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: Importation of sediment/water samples collected from hydrothermal seamounts outside New Zealand Territorial waters containing Extremophilic microorganisms

Applicant Organisation: Institute of Geological & Nuclear Sciences

ERMA Office use only

Application Code: ☐☐☐☐☐☐☐☐

Formally received:____/____/____

ERMA NZ Contact: _____

Initial Fee Paid: $

Application Status:

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 > Institute of Geological & Nuclear Sciences

Postal Address > Private Bag 2000, Taupo, NZ.

Physical Address > Institute of Geological and Nuclear Sciences
114 Karetoto Road, SH1
Wairakei, New Zealand

Phone > 07-374-8211

Fax > 07-374-8199

E-mail >

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 > Matthew Stott

Position > Scientist - Microbiologist

Address > Institute of Geological and Nuclear Sciences
114 Karetoto Road, SH1
Wairakei, New Zealand

Phone > 07-376-0132

Fax > 07-374-8199

E-mail > m.stott@gns.cri.nz

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 > N/A

Position >

Address >

Phone >

Fax >

E-mail >

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).

This application is for the importation of sediments and fluids that may contain unidentified and potentially novel microorganisms from hydrothermal marine vents and adjacent areas, for the purpose of biodiversity, ecology and biotechnology studies.

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.

The Microbial Biodiversity Research Group (MBRG) at the Institute of Geological & Nuclear Sciences (GNS) is undertaking research chiefly based around the enrichment and isolation of novel New Zealand indigenous extremophilic microorganisms from their natural environments. Extremophilic organisms live in environments not normally inhabitable by the majority of life, such as bubbling hot springs, highly salty brine water, deep underground oil reserves, acidic or alkaline waters or near hydrothermal vents at the bottom of the ocean. Expeditions on underwater volcanic ridges have found that new and totally independent self sustaining ecosystems exist in these areas and are based primarily around the extremophilic microorganisms that grow in the fluids ejected from the vent systems. A number of hydrothermal sites on the offshore Kermadec Arc (north east of NZ) will be visited for the first time by the Institute of Geological and Nuclear Sciences and their research partners, and it is anticipated that these sites may contain unique, and possibility undiscovered, microbial populations.

We hope that by studying these unique environments and the microorganisms that inhabit them, we will learn more about how these cells are able to survive in these harsh conditions and how they interact with the food chain and surrounding geochemical/mineral environments. Understanding the ecology of these systems has implications for understanding the origins of life, life on other planets and the similarities between extreme microbial survival and pathogen evasion techniques. Discovering new microbes opens up possibilities of developing potentially new beneficial technologies such as bioremediation of pollutants, generation of environmentally sustainable energies, medicine and drug discovery, and efficient industrial processes.

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:

Latin binomial, including full taxonomic authority:
Common name(s), if any:
Type of organism (eg bacterium, virus, fungus, plant, animal, animal cell):
Taxonomic class, order and family:
Strain(s) if relevant:
Other information, including presence of any inseparable or associated organisms:

The identities of the microbial biotypes present in the soils/sediments and fluids taken from the hydrothermal vent systems will, at the time of importation, be unknown. We are only expecting to enrich and isolate prokaryotic microorganisms and not Eukaryotes. A list of prokaryotic microorganisms that have previously been isolated from other submarine hydrothermal vent systems have been listed in Appendix 1. It is expected that the microbial biotypes found will be thermophilic and will belong to the bacterial and archaeal domains.

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).

As the soil/sediment and fluid samples have yet to be examined, it is impossible to state the exact microbial types contained (and even if any are present at all). Hydrothermal vent environments, due to their inhospitable nature, house a minimal microbial biodiversity. The microbes present will primarily be chemolithotrophic microorganisms which metabolise reduced inorganic compounds ejected from the vent systems (see reviews from Querellou *et al*., 2001; Canganella, 2001, Colaço *et al.,* 2002). However, the biotypes enriched and isolated for these submarine systems appear to be (to some extent) conserved, such that it is likely that some or all of the microorganisms listed in Appendix 1 *may be* resident in the samples. Note that the microorganisms included in Appendix 1 are described to their Risk classification according to AS/NZ Standard 2243:3:2002. Only Risk Group 1 are expected to be present in any soil/sediment and fluids samples taken from these hydrothermal vent systems: *Risk group 1 (low individual and community risk);* a microorganism or microbial culture that is unlikely to cause human, plant or animal disease (AS/NZ Standard 2243:3:2002). It should also be noted that many of genera and species of microorganisms that may populate these soil/sediment samples have already been approved form importation in previous ERMA permit applications (see NOC99021 & NOC99013 for examples).

