FORM HS3

Application for approval to

IMPORT OR MANUFACTURE ANY
HAZARDOUS SUBSTANCE IN
CONTAINMENT

under section 31 of the
Hazardous Substances and New Organisms Act
1996

Name of Substance(s): Yersinia entomophaga

Applicant: AgResearch, Lincoln Science Centre, Gerald Street, Private Bag 4749, Christchurch 8140, New Zealand

Office use only

Application Code: □□□□□□□□□□ Date
received:____/____/____

ERMA NZ Contact: ________________ Initial Fees Paid: $

Application Version No: ________
IMPORTANT

1. Before you fill in this application form, you may find it helpful to consult the *User Guide to Hazardous Substance Applications under the HSNO Act 1996*. This User Guide can either be downloaded from our website or purchased from ERMA New Zealand.

2. Part E of the User Guide covers applications under Section 31 of the Act and all of the cross references to this guide that are in this application form relate to Part E.

3. You can also talk to an applications officer 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.

4. This application form may be used to seek approvals for more than one hazardous substance where the substances are related, for example a concentrated compound (active ingredient) and its related formulations, or a range of substances for similar purposes to be tested in a field trial.

5. Any extra material that does not fit in the application form must be clearly labelled, cross-referenced, and included in an Appendix to the application form.

6. Commercially sensitive information must be collated in a separate Appendix.

7. Applicants must sign the form and enclose the correct application fee. The initial application fee can be found in our published *Schedule of Fees and Charges*. Make sure that you have an up to date copy of the Schedule. Please check with ERMA New Zealand staff. We are unable to process applications that do not contain the correct fee.

8. Unless otherwise indicated, all sections of this form must be completed for the application to be progressed. Where an applicant is unable to complete the sections marked optional, this information may be derived by ERMA New Zealand and the costs of doing so will be recovered from the applicant as part of the processing costs.

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

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NEW ZEALAND
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www.ermanz.govt.nz
Section One – Applicant Details

See comments under “Section One of Application Form” in the User Guide for guidance.

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

Name: AgResearch
Address: Lincoln Science Centre, Gerald Street, Private Bag 4749, Christchurch 8140, New Zealand
Phone: +64 3 321 8800
Fax: +64 3 325 9946

1.2 The applicant’s location address in New Zealand (if different from above):

Address:

1.3 Name of the contact person for the application:

This person should have sufficient knowledge to respond to queries and either have the authority to make decisions on behalf of the applicant that relate to processing the application, or have the ability to go to the appropriate authority.

Name: Dr Sue Zydenbos
Position: Senior Scientist/Team Leader
Address: Lincoln Science Centre, Gerald Street, Private Bag 4749, Christchurch 8140, New Zealand
Phone: +64 3 325 9909
Fax: +64 3 325 9946
Email: sue.zydenbos@agresearch.co.nz
Section Two – Application Type and Related Approvals Required

This form is only to be used for an application to import a hazardous substance into containment or manufacture a hazardous substance in containment.

If you are making the application for some other reason, you will need a different form.

2.1 Is this application to manufacture or import a hazardous substance in containment for any of the following purposes:
- Containment applications can only be made for a limited range of purposes. In particular it is not intended for commercial manufacture or sale. (See comments under “Section 2.1 of Form” in the User Guide)
- Small amounts of any hazardous substance for use as an analytical standard where approval to import or manufacture that substance has been declined? Yes/No
- Research on any hazardous substance to acquire information for use in assessing that substance for a HSNO approval? Yes/No
- Research and development on any hazardous substance? Yes/No
- Use in an emergency? Yes/No
- Other purposes? Yes/No

2.2 If you answered yes to one of the purposes listed above, please provide some supporting detail. If you answered yes to “other purpose”, describe the purpose and explain why this purpose is appropriate to a containment application. (See comments under “Section 2.2 of Form” in the User Guide)

This application is for approval to operate containment field trials of the bacterium *Yersinia entomophaga*, an organism that was isolated from the New Zealand grass grub. This microorganism has bio-insecticidal activity, and research under laboratory conditions has shown that it is effective against a number of insect pests that are important in New Zealand agriculture and horticulture. A containment application was deemed necessary at this stage because of the ecotoxic properties of the organism.

