containment any new organism that is not genetically modified, under Section 40 of the Hazardous Substances and New Organisms Act 1996

1.3 If the applicant is an organisation or individual situated overseas, provide name and contact details of the agent authorised to transact the applicant's affairs in relation to the application

This person should have sufficient knowledge to respond to queries and have the authority to make decisions that relate to processing of the application.

Name >

Position >

Address >

Phone >

Fax >

E-mail >

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Section Two – Purpose of the Application

This form is to be used for an application to import into containment any new organism that is not genetically modified. For an application to import into containment **a genetically modified organism**, use **Form NO2G**.

2.1 Give a short summary statement of the purpose of this application to be used on ERMA New Zealand's public register. (Maximum of 255 characters).

Briefly describe the organism(s) to be imported into containment, and the purpose(s) for which you wish to import the organism(s).

> To import into containment samples of Antarctic water (both fresh and marine in all states) and soil, sediments and rocks containing unidentified micro-organisms for identification and long term culture

2.2 Provide a short description of the background and aims of the project suitable for lay readers.

Describe the purpose of the importation and rationale for the overall project these organisms are to be used in so that people not directly connected with the research can understand why these organisms are required.

Micro-organisms are the major source of productivity in the Southern Ocean. They provide the primary energy source for the food web, they degrade organic compounds, and recycle nutrients for consumption by other organisms. They are not only the most important assemblage in the food web but also the most diverse group of life forms found in Antarctica. About 99% of the micro-organism species on the planet remain un-described.

If an approval is granted, micro-organisms (bacteria, algae, phytoplankton, zooplankton, protozoa, foraminifera, and micro invertebrates) will be collected from Antarctica and imported into containment at Victoria University of Wellington where we will employ DNA-identification technologies to generate "barcodes" for Antarctic microbial diversity. Our work will monitor and document natural changes or cycles in microbial communities in the sea ice, in the water below the ice and in the benthic sediments. This data will be compiled into a bio-inventory of the micro-organisms in ice covered regions of the Ross Sea. We will include geographical distributions, weather data and satellite imagery of ice cover. In addition we will use the cultures to study physiological growth parameters in relation to ecological and climate change issues in the Antarctic, and train students and staff in the safe containment of new organisms. Detailed knowledge of the biodiversity and ecology of micro-organisms in relation to their environmental conditions will lead to the better understanding of the needs for long-term sustainability, and environmental protection for Southern Ocean marine ecosystems.

Perhaps more than any other country, New Zealand is uniquely able to operate cost effective research in Antarctica. New Zealand is also responsible for the management of the Ross Dependency and we have an international obligation to take advantage of our geographic position to help maintain its pristine nature. Our research on micro-organisms in the Southern Ocean will contribute to New Zealand's international obligations in the Antarctic by documenting biodiversity and helping to predict consequences of environmental changes.

Section Three – Information on the Organism(s) to be imported

If the application is for importation of more than one organism, this section must be completed separately for each organism. If there are commercial reasons for not providing full information here, alternative approaches must be discussed with and agreed by ERMA New Zealand.

3.1 Give the unequivocal identification of the organism(s) to be imported

These names will be on the public register and should clearly identify the organisms. Please provide details of the following:

We wish to import into containment unknown marine micro-organisms from Antarctica. The ERMA New Zealand Protocol Number 3, series 2: Interpretations and Explanations of Key Concepts provides guidance for importation of unknown organisms for identification. In such cases, the description should clearly define the bounds of what is included and what is not. Depending on the case, this should include information on the type of organism (e.g. a fungus), the source of the organism (e.g. from a named forest in a named country), the nature of any substrate (e.g. soil) or host material (which may be an organism or from an organism, e.g. wood); any potentially associated organisms (e.g. parasites) and any information about the risk presented by the organism based on its behaviour or effects in its usual environment.

Type of organism

In accordance with this policy the organisms are samples containing algae, bacteria, cyanobacteria, fungi, protozoa and other micro-invertebrates and micro-zooplankton sourced from Antarctica. As these samples have yet to be examined it is impossible to state the range of organisms that they will contain. Samples will be used for the isolation of micro-organisms from the following groups: Archaeabacteria, Eubacteria, Cyanobacteria, Chlorophyta, Heterokontophyta, Haptophyta, Rhodophyta, Dinophyta, Euglenophyta, Cryptophyta, Glaucophyta, Chlorachniophyta, Protista, Foraminifera, Porifera, Cnidaria, Mollusca, Echinodermata. Organisms from other taxonomic groups collected alongside those listed above will be destroyed.

Source, Substrate, and associated organisms

The samples will be collected from Antarctic soils, sea ice, snow, sea water, small stones and sediments from the sea floor, freshwater habitats and associated aquatic materials namely sediments, stones, underlying soil and rock, and microbial mats. Sea water samples may contain zooplankton species such as copepods and krill. Sediment samples may contain mud and small stones, and possibly small sedentary invertebrates.

Risks

It is anticipated that none of the organisms likely to be isolated present a known risk to human, plant or animal health. The algae present no significant risk so far as is known. All samples will contain bacteria, which along with fungi or protozoa, may present a risk to humans under exceptional circumstances such as in an individual with a greatly compromised immune system. This is no different from the situation with samples that could be collected in the NZ environment.

Types of organism

In the lists below, generic names for which no species name is given, e.g. *Chaetoceros sp*., are organisms for which the species has not been identified.