The growth requirements of microorganisms living in the vicinity of submarine hydrothermal vents are best described as extreme. Not only do temperatures fluctuate between 4-350˚C (ambient sea water temperature to vent fluid temperatures), but the atmospheric pressures often can exceed 200bar. In addition, the environment is microaerobic to anaerobic, saline and

commonly devoid of organic carbon sources. These conditions restrict the types of organisms able to survive, let alone proliferate. However, those that do proliferate in these environments have specific growth requirements that generally prevent their growth in what is considered "normal growth conditions" for higher forms of life, like ourselves. The types of microorganisms to be found in the hydrothermal systems include methanogens, barophiles, thermophiles, hyperthermophiles, halophiles and possibly acidophiles. The types listed (with the exception of methanogens) are not able to infect or form symbiotic relationships with "standard" flora and fauna because of their specific growth requirements. Methanogens have been isolated from cattle rumen and sewage, but are not known to cause disease. However, the methanogens enriched from hydrothermal vents are expected to be obligate thermophiles (and probably halophilic) growth requirement.

The majority of microorganisms isolated and described from hydrothermal vents are *Archaea* that have, to this point, never been identified as pathogens (Reeve, 1999). Furthermore, all of the bacterial species so far isolated from hydrothermal systems (see Appendix 1) have been classified as Risk Group 1 and generally have a chemolithotrophic nature. It can also be assumed that the majority of hydrothermal vents have remained un-exposed to human activity (Canganella, 2001) and therefore all microorganisms situated there have had not had the time or the contact to evolve life-threatening traits. Likewise, the ability of human pathogens to translocate to, and then survive in these environments is remote due to the geographical isolation and selective pressures of the extreme conditions associated with hydrothermal vent systems (Querellou *et al*., 2001).

Of all the potential candidate microorganisms that have been isolated from hydrothermal vent systems, only *Clostridium caminithermale*, *Thermoanaerobacter siderophilus* and members of the genera *Tepidibacter* are capable of endospore generation (Brisbarre *et al*., 2003; Slobodkin *et al.,* 1999; Slobodkin *et al.,* 2003; Urios *et al*., 2004). *Clostridium caminithermale, T. formicigenes* and *T. thalassicus* have yet to be allocated an official EU Risk classification (http://www.dsmz.de/bactnom/nam0815.htm#8192, http://www.dsmz.de/bactnom/nam8221.htm#8220). However, due to their requirements for moderately thermophilic temperatures and growth in saline conditions, they are likely to be included in Risk Group 1 (note that other thermophilic members of the genera *Clostridium* (*C. thermoautotrophicum, C. thermosaccharolyticum, C. thermosulfurogenes C. thermohydrosulfuricum, C.stercorarium, C. therocellum and C. thermolacticum*) have been classified as Risk type 1 in ERMA permit NOC99021). While endospores generated prokaryotic Gram positive microorganisms are highly chemical and desiccation resistant, the generation of endospores (unlike spores from *Actinomycetes*, fungi and algae or cysts from Protozoa) are not part of the *normal* cell cycle. Endospores only form when conditions become non-conducive to growth. Therefore, where cells are routinely subcultured, the formation of endospore-inducing conditions is avoided. Note that is expected that NO fungi will be enriched from these samples and therefore no fungal spores will be generated.

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 MBRG containment facilities have been registered as a PC1 type laboratory with MAF according to the MAF Biosecurity Authority Standard 154.03.02. An inspection of the containment facilities has been conducted by a MAF officer (Mike Aitkenhead, Mike.Aitkenhead@maf.govt.nz**,** 29/07/2004,) and provisional approval was given to operate (official approval is awaiting a police security check of the operator responsible for the facility).