The proposed containment trials will test efficacy of *Yersinia entomophaga* in the field against a range of insect pests on various host crops, as described in the application. If efficacy can be demonstrated, the organism could be developed into a bio-insecticide suitable for use in both organic and conventional production systems and would be an environmentally-sustainable alternative to existing chemical insecticides.
2.3 Is the information in this application relevant to import, manufacture or both?  
(See comments under “Section 2.3 of Form” in the User Guide)

- Import the substance(s) only? Yes/No
- Manufacture the substance(s) only? Yes/No
- Import and manufacture the substance(s)? Yes/No
- If import only, indicate whether or not manufacture is likely in New Zealand Yes/No

2.4 If the information in the application relates to manufacture of the substance(s) in New Zealand, provide information on the proposed manufacturing process and any alternatives.  
(See comments under “Section 2.4 of Form” in the User Guide)

*Yersinia entomophaga* for testing in field trials will be produced by fermentation under PC2 conditions at AgR Lincoln Microorganism Containment Facility #480. Fermentation will be in volumes of up to 10 litres, which is within the limits for this type of work under existing ERMA approvals for the laboratory. All equipment and materials used for fermentation will be cleaned and sterilised after use, as per standard laboratory protocols for PC2 containment. No other alternatives are proposed.

2.5 If this substance(s) needs an approval under any other legislation, has an application for this approval been made?  
(Optional)  
(See comments under “Section 2.5 of Form” in the User Guide)

<table>
<thead>
<tr>
<th>Name of Approval</th>
<th>Application made</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agricultural Compounds and Veterinary Medicines Act 1997</td>
<td>Yes/No/NA</td>
</tr>
<tr>
<td>Food Act 1981</td>
<td>Yes/No/NA</td>
</tr>
<tr>
<td>Medicines Act 1981</td>
<td>Yes/No/NA</td>
</tr>
<tr>
<td>Chemical Weapons (Prohibition) Act 1996</td>
<td>Yes/No/NA</td>
</tr>
<tr>
<td>Radiation Protection Act 1965</td>
<td>Yes/No/NA</td>
</tr>
<tr>
<td>Biosecurity Act 1993</td>
<td>Yes/No/NA</td>
</tr>
<tr>
<td>Resource Management Act 1991</td>
<td>Yes/No/NA</td>
</tr>
<tr>
<td>Other (please specify):</td>
<td>Yes/No</td>
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</tbody>
</table>


Section Three – Information on the Substance(s)

Note all information that is commercially sensitive must be attached as an Appendix. The application form should be cross-referenced to the Appendix but should be able to be read as a stand-alone document which will be publicly available.

If approval is being sought for more than one hazardous substance, this section must be completed separately for each hazardous substance.
3.1 State the unequivocal identification of the substance(s):

The active substance is a micro-organism: *Yersinia entomophaga* (type strain MH96T) (Proteobacteria: Enterobacteriaceae) (Hurst et al. 2010). This bacterium was isolated from a diseased larva of the New Zealand grass grub, *Costelytra zealandica* (Coleoptera: Scarabaeidae), found in 1996 in New Zealand. *Yersinia entomophaga* has been classified by ERMA as being “not a new organism to New Zealand”.

The bacterium was isolated as a cream-coloured colony growing on Luria-Bertani (LB) agar at 30°C. It is able to grow at 25-42°C, but not at 45°C. No hemolysis is detected at 25°C or 37°C on Columbia sheep blood or Columbia horse blood agar. API 20E (bioMérieux) tests indicated the following properties: Positive for ornithine-decarboxylase and grows in the presence of meliobiose, glycerol, trehalose, cellobiose, lactose, raffinose, sucrose and citrate; Negative for lysine-decarboxylase and urease, and does not utilise rhamnose, L-Arabinose, D-Xylose, salicin, inositol, sorbitol, sorbose, aesculin, malonate and indole. Although API tests indicate a strong similarity (96.3% database match) to *Serratia marcescens*, colony morphology and subsequent biochemical tests (DNase agar and caprylate thallous agar) show this is a different organism. *Yersinia entomophaga* is a Gram-negative, rod-shaped bacterium with 1-3 peritrichous flagella. Standard rods are 0.7 μm wide and 1.7 μm long, but larger elongated cells ranging from 1 μm in width and 20-60 μm in length are also observed. *Yersinia entomophaga* is oxidase negative, catalase negative and able to reduce nitrate. At a DNA level the G+C content of MH96T is at 49.3 mol%.