Marine Eukaryotes
A number of algal micro-organisms have been already identified from preserved samples previously collected from Antarctic sea ice samples (Ryan et al 2004 and unpublished observations) and may be present in some of the samples to be collected. These preserved samples yielded the following algal organisms and it is likely that we will find the same species in some of our live samples. This is not an exclusive list of alga in the Southern Ocean, and we expect to find other known and also new genera. (See also Garrison 1991)

Taxonomic Class Phylum Heterokontophyta, Class Bacillariophyceae
Type of organism Marine Diatoms (single cell algae)

Latin binomial

Asteromphalus parvus
Berkelya adeliensis
Chaetoceros sp
Corethron cryophillum
Corethron dichaeta
Cylindrotheca cloisterium
Cylindrotheca subcurvatum
Dictyoche speculum
Entomoneis kjellmannii
Fragillariopsis curta
Fragillariopsis cylindrus
Manguiniana sp.
Navicula glaciei
Nitzschia stellata
Nitzschia laecointii
Odontella wiesfogii
Polarella sp
Probiscia truncata
Pseudo-nitzschia sp
Pleurosigma directum
Pinnularia sp
Porosira psuedodenticulata
Rhozolenia styleferum
Stellarima microtrias
Thalassiosira dichotomica
Thalassiosira australis
Thalassiosira gracilis

Taxonomic Class Phylum Haptophyta,
Type of organism Marine phytoflagellates

Phaeocystis antarctica
Phaeocystis pouchetii

Taxonomic Class Phylum Chlorophyta, Class Prasinophyceae
Type of organism Marine phytoflagellates
Pyramimonas sp
Pyramimonas tychotreta

Taxonomic Class Phylum Dinophyta, Class Dinophyceae
Type of organism Marine dinoflagellates
Protoperidinium metetana

Taxonomic Class Phylum Cryptophyta, Class Cryptophyceae
Type of organism Marine phytoflagellates
Cryptomonas sp
Chroomonas sp

Freshwater Eukaryotes

The following list is copied directly from the NOCO1005 application from University of Canterbury and comprises all algae collected from fresh water habitats by Dr Paul Broady. None of these organisms have had risk groups assigned, and there is no information on their ability to form vertebrate toxins.

Taxonomic Class Phylum Heterokontophyta, Class Bacillariophyceae
Type of organism Freshwater diatoms

Botrydiopsis alpina	coccoid unicell
Botrydiopsis arhiza	coccoid unicell
Botrydiopsis callosa	coccoid unicell
Botrydiopsis constricta	coccoid unicell
Botryochloris antarctica	unicells in mucilaginous colonies
Bumilleriopsis sp.	short filaments
Chlorellidium sp.	unicells, diads and tetrads of cells in clusters
Chlorellidium pyrenoidosum	unicells, diads and tetrads of cells in clusters
Chlorellidium tetrabotrys	unicells, diads and tetrads of cells in clusters
Chloridella sp. ?	unicell, coccoid, tentative identification
Ellipsoidion sp. ?	ellipsoidal unicell, tentative identification
Heterococcus sp.	branching filaments and cell aggregates
Heterococcus pleurococcoides	short branching filaments and cell aggregates
Xanthonema cf. bristolianum	readily fragmented unbranched filaments
Xanthonema debile	readily fragmented unbranched filaments
Xanthonema cf. hormidioides	readily fragmented unbranched filaments
Xanthonema montanum	readily fragmented unbranched filaments
Xanthonema cf. pascheri	readily fragmented unbranched filaments
Xanthonema sessile	readily fragmented unbranched filaments, holdfast on terminal cell
Xanthonema tribonematoides	readily fragmented unbranched filaments

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Taxonomic Class	Phylum Heterokontophyta, class Eustigmatophyceae
Type of organism	no common name
Eustigmatos sp.	coccoid unicell, stalked pyrenoid on chloroplast

Taxonomic Class	Phylum Chlorophyta
Type of organism	"green algae"

Ankistrodesmus angustus	spindle-shaped unicells
Ankistrodesmus antarcticus	spindle-shaped unicells
Binuclearia sp.	unbranched filament
Bracteacoccus sp.	coccoid unicell, disc-shaped, parietal chloroplasts
Bracteacoccus aerius	coccoid unicell, disc-shaped, parietal chloroplasts
Bracteacoccus cf. medionucleatus	coccoid unicell, disc-shaped, parietal chloroplasts
Bracteacoccus minor	coccoid unicell, disc-shaped, parietal chloroplasts
cf. Characium sp.	cylindrical, coccoid cell with short holdfast
Chlamydomonas sp.	coccoid unicell, biflagellate
Chlamydomonas chlorostellata	coccoid unicell, biflagellate
Chlorella sp.	coccoid unicell
Chlorella lobata	coccoid unicell
Chlorella reisiglii	coccoid unicell
Chlorella cf. reniformis	coccoid unicell
Chlorella ellipsoidea	coccoid unicell
Chlorella emersonii	coccoid unicell
Chlorella luteoviridis	coccoid unicell
Chlorella protothecoides	coccoid unicell
Chlorella saccharophila	coccoid unicell
Chlorella vulgaris	coccoid unicell
Chlorococcum humicolum	coccoid unicell
Chlorococcum victoriense	coccoid unicell
Chlorococcum elkhartiense	coccoid unicell
Chlorococcum infusionum	coccoid unicell
Chlorococcum tatrense	coccoid unicell
Chloromonas palmelloides	coccoid unicell, biflagellate
Coccobotrys mucosus	short branching filaments emerging from cell aggregates
Coccomyxa sp.	ellipsoidal, coccoid cells in mucilaginous colonies
Coccomyxa curvata	ellipsoidal, coccoid cells in mucilaginous colonies
Coccomyxa gloeobotrydiformis	ellipsoidal, coccoid cells in mucilaginous colonies
Coccothrix chlorolobata	readily fragmented, unbranched filaments
Coenochloris bilobata	spherical coccoid cells in mucilaginous colonies
Coenochloris signiensis	spherical coccoid cells in mucilaginous colonies
Coenocystis oleifera	spherical coccoid cells in mucilaginous colonies
Coleochlamys oleifera	pyriform coccoid unicell
Desmococcus endolithicus	short branching filaments emerging from cell aggregates
Desmococcus olivaceus	short branching filaments emerging from cell aggregates
Dictyosphaerium minutum	coccoid unicells remaining attached to star-shaped sporangium walls