All proposed work using the soil/sediment and fluid samples described in this application will be conducted in the afore mentioned containment facility according the protocols described in the MBRG Containment and Quarantine Manual (a copy of the manual has been included in this application). While specifics for the maintenance of a containment facility are described in detail in the manual, the overarching premises for containment regime are as follows;

- Only authorised, trained personnel will be permitted to conduct research within the containment facility. All staff with be trained in the correct and safe handling of microbial cultures
- All trained personnel are (or will be) required to ensure that work practices comply with the requirements of the Biosecurity and HSNO Acts and any additional conditions stipulated by ERMA
- All personnel are (or will be) required to understand and implement contingency plans in the unlikely situation where containment is compromised
- All microbiological material, irregardless of whether sourced from within New Zealand or classified as a “new organism” (see HSNO Act 1996) will be destroyed (using the appropriate methods described in the MRBG Containment and Quarantine Manual (and AS/NZ Standard 2243:3:2002) before leaving the containment facility
- A register of all HSNO-restricted microbial cultures will be maintained in the MRBG Containment and Quarantine Manual
- Transfer of HSNO-restricted microbial cultures 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

PATHWAYS OF ESCAPE for microorganisms

The laboratory will be run in accordance with the principles of AS/NZS 2243.3:2002 "Safety in Laboratories: Microbiological aspects and containment facilities", as required by MAF Standards 154.03.02 and 152.04.03F and the HSNO act (1998). If the procedures described in the Standard Work Practices (Waste Disposal and Treatment) are followed (Section 6.3, MBRG Containment Manual), the likelihood of this occurring is remote. Not-withstanding, the following areas have been identified as *possible* points of escape;

- *During transit between containment facilities*. Correct IATA packaging will prevent the escape of the microbial culture beyond the secondary packaging (see IATA packing instruction No: 650)
- *Accidental release of viable material into storm water without sterilisation*. Drain traps have been installed such that if release is suspected, the contaminated waters can be correctly treated with a chemical disinfectant.
- *Sabotage, lapse of security or fire*. Up-to-date safety equipment, and in-house and contracted security at the containment facility means that any accidental release of the facility by sabotage, lapse of security or fire is unlikely and will be minimised.

As mentioned is Section 3.2, a small number of Gram-positive endospore-forming *Bacteria* have been enriched from hydrothermal features. Endospores are chemical and desiccation resistant. However, endospore generating conditions are not part of the *normal* reproduction cycle and only occur when conditions are non-conducive, for example, where an energy source is spent. Growth of these cells will always be in closed containers that can be autoclaved and therefore the risk of aerosol generation is minimal if endospore stimulating conditions were to occur. It should be noted that aerosol formation may occur where a culturing vessel is broken or during sonication/vortexing. In all cases the quantity of endospores released would be too low to form a self-sustaining community (see section 5.1). Sonication and vortexing are conducted in closed vessels, so the spread of endospores can be contained. Where a vessel is broken, the spill would be chemically treated according to AS/NZS 2243.3:2002 "Safety in Laboratories: Microbiological aspects and containment facilities" (and as described in the MBRG Quarantine and Containment Manual).

In the advent of escape from the containment facilities, there is negligible probability that the microbial cultures enriched and isolated from the hydrothermal soil/sediment and fluid samples would be able to proliferate and out-compete indigenous microflora due to their specific growth requirements. As no known natural terrestrial features in New Zealand mimic the chemistries and conditions of hydrothermal vents, the possibility of establishing a self-sustaining microbial community would be essentially non-existent. In addition, due to their type Risk 1 profile, no microorganisms within these samples are likely to pose any threat to human, flora or fauna.

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.>

As outlined in section 4, in the advent of escape from the containment facilities, it is highly improbable that any microbial populations from the hydrothermal soil/sediment and fluid samples would be able to proliferate and out compete indigenous microflora due to their specific growth requirements, primarily temperature and salinity, but also their need for specific energy sources and terminal electron acceptors. Only small populations of microorganisms will be used in routine work (≤1L at maximum densities of 10^9 microorganisms/ml). At these levels, there are no predicted environmental perturbations because the numbers in the environment are usually much greater[1].

The primary route of escape from the facility, *if* containment protocols *and* back-up safeguards failed would be via wastewater drains to a sump. Conditions within the sump are non-conducive to growth due to the lack of elevated temperatures and energy sources/nutrients. Any indigenous microbial populations within the sump would out-compete the escaped microflora (or microflora generated from endospores). Likewise, spillage or transport and release of these microbial populations outside the containment facility would require the cultures to be transported and released *specifically* to a geothermally heated environment in large volumes. The microorganisms would then need to be in large enough population to out-compete

an established population[1] and to survive the specific environment. The likelihood of this occurring is essentially non-existent. Furthermore, there are no known natural terrestrial features in New Zealand that mimic the chemistries and physical conditions of marine hydrothermal vents and therefore the possibility of establishing a self-sustaining microbial community would be essentially non-existent.