*Yersinia entomophaga* is intended to be used as a bio-insecticide but it will need to be formulated for application to the target pest species and production systems. In the proposed containment trials the formulation components listed in Appendix 1 are expected to be used. Formulation requirements will be specific for each pest system and different components will need to be tested. Therefore the components listed and their anticipated % composition shown in the Appendix 1 are likely to vary for individual experiments. Full details will be given in actual trial protocols submitted to ERMA before the start of each trial. All components used in formulations will be taken from the “Generally Regarded as Safe” or “List of Inert Pesticide Ingredients” lists. Many components used are part of the bioshield™ product that has recently received organic certification (Appendix 2).

For mixtures, in addition to the above information being provided on the actual mixture, information is also required on the composition of the mixture i.e. the chemical name, CAS number, function (e.g. active ingredient, emulsifier, surfactant, filler, dispersant) and percentages of ALL components of the mixture (including non-hazardous components and impurities) should be provided. This information may be best expressed in tabular form. If the composition is variable, please ensure to state the limits.

If there are commercial reasons for not providing full information in the main part of the form, alternative approaches must be discussed with and agreed by ERMA New Zealand. These must include the provision of a unique identifier of some kind.
3.2 Provide information on the chemical and physical properties of the substance(s).

Provide as much information as possible on the chemical and physical properties of the substance(s) (at 20°C and 1 atmosphere unless otherwise stated) eg

- Appearance (colour, odour, physical state or form)
- pH
- Density
- Vapour pressure
- Boiling/melting point
- Solubility in water
- Water/octanol partitioning co-efficient

None of the formulation components are considered hazardous in the quantities being used. Details of the biological properties of *Yersinia entomophaga* have been given in Section 3.1. A description of chemical and physical properties of the organism is not applicable.

Below is a description of the *Yersinia entomophaga* formulations that would be used in field trials.

**Gel**

Appearance (colour, odour, physical state or form):

- An off-white to light yellow/brown opaque viscous water dispersible liquid.

  - pH: 5-8
  - Density: ~1 g/mL
  - Vapour pressure: ~ 2.3 kPa
  - Boiling: ~100°C
  - Solubility in water: Freely soluble
  - Water/octanol partitioning co-efficient: NA

**Bait (Extruded prill)**

Appearance (colour, odour, physical state or form):

- A light to dark brown rod like structure approximately 0.5-4 mm in diameter and 0.5-2 cm in length.

  - pH: NA
  - Density: 1-5 g/mL
  - Vapour pressure: NA
  - Boiling: NA
  - Solubility in water: Dispersible
  - Water/octanol partitioning co-efficient: NA

**Granules**

Appearance (colour, odour, physical state or form):

- A light to dark brown granule approximately 1-8 mm in diameter.

  - pH: NA
  - Density: 1-5 g/mL
  - Vapour pressure: NA
  - Boiling: NA
  - Solubility in water: Dispersible
  - Water/octanol partitioning co-efficient: NA

For mixtures, information is required on the chemical and physical properties of the mixture itself. However, if this information is not available, you should provide information on the chemical and physical properties of EACH hazardous component of the mixture.
3.3 Provide information on the hazardous properties of the substance(s).
Information should be provided on the hazardous properties of the substance(s) known to the applicant.

As outlined above, none of the formulation components are considered hazardous in the quantities being used, therefore information on the hazardous nature of the substance is limited to the organism itself.

Yersinia entomophaga is a biological organism and the only potentially hazardous property is ecotoxicity. Details of potential ecotoxicity for each of the four sub-classes are listed below.

1. Aquatic (subclass 9.1) – There is currently no available information on the toxicity of Yersinia entomophaga to aquatic organisms. Data are currently being collected by a US-based industry partner and should be available early in 2011. None of the field containment trials will be carried out near open water and there is little likelihood of the organism entering an aquatic environment. This is not considered to be a significant risk for the containment trials proposed.