Dilabifilum prostratum	branching filaments emerging from cell aggregates
Diplosphaera mucosa	sarcinoid aggregates of cells, sometimes mucilaginous
Elliptochloris reisiglii	coccoid unicell
Elliptochloris reniformis	coccoid unicell
Gloeobotrys terrestris	coccoid unicells in mucilaginous colonies
Hemichloris antarctica	coccoid unicell
cf. Hemichloris	coccoid unicell
Hormidiospora verrucosa	unbranched filaments, verrucose spores
Kentrosphaera sp.	large coccoid unicells, irregular shapes
Klebsormidium flaccidum	long, unbranched filaments
Koliella sp.	spindle-shaped coccoid unicells
Koliella sempervirens	spindle-shaped coccoid unicells
Macrochloris cohaerens	coccoid unicell
Macrochloris multinucleatum	coccoid unicell
Microthamnion kuetzingianum	narrow, branching filaments
Muriella terrestris var. reticulata	coccoid unicell
Muriellopsis sphaericum	coccoid unicell
Myrmecia bisecta	coccoid unicell, pyriform
Myrmecia macronucleata	coccoid unicell, prominent nucleus
Neospongiococcum gelatinosum	coccoid unicell
Oocystis minuta var. ellipsoidea	ellipsoidal coccoid unicell
Planophila sp.	coccoid unicell and cell pairs
Prasiococcus calcarius	sarcinoid cell aggregates
Prasiola calophylla	ribbon-shaped, multiseriate filaments
Prasiola crispa	unbranched filaments and multicellular, sheet-like
Pseudococcomyxa simplex	pyriform, coccoid unicells
Radiosphaera lobata	coccoid unicell
Raphidonema sp.	short, cylindrical cells forming readily fragmented filaments
Raphidonema pyrenoidifera	spindle-shaped unicells and cell pairs
Raphidonemopsis sessilis	spindle-shaped unicells and cell pairs, attached by one apex
Schizochlamydella minutissima	coccoid unicells with thin mucilage coating
Scotiellopsis terrestris	ovoid coccoid unicells, ribbing along cell wall
Stichococcus sp.	short cylindrical cells forming readily fragmented unbranched filaments
Stichococcus allas	short cylindrical cells forming readily fragmented unbranched filaments
Stichococcus bacillaris	short cylindrical cells forming readily fragmented unbranched filaments
Stichococcus exiguus	short cylindrical cells forming readily fragmented unbranched filaments
Stichococcus minutus	short cylindrical cells, single and in pairs
Tetracystis sp.	coccoid unicells and tetrads
Tetracystis antarctica	coccoid unicells and tetrads
Tetracystis fissurata	coccoid unicells and tetrads
Trebouxia corticola	coccoid unicell
Trebouxia crenulata	coccoid unicell

Trichosarcina mucosa uniseriate becoming pluriseriate filaments
Chaetophorales? short branched filaments emerging from cell aggregates

Taxonomic Class Phylum Cyanophyta Class cyanophyceae
Type of organism Cyanobacteria or "blue-green algae"

Cylindrospermum sp. unbranched filaments with terminal heterocyst
Leptolyngbya fragilis unbranched filament lacking heterocysts
Nostoc sp. unbranched filament with heterocysts, embedded in mucilage
unidentified members of the order Oscillatoriales; unbranched, non-heterocystous filaments
Tolypothrix sp. filaments with basal heterocyst, false-branching

End of University of Canterbury list

Prokaryotes
The following is a list of prokaryote genera, which have been found in Antarctica (Brinkmeyer et al., 2003; Christner et al., 2003; Sjoling and Cowan, 2003). This is not an exclusive list and we may find other known as well as new genera.
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Taxonomic Class Phylum Cyanophyta,
Type of organism Blue green algae
Synechococcus sp
Proclorococcus sp

Taxonomic Class Kingdom Eubacteria α-Proteobacteria
Type of organism Bacteria
Roseobacter. sp
Sphingomonas sp