In the unlikely case where an "as of yet undiscovered" geothermal feature identically mimicking the geochemical and geophysical conditions found in a specific marine hydrothermal vent AND a large volume of restricted microorganisms were then transported to this site and released, then the establishment of a self-sustaining population within a geothermal environment would be difficult to eradicate. However, in this case, it would be highly probable that a similar, if not identical indigenous microorganism would also be present.

[1]ESR Ltd GMC99004: In this approved application it states. "Such small amounts of organism being used in individual experiments….means that there would be no predicted environmental perturbation as the number already in the environment are much greater." Thus, GMC99004 is referenced as a precedent of the argument and for its documentation.

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)

No potential adverse effects on the environment have been identified within containment or if the microbial cultures were to escape containment. It is a common premise in environmental microbiology that the environment dictates the type of microbial population. Therefore in the event of escape, the geographical distance to an environment conducive to growth makes establishment improbable. However, if this were to occur, it is highly likely that a similar or identical microbial species will inhabit the environment (H. Morgan, personal communications). If this case were ever to occur, then no perceived adverse effects on the environment can be envisaged.

It is not known whether any of the microorganisms within these samples are inseparable. It is possible that some of the samples may contain bacteriophage populations. Only one viral particle (bacteriophage) has so far been isolated from a hydrothermal vent system (Geslin *et al.,* 2003). The viral strain appears to only replicate in the host microorganism it was isolated from, *Pyrococcus abyssi*. Again, as outlined previously, it is unlikely that any non-separable strains would be able to survive outside containment.

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

Risk to public health (including MBRG personnel) will be negligible because the microorganisms likely to be enriched from the hydrothermal sediment/soil and fluids samples are not human pathogens.

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.

No evidence of any adverse effects on the relationships of Māori and their culture and traditions with their ancestral lands, water, sites, waahi tapu or valued flora and fauna and other taonga have been identified. As outlined in section 5.2A, in the unlikely event of escape into the conducive environment, no adverse effect would be noted. The risks are no greater than outlined above.

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

I can conceive of no way in which the escape of these microorganisms could have any adverse effects on NZ's international obligations, nor any negative social or economic effects or ethical issues.

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 risk of escape and establishment of a self-propagating microbial population are minimal to non-existent and therefore the value associated with this is negligible.

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

N/A

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.

N/A

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

N/A

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.

We hope that by studying these unique environments and the microorganisms that inhabit them we will learn more about how these cells are able to survive in these harsh conditions and how they interact within food chain and surrounding geochemical/mineral environments. Therefore, we believe that by importing these soil/sediment and fluid samples from hydrothermal vent systems we will firstly, and most importantly, increase scientific knowledge. Understanding the ecology of these systems has implications for understanding the origins of life, life on other planets and the similarities between extreme microbial survival and pathogen evasion techniques. Furthermore, the discovery of new microorganisms open up possibilities of developing potentially new beneficial technologies such as bioremediation of pollutants, generation of environmentally sustainable energies, medicine and drug discovery and efficient industrial processes. It is proposed that these samples will be collected with world-recognised international collaborators; therefore the study of these microorganisms will bolster New Zealand's international science reputation.

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.

While it is difficult to quantify benefits for increasing scientific knowledge, there is no doubt that if novel microorganisms were isolated form these samples, then there is potential for medical and environmental biotechnologies to be identified. Likewise, there is potential for the development of environmentally sustainable technologies such as eco-friendly fuels or pollutant bioremediation out of microbial cultures enriched form these samples. Again, it is difficult to quantify these benefits.

A list of scientific publications my members of the research team at GNS have been provided in the references list.

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 benefits of permitting the soil/sediment and fluid samples to be held and processed in containments clearly outweigh the adverse effects of the organism and any associated organisms. By studying the biodiversity and isolating any microorganisms from these samples we are increasing the scientific knowledge of the ecology in these unknown environments. In doing so we, may gain some insights to the origins of life, perhaps life on other planets and the similarities between extreme microbial survival and pathogen evasion techniques. Furthermore, the discovery of new microorganisms may open up possibilities of developing potentially new beneficial technologies such as bioremediation of pollutants, generation of environmentally sustainable energies, medicine and drug discovery and efficient industrial processes. The

benefits for New Zealand and science if any of these technologies were realised would potentially be huge.