2. Soil (subclass 9.2) – Laboratory trials indicate that Yersinia entomophaga added to soil at levels greater than $10^6$ colony forming units (cfu)/g declined to below a threshold of $10^3$ cfu/g after 15 days, which is a decrease of over 99.9% of viable cells (M.R.H. Hurst, unpublished data; see Appendix 3). Yersinia entomophaga inoculated into soil does not affect development of two earthworm species (Aporrectodea caliginosa and Eisenia fetida) (Glare et al. 2010). The effect of Yersinia entomophaga on soil microbial communities is unknown, but due to Yersinia entomophaga having limited survival in the soil this is not considered to be a significant risk.

3. Terrestrial vertebrate (subclass 9.3) – A study has been carried out by Valley Animal Research Centre Ltd, an accredited testing laboratory, to investigate terrestrial vertebrate toxicity of Y. entomophaga (Goldenthal & Vallance 2007). No adverse side-effects were detected when bacteria were injected intraperitoneally into mice (report summary in Appendix 4). No further terrestrial vertebrate toxicity testing has been carried out to date, but this work will be done if results from the containment trials indicated that the organism has potential as a bioinsecticide.

4. Terrestrial invertebrate (subclass 9.4) – Yersinia entomophaga causes mortality of a range of insect species including C. zealandica, Tasmanian grass grub (Acrorius tasmaniae), redheaded cockchafer (Adoryphorus couloni), Chafer beetle (Odontria sp.), bronze beetle (Encolapisspp.), black beetle (Heteronychus arator), porina (Wiseana cervinata, Wiseana spp.), diamondback moth (Plitella xystalatha), white cabbage butterfly (Pieris rapae), cotton bollworm (Helicoverpa amigera), codling moth (Cydia pomonella), painted apple moth (Teia anartoides), light brown apple moth (Epiphyas postvittana), brownheaded leafroller (Ctenoptusis spp), greenheaded leafrollers (Planotortis excessana and P. notophaea), greater wax moth (Galleria mellonella), the migratory locust (Locusta migratoria), Darwin’s ant (Doleromyrma darwiniana) and clover root weevil (Sitona lepidus).

A study was conducted where caged honey bees were fed a sugar solution without or with Yersinia entomophaga ($10^7$/ml or $10^9$/ml) or given no sugar solution (Taylor et al. 2007). There was no significant difference in mortality between honey bees fed sugar solution only or sugar solution containing $10^7$ Yersinia entomophaga/ml, which is in the order of magnitude of target field applications of the organism. At very high concentrations ($10^9$ Yersinia entomophaga/ml sugar solution), which would only be found as stock solutions for preparing formulations, mortality was similar to honey bees that were starved (i.e. given no access to sugar solution). These results are for direct oral ingestion and any exposure through contact is expected to have considerably less impact on mortality.
Some insects, such as Argentine stem weevil (*Listronotus bonariensis*), a native amphipod (possibly *Parachestria tenuis*), the black field cricket (*Teleogryllus commodus*) and American cockroach (*Periplaneta americana*), are not affected by *Yersinia entomophaga*.

In addition, as outlined above, development of the earthworms (*Aporrectodea caliginosa*) was not affected when soil was inoculated with *Yersinia entomophaga*.

Full details of these laboratory experiments are summarised in a technical report and two scientific papers (Brownbridge et al. 2008; McNeill & Hurst 2008; Glare et al. 2010).

You should consider each of the six hazardous properties below and provide information on those hazardous properties. This information is needed in order to assess risks and determine whether or not and how the substance can be adequately contained.

- explosiveness
- flammability
- oxidising properties
- corrosiveness
- toxicity
- ecotoxicity

If your substance is a mixture and you cannot provide direct information on its hazardous properties, you can apply mixture rules to the hazardous components of the mixture. If you do this, then you will need to provide information on the hazardous properties of each hazardous component of the mixture, and show your workings.
3.4 Provide information on what will happen to the substance throughout its whole life from its introduction into New Zealand, its uses, through to disposal.

Yersinia entomophaga for testing in field trials will be produced by fermentation under PC2 conditions at AgR Lincoln Microorganism Containment Facility #480. Fermentation will be in volumes of up to 10 litres, which is within the limits for this type of work under existing ERMA approvals for the laboratory. All equipment and materials used for fermentation will be cleaned and sterilised after use, as per standard laboratory protocols for PC2 containment.