Taxonomic Class Kingdom Eubacteria ß-Proteobacteria
Type of organism Bacteria

soil ultramicrobacterium strain ND5
Pseudomonas sp

Taxonomic Class Kingdom Eubacteria γ-Proteobacteria
Type of organism Bacteria

Acidobacter sp
Acinetobacter sp
Actinobacteria sp
Brachybacterium sp

Cellulophaga sp
Coliwellia sp
Cytophaga sp
Desulforho sp
Flavobacterium sp
Gemmimonas sp
Glaciecola sp
Gloeobacter sp
Halomonas sp
Marinobacter sp
Marinomonas sp
Methylophaga sp
Microbacterium sp
Nesterenkonia sp
Oceanospirillum sp
Octadecabacter sp
Pelobacter sp
Planctomycetes sp
Polaribacter sp
Pseudoalteromonas sp
Psychrobacter sp
Psychroflexus sp
Psychromonas sp
Psychroserpens sp
Salegentibacter sp
Shewanella sp
Terredinibacter sp
Verrucomicrobia sp
Vibrio sp

Taxonomic Class Kingdom Archaebacteria
Type of organism Archaea
Crenarchaeota sp

Other information, including presence of any inseparable or associated organisms:

> Some organisms such as small sedentary molluscs or anemones may have symbiotic algae or bacteria associated with them. These organisms will be also collected.

3.2 Characteristics of the organism(s) to be imported

Provide information on the biology, ecology and the main features or essential characteristics of each organism(s) to be imported. For example, note production of spores/seeds/pollen, conditions for growth and reproduction. Also provide information on affinities of the organism(s) with other organism(s) in New Zealand. This information should be relevant to the identification of the risks of the organism (section 5).

>The micro-organisms will be collected from the following habitats

1. Sea ice brines. Micro-organisms grow on, and within sea ice, in small channels of liquid sea brine between crystals of pure ice. The salinity of this brine varies from close to sea water concentration to several times more concentrated than sea water. The temperature of the brine varies from about $-2^{o}C$ to $-15^{o}C$. Only specially adapted species of bacteria and algae can survive in this environment, which surely represents the coldest habitat for growing organisms on the planet. Reproduction of these organisms is primarily by simple cell division, and growth rates can be surprisingly high in algae (doubling in biomass in a matter of days). To my knowledge, no species of ice algae are known to exist in temperate waters. The relationships of bacteria to temperate species are unknown.
2. Sea water. Sea water temperature under sea ice is -1.8 to $-1.9^{o}C$. A greater variety of micro-organisms (bacteria, cyanobacteria, plant and animal plankton) exist in this habitat. As for sea ice organisms, the species found here are likely to be unique to the polar environment.
3. Sea floor. Benthic samples will also be exposed to similar temperatures as the sea water organisms. The species found here are likely to be different from those above and again unique to the polar environment.
4. Fresh water habitats. The samples will be taken from streams, lakes and pond water and will comprise freshwater aquatic materials, namely: snow, microbial mats, stones, underlying soil rock and underlying sediment.
5. Soils, particularly those around bird colonies. Soils will be sampled for soil borne micro-organisms such as bacteria. The temperatures of these soils will range from $-40^{o}C$ to about $+5^{o}C$.

A range of invertebrates will be collected from these habitats, for screening for symbiotic algae and bacteria, and physiology work. Invertebrate groups collected will largely be sponges, cnidarians, foraminiferans, and echinoderms, though molluscs may also be collected.

Section Four – The Proposed Containment System and its Effectiveness

4.1 Describe the proposed containment system (physical and operational) and the ability of the organism(s) to escape from this system. The adequacy of the containment regime is a principal consideration so you need to provide comprehensive information on the containment system. Containment facilities must be registered by MAF, and you should provide documentary evidence of this. Refer to relevant containment manuals as appropriate. Please also ensure that ERMA New Zealand has an up-to-date copy of the containment manual relating to this facility. Identify possible pathways of escape of the organism(s) from containment, including through lapses of security or sabotage. Describe the biological features of the organism(s) that might affect its ability to escape from containment.

The micro-organism samples will be contained to a minimum of PC1 standard. The proposed research will be carried out in the Transitional Facilities for Biological Products and Containment Facilities for Microorganisms at the School of Biological Sciences, Victoria University of Wellington. The School's containment facilities have been registered as PC1 and PC2 type laboratories with MAF according to the MAF Biosecurity Authority Standards 154.02.17 and 154.03.02. Copies of recent MAF audits are enclosed.

All proposed work described in this application will be conducted in our containment facility according to the protocols described in the Safety in Laboratories Manual Part 3: Microbiological aspects and containment facilities (2002). The specifics for the maintenance of this containment facility are described in detail in the manual. An additional precaution requires that only authorised personnel be allowed into the facility. The doors of the facility are kept locked and entry requires a security swipe card. All personnel are required to attend a PC2 training course and are given a briefing on the safety procedures in the laboratory prior to commencing work in the lab.

While the majority of bacterial and fungal species isolated to date from Antarctica, are not spore-formers, there is the possibility that spore forming species will be isolated. While endospores generated from prokaryotic gram positive micro-organisms are highly chemical and desiccation resistant, the generation of endospores are not part of the normal cell cycle. Endospores only form when conditions become non-conducive to growth. Therefore, where cells are routinely sub cultured, the formation of endospore-inducing conditions is avoided. Nevertheless, to avoid release of spores, all Antarctic samples will be maintained in sealed containers. All handling of samples will be to a minimum of PC1 standard. Transfer of bacterial samples to other containment facilities will only be conducted with the written approval of MAF and according to the transport container requirements of the IATA Dangerous Goods Regulations.

It is expected that no fungi will be enriched from these samples under our culture conditions and therefore no fungal spores will be generated.