Conversely, the risks associated with cultivating these microorganisms are minimal to non-existent. Any microorganisms enriched from these samples clearly require specific conditions that will prevent the establishment of a self-propagating community if containment were ever to be breached. In addition, there is no risk to public health for a containment failure or occupational exposure, due to the microorganism's lack of pathogenesis and inability to grow in all but extreme conditions.

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.

No approvals are required for the Animal Welfare Act 1999 or CITES. The Biosecurity Act 1993 requires that a containment facility be established according to the directions dictated by MAF Standards 154.03.02 for the importation of HSNO restricted microorganisms (ERMA). This has been done and is being processed by MAF currently (see section 4.1 for details). The Biosecurity Act also requires environmental samples containing microorganisms to be imported under permit from MAF. This will be done following any ERMA decision relating to this application.

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)?

N/A. It is impossible to determine what microorganisms will be contained within the soil/sediment and fluid samples. However, Appendix 1 details the microorganisms previously isolated from other hydrothermal vent systems. It is possible that some or all of the microorganisms isolated from the samples in question are included in Appendix 1.

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

No

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

- **Extremophilic microorganisms** require 'extreme' physio-chemical conditions for optimal growth. These conditions are usually outside those that permit the majority of life to exist;
    - acidophiles (acidity pH <3.0)
    - thermophiles (Temperatures >45˚C)
    - hyperthermophiles (Temperatures >80˚C)
    - methanogens (microorganisms that generate methane and are strict anaerobes)
    - barophiles (requirement of a atmospheric pressure greater than 1atm for growth)
    - halophiles (requirement of a minimum NaCl requirement, usually considered greater than that of seawater)
- Metabolic descriptions

- **Autotroph**; a microorganism that obtains its carbon source from carbon dioxide (ie not an organic compound)
- **Heterotroph**; a microorganism that obtains its carbon source from an organic compound
- **Chemolithotroph**; a microorganism that grows (gains energy) by the oxidation of inorganic compounds. Generally chemolithotrophic microorganisms are autotrophic
- **Chemoorganotroph**; a microorganism that grows (gains energy) by the oxidation of organic compounds. Generally chemoorganotrophic microorganisms are heterotrophic
- **Terminal electron acceptor;** a terminal electron acceptor (or oxidant) is required to gain energy from the oxidation of an energy source. In the case of aerobic organisms (like ourselves) oxygen is the terminal electron acceptor, whereas in the case of anaerobic organisms such as a methanogen, carbon dioxide is the terminal electron acceptor.
- **Anaerobe**; a microorganism that grows in the absence of free oxygen, usually using an inorganic compound in place of oxygen as the terminal electron acceptor
- **Aerobe**; a microorganism that grows in the presence of atmospheric oxygen (as the terminal electron acceptor).
- **Microaerobe**; a microorganism that grows in the presence of reduced concentrations of atmospheric oxygen, but is damaged by normal oxygen concentrations

- A **new microorganism** is (see HSNO Act 1998);
  - An organism belonging to a species that was not present in New Zealand immediately before 29 July 1998;
  - An organism belonging to a species , subspecies, infrasubspecies, variety, strain, or cultivar prescribed as a risk species, where that organism not present in New Zealand at the time of promulgation of the relevant regulation;
  - A genetically modified organism
- **NZ EEZ**; New Zealand Exclusive Economic Zone
- **Bacteriophage;** a virus that infects prokaryotic microorganisms
- **Microbial classifications;**
  - **Prokaryote;** single cellular organisms encompassing the bacterial and archaeal domains. Prokaryotic DNA is not enclosed by a membrane (nucleus).
  - **Eukaryote;** Cells that contain a membrane enclosed nucleus. The cells are generally larger than prokaryotes and in many eukaryotes are multicellular organisms (plants and animals). Eukaryote cells encompassing protista, algae, fungi, plants and animals
  - ***Archaea*;** a prokaryotic cell distinct from *Bacteria*. Archaeal cells lack a muramic acid in their cell wall structure, have ether-linked membrane lipids and are insensitive to many antibiotics. *Archaea* tend to be the dominant microbial type in so called extreme conditions
  - ***Bacteria***; All prokaryotes that are not of the domain *Archaea.* Bacterial cells contain muramic acid in their cell wall structure, have ether-linked membrane lipids and are insensitive to many antibiotics.
  - **Gram positive/Gram negative;** all prokaryotic cells can be classified by a cell wall strain known as a Gram stain. Cells either stain positive or negative and are characterised by a single plasma membrane and a thick peptidoglycan envelope or a double membrane and a thin peptidoglycan layer in between respectively.
  - **Algae;** Photosynthetic unicellular eukaryotes
  - **Fungi;** Non-photosynthetic unicellular or multicellular eukaryotes with rigid cell walls
  - **Acintomyces;** Gram-positive aerobic bacteria that form branching filaments or hyphae and asexual spores