Formulations for application to the field sites will be prepared under PC2 conditions and packaged in accordance with IATA packaging instruction 650. They will be transported to field sites in suitably labelled double sealed containers, the outer being constructed of rigid plastic.

During application of Yersinia entomophaga to the field sites operators will follow standard procedures for pesticide application. The application may use one of the following methods for application of the organism to the field site: liquid spray, broadcast granules, coated seed, or formulated bait. Equipment used to apply Yersinia entomophaga formulations will be cleaned and sterilised on site or taken back to a PC2 laboratory for cleaning and sterilisation. Specific details of the type of application and formulation will be included in the experimental protocol submitted to ERMA before initiation of a field trial. The experimental protocol for the first proposed field trial is attached in Appendix 5.

Field sites are contained in the following manner:

- Sites where Yersinia entomophaga is applied will be fenced to exclude mammals and birds and appropriate signage erected to prevent access by unauthorised persons. Trial plots will be netted to prevent ingress by birds. Sites will be within fenced properties and are likely to be on private land or research farms and therefore not readily accessible by unauthorised persons.
- Samples will be taken from treated foliage at regular intervals (e.g. 1-3 days) to assess Yersinia entomophaga survival by standard plating methodology and persistence of insecticidal activity by bioassay. Containment will be maintained until bacteria and insecticidal activity are no longer detectable on the trial site.
- In the unexpected event that Y. entomophaga is still detectable after one month from treatment, there are several options to ensure that the Y. entomophaga is removed from the site. For example, all plant material to which Yersinia entomophaga formulations have been applied will be removed and destroyed by autoclave or burning. In the case of application to pasture the trial area will be cultivated and foliage buried to a depth of at least 100 mm. In addition, the treated area could be sprayed with broad-spectrum insecticide to prevent movement of insects outside of the trial area.

The information provided needs to reflect the containment character of the application. It will be used in the development of exposure scenarios and the assessment of risks and hence the specification of the containment conditions.
3.5 Provide information on the quantity of the substance proposed to be imported or manufactured.

Formulations for each of the field trials will be produced from single fermentations of up to 10 litres.
Section Four – Information on the Proposed Containment System

Provide information on the proposed containment system.

It is essential that good information is provided on the containment system because the adequacy of containment in conjunction with the hazardous properties of the substance will have a major impact on whether or not approval is given.

*Yersinia entomophaga* will be tested in containment field trials for efficacy against the following key pests:

- bronze beetle (*Eucolapsis* spp.)
- porina (*Wiseana cervinata, Wiseana* spp.)
- diamondback moth (*Plutella xylostella*)/white cabbage butterfly (*Pieris rapae*)
- black beetle (*Heteronychus arator*)
- grass grub (*Costelytra zealandica*)

The containment field trials will be run in three major production systems:

- perennial fruit crops (e.g. apples, kiwifruit)
- perennial pasture
- annual field crops (e.g. brassicas, maize)

Specific protocols will be provided to ERMA before each field trial. These will include details such as the site of the trial, the dates of operation of the trial, the names and experience of the people operating the trial and any details that are specific to that site. A trial design and management protocol for the first trial is presented in Appendix 5.

Generic details for the three types of field trial are provided below.

You will need to provide a description of the containment proposed AND information on how you intend to address the following issues (proposed controls):

- methods for preventing the escape of the contained hazardous substance and preventing the contamination of the facility.

The *Yersinia entomophaga* formulations will be transported from the PC2 production facility to the field site in double sealed containers as described above (section 3.4). The treatments will be applied to the field plots as per the trial protocol (e.g. spray, broadcast, drill, bait). Survival of the organism is expected to be much less than 15 days on plant (Monjaret & Glare 2004) or soil surfaces (Appendix 3) and therefore the site will be fenced to contain *Yersinia entomophaga* during the trial period. All trial sites will be located well away from ponds or waterways to prevent movement of the *Yersinia entomophaga* into these systems. As described in section 3.4, measurements of residual *Yersinia entomophaga* activity will be made at the end of the trial and appropriate action taken to maintain containment until *Yersinia entomophaga* is no longer detectable or remove the bacterium from the site.