It is unlikely that samples of sponges will survive for long when back in NZ due to their fragility, however these and the other invertebrates will be maintained in closed seawater aquaria in PC1 standard cool rooms at Victoria University. Symbionts extracted from these host invertebrates will be cultured in closed systems and maintained in incubators in a PC1 lab.

To eliminate the risk of escape of bacteria and other micro-organisms, sterilisation of liquid cultures by overnight incubation in bleach water (1:8) will be performed prior to their disposal. Solid biological waste such as disposable plastics, agar growth media, examination gloves, will be decontaminated by autoclaving prior to disposal. Any spore forming organisms, whether from liquid or solid culture, will be destroyed by autoclaving. All un-wanted Antarctic material including micro-invertebrates and zooplankton will be destroyed by autoclaving prior to disposal.

Section Five - Identification and Assessment of Risks, Costs, and Benefits

This section must include information on the beneficial and adverse effects referred to in the HSNO Act. It is easier to regard risks and costs as being adverse (or negative) and benefits as being positive. You should consider costs and benefits with respect to both non-monetary and monetary (dollar) terms and

also consider the distribution of this incidence. Provide a brief description of where the information in the application has been sourced from, e.g. from in-house research, independent research, technical literature, community or other consultation.

5.1 Ability of organism(s) to establish a self-sustaining population.

Discuss the ability of the organism(s) to establish an undesirable self-sustaining population, should an escape from containment occur, and the ease with which such a population could be eradicated. You should consider the ability of the organism(s) to survive and reproduce if it did escape from containment.

The micro-organisms isolated will be "cold-loving" (psychrophilic) with a variable requirement of low temperature for growth. Samples will be isolated from habitats ranging from-20 to +5^{o}C. Therefore their ability to survive in N.Z. climatic conditions is considered unlikely. In addition, the marine samples will be isolated from saline or hypersaline water, which eliminates growth in any environment in New Zealand apart from our marine environment. There is some evidence that some of the algae that have been transported in icebreaker ballast water tanks can survive in sea water at higher temperatures (Lewis et al 2003). These authors note that while a potential risk exists that these organisms could survive, there is no evidence that this has or will occur. In the event of their survival their adaptation to cold will make them unsuitable for the warm temperature environment they would encounter in New Zealand and are unlikely to compete well against existing populations.

The terrestrial and freshwater biota of Antarctica is limited in diversity (Vincent 1988) but generally widespread so that it is likely that some of the organisms listed in section 3.1 above may be in the samples. Aerial borne spores may be generated from some of the soil samples and these will all be contained within PC1 facilities at all times. As with the marine samples, the ability of these organisms to establish a self-sustaining population in New Zealand is low. Their temperature requirements for growth will make them unlikely to compete well against existing populations, and it is unlikely that any suitable environment for their sustained growth exists in New Zealand.

In the unlikely event that a population of marine Antarctic micro-organisms were to establish in coastal waters of New Zealand, eradication of the organisms would be very difficult because we could never be certain that we have removed every cell of the offending species from all of the contaminated waters.

5.2 Identify all potential adverse effects of the organism(s).

Identify potential adverse effects associated with the organism(s) and with any inseparable organisms, both within containment, and outside of containment (should an escape occur). Consider effects on the environment, and human health and safety (e.g. of workers in the containment facility), and any ethical and cultural effects. It is important to think about the source of the risk, i.e. the way in which the risk is created (the exposure pathway), and then the consequences of exposure. Adverse effects should be identified for the following categories:

A. Potential adverse effects on the environment, in particular on ecosystems and their constituent parts (e.g. adverse effects on: life supporting capacity of air, water, soil and ecosystems; native and valued introduced flora and fauna; natural habitats and the intrinsic value of ecosystems; New Zealand's inherent genetic diversity; animal or plant health)

If a micro-organism was released from containment into the New Zealand environment the potential risk is that it could adversely effect NZ ecosystems. Possible pathways include potential bloom events of Antarctic algae in NZ temperate waters, or pathogenicity to native plants, animals or humans.

B. Potential adverse effects on public health (including occupational exposure)
> There is a risk that an introduced micro-organism could be pathogenic to humans.

C. Potential adverse effects on the relationship of Māori and their culture and traditions with their ancestral lands, water, sites, waahi tapu, valued flora and fauna and other taonga (taking into account the principles of the Treaty of Waitangi). For example, you should consider whether the organism(s) would have an effect on specific native flora or fauna if they escaped from containment.

> None

D. Other potential adverse effects (such as New Zealand's international obligations, social or economic adverse effects, ethical issues)

> None

5.3 Provide an assessment of the adverse effects identified in Sections 5.2.

The assessment should include the nature, likelihood or probability of occurrence, and magnitude of each adverse effect (i.e. **the risk**), and the value (in monetary or non-monetary terms) of a particular adverse effect (i.e. **the cost**). The uncertainty bounds of the information contained in the assessment should also be discussed.
Adverse effects should be assessed in relationship to:

A. Potential adverse effects on the environment, in particular on ecosystems and their constituent parts (e.g. adverse effects on: life supporting capacity of air, water, soil and ecosystems; native and valued introduced flora and fauna; natural habitats and the intrinsic value of ecosystems; New Zealand's inherent genetic diversity; animal or plant health)

>The Antarctic micro-organisms will pose a negligible risk to the NZ environment because they are maintained in approved containment facilities maintained by properly trained operators. Our facilities are certified containment facilities (minimum PC1) and all unwanted liquid cultures will

be disposed of by the addition of bleach, while solid cultures and spore forming micro-organisms will be disposed of by autoclaving. Uncultured samples will be stored in a locked deep-freeze.