- **Protozoa;** Unicellular eukaryotes that lack a cell wall

- **Hydrothermal vent/feature;** Hydrothermal vents arise from seawater being heated by magmatic features near to the earths surface. Hydrothermal vents are usually associated with marine systems and are most readily located is the vicinity of tectonic plate edges.

6.5 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.

See over page

Appendix 1. List of prokaryotic microorganisms isolated and described from submarine hydrothermal vent systems

Microbial genus and species name		Domain	Risk classification*	Optimal growth temp.	Culture collection reference	reference
Aquifex	*pyrophilus*	*Bacteria*	1	85˚C	DSM 6858	Huber *et al.,* 1992
Balnearium	*lithotrophicum*	*Bacteria*	1	70°C	DSM 16304	Takai *et al.,* 2003
Caminibacter	*hydrogeniphilus*	*Bacteria*	1	60°C	DSM 14510	Alain *et al.,* 2002
	profundus	*Bacteria*	1	55°C	DSM 15016	Miroshnichenko *et al.,* 2004
Caminicella	*sporogenes*	*Bacteria*	1	60˚C	DSM 14501	Alain *et al.,* 2002
Carboxydothermus	*restrictus*	*Bacteria*	1	65°C	DSM 7242	Svetlichnyi *et al.,* 1994
Catenococcus	*thiocyclus*	*Bacteria*	1	25°C	DSM 9165	Sorokin, 1994
Clostridium	*caminithermale*	*Bacteria*	1	42°C	DSM 15212	Brisbarre *et al.,* 2003
Deferribacter	*abyssi*	*Bacteria*	1	55°C	DSM 14873	Miroshnichenko *et al.,* 2003
	desulfuricans	*Bacteria*	1	62°C	DSM 14783	Takai *et al.,* 2003
Desulfacinum	*hydrothermale*	*Bacteria*	1	60°C	DSM 13146	Sievert and Kuever, 2000
Desulfonauticus	*submarinus*	*Bacteria*	1	45°C	DSM 15269	Audiffrin *et al.,* 2003
Desulfurobacterium	*crinifex*	*Bacteria*	1	60°C	DSM 15218	Alain *et al.,* 2003
	thermolithotrophum	*Bacteria*	1	70°C	DSM 11699	L'Haridon *et al.,* 1998
Ferroglobus	*placidus*	*Archaea*	1	85°C	DSM 10642	Hafenbradl *et al.,* 1997
Geothermobacter	*ehrlichii*	*Bacteria*	1	50-55°C	DSM 15274	Kashefi *et al.,* 2003
Halomonas	*neptunia*	*Bacteria*	1	30˚C	DSM 15720	Kaye *et al.,* 2004
	sulfidaeris	*Bacteria*	1	30˚C	DSM 15722	Kaye *et al.,* 2004
	axialensis	*Bacteria*	1	30˚C	DSM 15723	Kaye *et al.,* 2004
	hydrothermalis	*Bacteria*	1	30˚C	DSM 15725	Kaye *et al.,* 2004
Halothiobacillus	*hydrothermalis*	*Bacteria*	1	35°C	DSM 7121	Durand *et al.,* 1997
	kellyi	*Bacteria*	1	37°C	DSM 13162	Sievert *et al.,* 2000
Hyperthermus	*butylicus*	*Archaea*	1	99°C	DSM 5456	Zillig *et al.,* 1990
Idiomarina	*loihiensis*	*Bacteria*	1	30°C	DSM 15497	Donachie *et al.,* 2003
Ignicoccus	*pacificius*	*Archaea*	1	90°C	DSM 13166	Huber and Stetter, 2000
Marinithermus	*hydrothermalis*	*Bacteria*	1	70°C	DSM 14884	Sako *et al.,* 2003
Marinitoga	*camini*	*Bacteria*	1	55°C	DSM 13578	Wery *et al.,* 2001
	piezophila	*Bacteria*	1	65°C	DSM 14283	Alain *et al.,* 2002