- methods for excluding unwanted organisms from the facility or to control organisms within the facility

As described above, the trial area will be securely fenced to prevent unwanted vertebrate organisms entering the facility. Cages or netting will be applied to treated areas (e.g. ...
branches of trees) to prevent access by birds and to minimise movement of invertebrates.

- **methods for excluding unauthorised people from the facility**

As mentioned above, trial areas will be fenced to prevent unintended access to treated areas. Where appropriate, signage will be posted to indicate that people should not enter the area and locations will be chosen in areas of limited access where possible.

- **methods for preventing unintended release of the substance by experimenters**

The key methods for preventing unintended release by researchers are good labelling of treatments in the PC2 facility and secure packaging during transport as described in Section 3.4. During application of treatments, standard equipment for application of pesticides in the field will be used (e.g. spray suits). All equipment used on site will be decontaminated on site or double bagged and returned to lab for appropriate cleaning or destruction. Standard pesticide procedures and appropriate controls will used to prevent and contain any onsite spillages.

- **methods for controlling the effects of any accidental release of the substance**

In the event of an accidental release, the procedure would be to contain the site by erecting fencing and bird netting for at least 15 days, after which the site would be tested for survival of the organism. In the unexpected case of *Yersinia entomophaga* survival the site would be sprayed with a broad-spectrum insecticide to prevent movement of any invertebrates that came in contact with the organism. Foliage would be removed and destroyed and soil would be cultivated to bury the surface layers.

- **inspection and monitoring requirements of the containment facility**

Before application of treatments, the researcher will report to a designated compliance manager stating that the containment system has been established as described in this application. The trial site will be visited by the researcher for three of the first five days to ensure that the site remains securely contained. Over the following 10 days, a further three visits will be made to monitor the containment.

A management plan may be attached as an appendix. This plan should specify the procedures for implementing the above methods for containing the substance(s), and provide details of the qualifications of the person responsible for implementing those controls. (See comments under “Section 4.1 of Form” in the User Guide.)
Section Five: Identification and Assessment of Risks

In completing this section, it is important that you take account of the proposed containment system you described in Section 4. We are particularly interested in knowing about risks that may still remain with the containment system in place. You will need to consider the effects on the environment and public health including any social effects. For more details see comments under “Section Five of Application Form” in the User Guide.

You should also take account of the quantity of material involved and the number of different locations that may be involved.

Complete this section as far as you can. If the analysis provided is incomplete, then it will be completed by ERMA New Zealand. However, the costs of doing this will be chargeable.
5.1 Identify all of the risks of the substance(s).

Potential areas of risk from the application of *Yersinia entomophaga* to a contained field trial site are listed below. All of these risks arise from potential ecotoxic effects of the organism. There are no other sources of risk.

- **the sustainability of native and valued introduced flora and fauna** – there is a risk that *Yersinia entomophaga* will affect susceptible non-target invertebrates that are present in the treated zone. However, because (i) the organism must be ingested for bioinsecticidal activity, (ii) the organism is only effective at densities greater than $1 \times 10^4$ cells per mm of leaf surface or g of soil and (iii) the cell residues decline rapidly to below these densities within 2-3 days from application, the risk is no more, and probably less than that from currently registered insecticides. In addition, any effect would be temporary as the sites are small (<1 ha) and would be easily re-occupied by the non-target species.

- **the intrinsic value of ecosystems** – the material applied is a naturally occurring, native bacterium. It will act as a “soft” material with little long-term effect and minimal environmental disruption as it will not persist at high levels in the treated ecosystem. This source of risk is considered to be low.

- **public health (including occupational exposure)** – there is no known hazard from the material to public health. Researchers applying the *Yersinia entomophaga* formulations will use standard practices for application of pesticides. This source of risk is considered to be insignificant.

- **the relationship of Maori and their culture and traditions with their ancestral lands, water, sites, waahi tapu, valued flora and fauna and other taonga** – the bacterium is a native bacterium that has evolved within Aotearoa. The bacterium has never been isolated from native flora and fauna with the exception of a single isolation from the endemic pest insect, the New Zealand grass grub. There is a very low risk of *Yersinia entomophaga* affecting Maori values, culture and traditions.

- **the economic and related benefits to be derived from the use of the hazardous substance** – if developed as a commercial control, the bacterium will have a significant role in underpinning New Zealand’s clean-green’, pesticide residue free production and provide a sustainable option for ongoing crop protection. *Yersinia entomophaga* is therefore considered a potential tool to limit the environmental effects from the application of other insecticides to productive ecosystems (i.e. it would decrease current environmental risk).