Marine samples
Even if marine organisms were to escape from our containment facility, they would need to travel from our building on Kelburn Parade through air or the drainage system to the sea, a distance of some 5kms. Since these organisms are derived from a saline or hypersaline environment, the possibility that cells would survive of this journey seems remote.

If they did survive this journey, the marine micro-organisms are unlikely to establish an undesirable, self-sustaining population because the micro-organisms have been isolated from the Antarctic where they were adapted to an environment of -1.8 ^{o}C (sea water temperature under ice) to -15^{o}C (temperature of concentrated sea water brine in sea ice). Therefore their survival in N.Z. climatic conditions is considered unlikely. Bloom events of such algae adapted to sub zero temperatures are highly unlikely as they are associated with melting of huge volumes of sea ice and upwelling of cold nutrient rich water at the ice edge, where they are the major source of energy for the Southern Ocean. Such conditions obviously do not exist in temperate waters. If a bloom were to occur, its impact is unknown, but it could stimulate local zooplankton and fish growth for a short time before naturally diminishing. Similarly, pathogenic attack by cold adapted Antarctic micro-organisms are also highly unlikely.

It is unlikely that the specimens of invertebrates will even survive the journey to NZ. This is especially true for sponges due to their extreme fragility. If they do survive the journey, then long term culture of these organisms is very difficult. In our case we are interested in the symbiotic micro-organisms they contain and once these are isolated the invertebrate host will be destroyed.

Terrestrial samples
Importation of terrestrial micro-organisms into containment is unlikely to significantly increase the risk already posed by these organisms. This is because of the ease of their dispersal by natural (wind, bird and insect flight) and human vectors (on clothes and shoes of Antarctic visitors) (Vincent 1998, Broady et al 1994). It is therefore highly likely that any terrestrial micro-organisms originating from Antarctica that would have the potential to cause problems would have been noticed already. Samples collected for culture will be contained and transported to PC1 culture conditions in a much more secure fashion than the casual transfer by Antarctic visitors. The soil algae are known to be readily dispersed by wind and numerous studies have captured diverse viable soil algae from the air. The freshwater algae appear to be cosmopolitan presumably because of the ease of their dispersal, even across oceanic barriers. Antarctic freshwater algae exist in habitats, which often dry out in late summer or over winter, e.g. streams, small ponds, lake margins. The algae survive there in a freeze-dried condition and are readily lofted into the atmosphere during strong winds. It is likely that a proportion of these reach the upper atmosphere where they are subjected to long distance dispersal. With the huge numbers of visitors to New Zealand from all continents, the tens of thousands of tonnes of freight brought into N.Z. each year, and with the thousand or more people who travel to and from Antarctica each year it is inevitable that viable propagules of many (or all?) of these micro-organisms will have reached New Zealand on many occasions. Most are capable of surviving the desiccation they would experience during transit on clothing and footwear and in dust on the surfaces of freight and its packaging as well as surviving

the high levels of UV radiation and low temperatures experienced during long distance dispersal by wind.

Broady et al (1994) has shown that many algae of N.Z. origin are dispersed by humans to Antarctica each year and dispersal in the reverse direction can be assumed It is likely that the natural terrestrial and freshwater algal flora of N.Z. contains many and possibly all of the species present in Antarctica. The Antarctic terrestrial and freshwater flora appears to consist of a limited suite of species capable of survival under the harsh environmental conditions of Antarctica and which have been able to survive equally harsh conditions during their dispersal to the Antarctic Continent, e.g. by winds in the upper atmosphere, i.e. they are derived from more temperate regions to the north. The fact that no effects have been observed indicates that establishment of these organisms has most likely not occurred.

The organisms are likely to be Risk Group 1 as defined in AS/NZ Standard 2243:3 2002 Safety in Laboratories Part 3: Micro-organisms, and therefore are unlikely to cause any environmental harm in the very unlikely event of escape from containment.

B. Potential adverse effects on public health (including occupational exposure)

> The organisms collected from similar samples would all be classed as Risk Group 1. Moreover, these organisms will be contained in a "closed system" where there is less human and environmental risk. The risk to human health is therefore considered to be minimal. Organisms functioning at very low temperatures and associated with saline environments are extremely unlikely to survive at body temperature, be pathogenic or show toxicity that would be a danger to public health. Also, there is little likelihood of any strains from the culture samples escaping from containment. (see Section 4, for details of treatment and storage of sample materials).

C. Potential adverse effects on the relationship of Māori and their culture and traditions with their ancestral lands, water, sites, waahi tapu, valued flora and fauna and other taonga (taking into account the principles of the Treaty of Waitangi). For example, you should consider whether the organism(s) would have an effect on specific native flora or fauna if they escaped from containment. If consultation with Maori has been undertaken, provide details of the process used and the outcome.

>It is difficult to envisage any way in which the samples collected and kept in culture could affect the relationship of Maori with taonga if they escaped from containment. The risks are no greater than those outlined in sections above. For this reason Maori groups have not been consulted.