Microbial genus and species name		Domain	Risk classification*	Optimal growth temp.	Culture collection reference	reference
Methanocaldococcus	*fervens*	*Archaea*	1	85°C	DSM 4213	Jeanthon *et al.,* 1999
	infernus	*Archaea*	1	85°C	DSM 11812	Jeanthon *et al.,* 1998
	vulcanius	*Archaea*	1	80°C	DSM 12094	Jeanthon *et al.,* 1999
	indicus	*Archaea*	1	85°C	DSM 15027	L'Haridon *et al.,* 2003
	jannaschii	*Archaea*	1	80-85°C	DSM 2661	Jones *et al.,* 1984
Methanotorris	*igneus*	*Archaea*	1	85°C	DSM 5666	Burggraf *et al.,* 1990
Methanopyrus	*kandleri*	*Archaea*	1	98°C	DSM 6324	Kurr *et al.,* 1992
Methanothermococcus	*okinawensis*	*Archaea*	1	65°C	DSM 14208	Takai *et al.,* 2002
	thermolithotrophicus	*Archaea*	1	65°C	DSM 2095	Huber *et al.,* 1984
Nanoarchaeum	*equitans*	*Archaea*	1	90˚C?	-	Huber *et al.,* 2003
Oceanithermus	*profundus*	*Bacteria*	1	60°C	DSM 14977	Miroshnichenko *et al.,* 2003
Palaeococcus	*ferrophilus*	*Archaea*	1	80-83°C	DSM 13482	Takai *et al.,* 2000
Persephonella	*marina*	*Bacteria*	1	70°C	DSM 14350	Götz *et al.,* 2002
	guaymasensis	*Bacteria*	1	70°C	DSM 14351	Götz *et al.,* 2002
	hydrogeniphila	*Bacteria*	1	70°C	DSM 15103	Nakagawa *et al.,* 2003
Pyrococcus	*horikoshii*	*Archaea*	1	95°C	DSM 12428	González *et al.,* 1999
	woesei	*Archaea*	1	97-100°C	DSM 3773	Zillig, 1988
	furiosus	*Archaea*	1	97-100°C	DSM 3638	Fiala and Stetter, 1986
Pyrodictium	*brockii*	*Archaea*	1	85-105°C	DSM 2708	Stetter *et al.,* 1984
	occultum	*Archaea*	1	85-105°C	DSM 2709	Stetter *et al.,* 1984
	abyssi	*Archaea*	1	98°C	DSM 6158	Pley and Stetter, 1991
Pyrolobus	*fumarii*	*Archaea*	1	103°C	DSM 11204	Blöchl *et al.,* 1999
Rhodothermus	*marinus*	*Bacteria*	1	65°C	DSM 4253	Alfredsson *et al.,* 1995
Staphylothermus	*marinus*	*Archaea*	1	85-90°C	DSM 3639	Fiala *et* al., 1986
	hellenicus	*Archaea*	1	85°C	DSM 12710	Arab *et al.,* 2000
Stetteria	*hydrogenophila*	*Archaea*	1	93°C	DSM 11227	Jochimsen *et al.,* 1997
Tepidibacter	*thalassicus*	*Bacteria*	1	50°C	DSM 15285	Slobodkin *et al.,* 2003
	formicigenes	*Bacteria*	1	45°C	DSM 15518	Urios *et al.,* 2004
Thermaerobacter	*marianensis*	*Bacteria*	1	75°C	DSM 12885	Takai *et al.,* 1999
	nagasakiensis	*Bacteria*	1	70°C	DSM 14512	Nunoura *et al.,* 2002
Thermoanaerobacter	*siderophilus*	*Bacteria*	1	70°C	DSM 12299	Slobodkin *et al.,* 1999