D. Other potential adverse effects (such as New Zealand's international obligations, social or economic adverse effects, ethical issues)

> No other effects likely

5.4 Identification of beneficial effects (benefits)

Identify and describe monetary and non-monetary benefits associated with importing the organism(s) into containment. Outline and discuss the purpose(s) for the importation and the potential use of the organism(s). Focus on the immediate benefits, as well as longer-term benefits. For example, "increase in scientific knowledge", "increased production of agricultural produce". Substantiate claims by reference to sources of information. Specify whether the benefits identified are environmental, public health or economic benefits; and/or are specific benefits to Maori.

>Benefits of introduction into NZ are non-monetary at least in the short term. As a direct result of this work we will generate a bio-inventory of the primary producer species (bacteria, algae phytoplankton, and zooplankton), which will allow the eventual establishment of policies focused on long-term sustainability and environmental protection in Antarctic marine ecosystems. This will help New Zealand interests in the Antarctic and may give fuel to conservation measures for important and irreplaceable Antarctic fisheries.

This work will contribute to New Zealand's treaty obligations in the international arena. Perhaps more than any other country, New Zealand is uniquely able to operate cost effective research in Antarctica. New Zealand is also responsible for the management and administration of the Ross Dependency and we have an obligation to international science to take advantage of New Zealand's geographic and political position in the region and to help maintain its pristine nature. Our research on micro-organisms in the Southern Ocean will contribute to New Zealand's international obligations in the Antarctic by documenting biodiversity and helping to predict consequences of environmental changes. This work will also accrue kudos in the scientific community to Victoria University via publication of our results in the international literature.

The threat to biodiversity in ecosystems may be the most serious consequence of climate change. The International Panel on Climate Change (White et al 2001) has noted that "climate change in the polar region is expected to be among the greatest of any region on earth". Under the Convention on Biological Diversity, New Zealand has a particular responsibility for conserving our ecosystems. The New Zealand Biodiversity Strategy requires that all ecosystems in our nation (and this includes the Ross Dependency) be maintained. Studies like ours that define the diversity in Antarctic waters and the threats to its conservation will contribute to this obligation.

Our work with the micro-organism cultures will analyse physiological parameters in relation to ecological issues in the Antarctic and will therefore contribute directly to increased scientific awareness of the functioning of marine ecosystems in polar environments. This study is central to the major themes defined in the Antarctic Research Strategy (Peterson 2004). In addition, Antarctic New Zealand is coordinating a latitudinal gradient approach to future research in the Ross Sea Region, and this work is an integral part of that research effort. The proposal also addresses major issues identified in the State of the Environment Report for the Ross Sea Region (Waterhouse 2001), where the lack of knowledge of the marine environment of Antarctica has been particularly highlighted.

The cultures will contribute also to the education of NZ students.

5.5 Provide an assessment of the benefits identified in Section 5.4.

Estimate the likelihood that the benefits will be realised, the magnitude of benefits associated with importing the organism(s) into containment, and any uncertainties associated with this assessment. You should also indicate who would receive the benefits and the expected time-course of delivery of the benefits.

> DNA barcodes have a high likelihood of being produced, and indeed is already being done in may groups of organisms (Blaxter 2004). DNA technology nowadays may be considered routine, and the generation of "barcodes" will essentially depend only on our time to do the work. Members of our group (eg Peter Ritchie) have produced a number of papers, which verifies our capabilities in this area (Ritchie et al 2004, Lambert et al 2002). Within 5 years we expect to have sufficient data to be able to generate policies for government environmental agency use. Student participation will fluctuate in the short term (1-2 years) depending on their availability, but in the long term (5 years+), student educational benefits will be assured.

Dr Ryan has had continuous funding from FRST in various areas since FRST was first set up in the mid 1980s, and has over 45 publications in international research journals. He has undertaken research on Antarctic micro-organisms (algae) since 1991, and a selection of his papers in this field is included in the reference list. Current FRST funded Antarctic research will continue until 2007, when further funding will be sought.

The realisation of benefits associated with international and national obligations, is harder to quantify. The generation of scientific knowledge is of general benefit to the scientific community, and we expect considerable use will be made of our data.

5.6 Overall evaluation of risks, costs, and benefits

This overall evaluation is the main task of the Authority. The Authority has to decide whether the beneficial effects of having the organism in containment outweigh the adverse effects of the organism and any associated inseparable organisms. The Authority must also be satisfied that the organism can be safely contained. You may wish to express a view on the relative importance of the different risks, costs and benefits and how they should be brought together in making a decision.

>The overall risks associated with the importation and containment of Antarctic micro-organisms may be considered to be extremely low, considering the environment from which they have been derived. Indeed our major problems may well be the difficulty in preventing the samples from dying while transporting them from Antarctica.

The risks posed by accidental release of marine micro-organisms from culture into NZ waters are minimal considering

- The organisms are adapted to sub zero and saline or hypersaline conditions
- The organisms are not known to harm marine plants or animals T
- Transfer from our laboratory to the NZ marine environment would involve transfer of cold adapted and saline dwelling cells through air or drainage systems.

The risks posed by terrestrial micro-organisms are also minimal considering

- The ready dispersal of propagules in a freeze dried state, over large distances means that there is a high likelihood that Antarctic terrestrial micro-organisms have already spread

to New Zealand. The fact that they have not already established indicates a low level of risk.

The over all risks associated with all samples are low considering

- The organisms are not known to cause human health problems
- The organisms are not known to be damaging to Maori or Maori taonga.
- The organisms will be contained in approved containment facilities by people who are well trained in their use.

The benefits are high both in generation of sensible policies for the management of coastal Antarctic ecosystems and in the training of students and staff in the safe use of micro-organism cultures. Benefits to New Zealand's international treaty obligations are real but less quantifiable.

Section Six – Additional Information

6.1 Do any of the organism(s) need approvals under any other New Zealand legislation or are affected by international obligations?

For example, indicate whether the organism is subject to other New Zealand legislation, e.g. the Biosecurity Act 1993, or Animal Welfare Act 1999; or if the organism(s) are listed in CITES, then approval is required from both the importing and exporting countries.

> Dr Ryan has an existing MAF permit to bring preserved laboratory specimens into New Zealand, and if this application is successful he will also apply for a permit to import live samples. No other approvals are required to my knowledge.

6.2 Have any of the new organism(s) in this application previously been considered in New Zealand or elsewhere?

For example, has the organism(s) been previously considered for import (e.g. under the Plants Act)?

>Yes, a similar proposal for importation into containment of unknown Antarctic marine organisms was approved in 2001 for the University of Canterbury.

6.3 Is there any additional information that you consider relevant to this application that has not already been included?

> None

6.4 Provide a glossary of scientific and technical terms used in the application.

Benthic. From the sea floor.
Eukaryotes, organisms which have a nucleus in each of their cells
Foraminifera, Small animal cells which secrete a shell of calcium carbonate
Micro-organisms. Bacteria, cyanobacteria, algae and animals that may only be observed under a microscope

Prokaryotes, bacteria and other primitive organisms without a nucleus
Propagule, reproductive unit from an organism, eg spore, cyst, dried cell
Protozoa, small microscopic animal cells
Cyanobacteria, microscopic prokaryotic cells capable of photosythesis.

List of appendices. List any appendices included with this application. Any information that is commercially sensitive, or additional material included with the application (such as details of consultations, referenced articles) should be contained in appendices. The main application should refer to the relevant appendices but be able to be read as a stand-alone document.

- A copy of our containment manual
- Copies of all scientific papers cited
- Copies of recent MAF audit of Containment and Transitional facilities

Application for approval to import into containment any new organism that is not genetically modified, under Section 40 of the Hazardous Substances and New Organisms Act 1996

Section Seven – Application Summary

Summarise the application in clear, simple language that can be understood by the general public. Include a description of the organism(s) to be imported into containment, and any risks and benefits associated with their importation. This summary will be used to provide information for those people and agencies who will be notified of the application (e.g Ministry of Agriculture and Forestry, Department of Conservation, Crown Research Institutes) and for members of the public who request information. Do not include any commercially sensitive information in this summary.

> Micro-organisms (bacteria, algae, phytoplankton, zooplankton, protozoa, foraminifera, and micro-invertebrates) will be collected from Antarctica and imported into containment at Victoria University of Wellington. Micro-organisms are the major source of productivity in the Southern Ocean. They provide the primary energy source for the food web; they degrade organic compounds, and recycle nutrients for consumption by other organisms. They are not only the most important assemblage in the food web but also the most diverse group of life forms found in Antarctica. About 99% of the micro-organism species on the planet remain un-described.

We will employ DNA technologies to generate "barcodes" for Antarctic micro-organisms. A DNA bio-inventory of the organisms and an understanding of their functioning at the base of the food web, will allow the development of policies focused on long-term sustainability, and environmental protection for Southern Ocean marine ecosystems. In addition we will use the cultures to study growth responses to environmental stresses (eg climate change effects), and train students and staff in the safe containment of new organisms.

The overall risks associated with the importation and containment of Antarctic micro-organisms may be considered to be extremely low, considering the environment from which they have been derived. In particular the organisms are adapted to sub zero saline conditions, and are not known to harm New Zealand's marine plants or animals, or cause human health problems. People who are well trained in their use will contain the organisms in approved containment facilities, and successful transfer from culture in our Kelburn laboratory to the NZ environment is highly unlikely.

The benefits are high both in generation of sensible policies for the management of coastal Antarctic ecosystems and in the training of students and staff in the safe use of micro-organism cultures. Benefits to New Zealand's international treaty obligations are also important. Perhaps more than any other country, New Zealand is uniquely able to operate cost effective research in Antarctica. New Zealand is also responsible for the management of the Ross Dependency and we have an international obligation to take advantage of our geographic position to help maintain its pristine nature. Our research on micro-organisms in the Southern Ocean will contribute to New Zealand's international obligations in the Antarctic by documenting biodiversity and helping to predict consequences of environmental changes.

Application for approval to import into containment any new organism that is not genetically modified, under Section 40 of the Hazardous Substances and New Organisms Act 1996

Checklist

Please check and complete the following before submitting your application:

All sections completed	Yes
Appendices enclosed	Yes
Confidential information identified and enclosed separately	NA
Copies of additional references attached	Yes
Cheque for initial fee (incl. GST) enclosed	Yes
If "yes", state amount:	$…….
Direct credit made to ERMA bank account:	Yes/No
If "yes" give date of direct credit …/…/… and amount deposited:	$…….
Application signed and dated	Yes
Electronic copy of application e-mailed to ERMA New Zealand	Yes

*NA – not applicable

Signed: **Date:**

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General

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*Copy of reprint will be forwarded when the paper is published

Dr Ryan has been working in Antarctic research for 13 years, and has undertaken 8 research field trips to study biodiversity and ecophysiology of Antarctic marine algae.