Microbial genus and species name		Domain	Risk classification*	Optimal growth temp.	Culture collection reference	reference
Thermococcus	*profundus*	*Archaea*	1	80°C	DSM 9503	Kobayashi and Horikoshi, 1995
	chitonophagus	*Archaea*	1	85°C	DSM 10152	Huber and Stetter, 1996
	alcaliphilus	*Archaea*	1	85°C	DSM 10322	Keller *et al.,* 1995
	peptonophilus	*Archaea*	1	85°C	DSM 10343	González *et al.,* 1996
	guaymasensis	*Archaea*	1	88°C	DSM 11113	Canganella *et al.,* 1998
	barophilus	*Archaea*	1	85°C	DSM 11836	Marteinsson *et al.,* 1999
	aegaeus	*Archaea*	1	90°C	DSM 12767	Arab *et al.,* 2000
	stetteri	*Archaea*	1	75°C	DSM 5262	Miroshnichenko, 1990
	celer	*Archaea*	1	88°C	DSM 2476	Zillig, 1983
	litoralis	*Archaea*	1	83°C	DSM 5473	Neuner *et al.,* 2001
	pacificus	*Archaea*	1	85°C	DSM 10394	Miroshnichenko *et al.,* 1998
	gorgonarius	*Archaea*	1	85°C	DSM 10395	Miroshnichenko *et al.,* 1998
	acidaminovorans	*Archaea*	1	85°C	DSM 11906	Dirmeier *et al.,* 2001
	siculi	*Archaea*	1	85°C	DSM 12349	Grote *et al.,* 2000
	waiotapuensis	*Archaea*	1	85°C	DSM 12768	González *et al.,* 1999
	aggregans	*Archaea*	1	85°C	DSM 12819	Canganella *et al.,* 1998
	fumicolans	*Archaea*	1	85°C	DSM 12820	Godfroy and Meunier, 1996
	atlanticus	*Archaea*	1	85°C	DSM 15226	Cambon-Bonavita *et al.,* 2003
	gammatolerans	*Archaea*	1	88°C	DSM 15229	Jolivet *et al.,* 2003
Thermodesulfatator	*indicus*	*Bacteria*	1	70°C	DSM 15286	Moussard *et al.,* 2004
Thermodesulfobacterium	*hydrogeniphilum*	*Bacteria*	1	70°C	DSM 14290	Jeanthon *et al.,* 2002
Thermosipho	*japonicus*	*Bacteria*	1	70°C	DSM 13481	Takai and Horikoshi, 2000
Thermotoga	*maritima*	*Bacteria*	1	80°C	DSM 3109	Huber *et al.*, 1986
	neapolitana	*Bacteria*	1	85°C	DSM 4359	Jannasch *et al.,* 1989
Thermovibrio	*ruber*	*Bacteria*	1	80°C	DSM 14644	Huber *et al.,* 2002
	ammonificans	*Bacteria*	1	75°C	DSM 15698	Vetriani *et al.,* 2004
Thiomicrospira	*crunogena*	*Bacteria*	1	25°C	DSM 12353	Jannasch *et al.,* 1985
Vulcanithermus	*mediatlanticus*	*Bacteria*	1	65°C	DSM 14978	Miroshnichenko *et al.,* 2003

* *Risk classification according to AS/NZ Standard 2243:3:2002.*

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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.

This application is for the importation into containment of soils/sediments and fluids that may contain unidentified and potentially novel microbial species from shallow marine, and deep hydrothermal marine vents and adjacent areas in the Kermadec volcanic arc (NE of NZ), for the purpose of biodiversity, ecology and biotechnology studies.

The Institute of Geological and Nuclear Sciences and their research partners will be visiting these sites and predict that may contain unique, and possibly undiscovered, microbial populations. We hope that by studying these unique environments and the microorganisms that inhabit them, we will learn more about how these cells are able to survive in these harsh conditions and how they interact within the food chain and surrounding geochemical/mineral environments. Understanding the ecology of these systems has implications for understanding the origins of life, life on other planets and the similarities between extreme microbial survival and pathogen evasion techniques. Discovering new microbes open up possibilities of developing potentially new beneficial technologies such as bioremediation of pollutants, generation of environmentally sustainable energies, medicine and drug discovery, and efficient industrial processes.

As these samples, at the time of importation, will not have been analysed for microorganisms, it is not possible to determine what types of cells may (if any) be present. However, a review of the microbial types isolated (so far) from other hydrothermal vent systems suggest that any microorganisms enriched from these samples will be low risk and non-pathogenic to humans, flora and fauna. Furthermore, as these samples will be taken from

extremely hot (from 50-300˚ C), marine environments, with very little organic energy sources, it highly unlikely any of the microorganisms present will be able to grow outside specialised laboratory conditions.

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 (*only selected references*)	yes
Cheque for initial fee (incl. GST) enclosed	Yes
If "yes", state amount:	$1125
Direct credit made to ERMA bank account:	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:**