- **New Zealand’s international obligations** – use of the bacterium is not in conflict with any of New Zealand’s international obligations. There is no risk from use of *Yersinia entomophaga* in this category.

Include information on potentially significant possible risks of the substance and whether or not these risks are likely to be significant. 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.
### 5.2 Provide an assessment of the potential risks identified in Section 5.1.

An explicit risk assessment only needs to be provided for those risks which might be significant. The assessment should consider whether the identified risks can be adequately managed by the proposed containment system and the substance(s) itself adequately contained.

As outlined in Section 5.1, risk is considered to be low and limited to potential ecotoxic effects on non-target invertebrates in the treated zone. Due to the limited survival of *Yersinia entomophaga* in soil or on leaf surfaces it is unlikely to persist in the containment area from 15 days after treatment application. This means that non-target invertebrates can safely move back and recolonise the treatment area after this time period. The site will be contained until *Yersinia entomophaga* can no longer be detected.

The assessment should include the nature, probability of occurrence and magnitude of each adverse effect. The uncertainty bounds of the information contained in the assessment should also be discussed.

(Optional) (See comments under “Section 5.2 of Form” in the User Guide).
Section Six – International Considerations

6.1   ERMA New Zealand is interested in whether this substance (or any of its components) has been considered by any other regulatory authority in New Zealand or by any other country. If you are aware of this, please provide details of the results of such consideration.

Yersinia entomophaga has previously been assessed by ERMA as “not a new organism to New Zealand”. The organism is currently being assessed by a commercial partner in the USA and it may then proceed to registration as a biopesticide under US Environmental Protection Agency Regulations.

(Optional) (See comments under “Section 6.1 of Form” in the User Guide).
## Section Seven – Miscellaneous

### 7.1 Provide a glossary of scientific and technical terms used in the application.
(See comments under “Section 7.1 of Form” in the User Guide)

### 7.2 Provide here any other information you consider relevant to this application not already included.
(See comments under “Section 7.2 of Form” in the User Guide)
Section Eight – Summary of Public Information

The information provided in this section may be used in the Authority’s public register of substances required under Section 20 of the HSNO Act. This summary information will be used to provide information for those people and agencies (e.g., Ministry for the Environment, Department of Conservation, Regional Councils, etc.), who will be notified of the application, and for potential submitters who request information. This information will also be used to prepare the public notice of the application. For these reasons, applicants should ensure that this summary information does not contain any commercially sensitive material.

8.1 Name of the substance(s) for the public register:

_Yersinia entomophaga_

Please use a maximum of 80 characters. (See comments under “Section 8.1 of Form” in the User Guide)

8.2 Purpose of the application for the public register:

This should include (in a maximum of 255 characters) an abstract giving information on the intended use of the substance and why an application is needed based on its hazardous properties.

_Yersinia entomophaga_ will be tested in the field for efficacy as a bioinsecticide against a range of insect pests found in productive ecosystems. If effective, the bacterium will be formulated and commercialised as an environmentally-sustainable pest control option.

(See comments under “Section 8.2 of Form” in the User Guide)

8.3 Use Categories of the substance(s):

ERMA New Zealand has adopted the system of use categories developed by the European Union, which identify various functional uses of substances. This information is pertinent to the assessment of exposure scenarios and the determination of risk and is also useful for building up a profile of the substance. There are three sets of use categories. Within each of these, applicants should state which use categories are relevant to all intended uses of the substance(s).

Main category: Wide dispersive use
Industry category: Agricultural industry
Function/Use category: Pesticides

Main category: There are four main categories - see User Guide for details.
Industry category: There are 16 industry categories - see User Guide for details.
Function/Use category: There are 55 function/use categories - see User Guide for details.

(Optional) (See comments under “Section 8.3 of Form” in the User Guide).
8.4 Executive Summary:

In this section, the applicant should provide a summary of information contained in this application, including:

- the identification of the substance, its hazardous properties, intended uses, and disposal
- an assessment of the adverse effects of the substance
- information on the proposed containment

This application is for field testing in containment the micro-organism Yersinia entomophaga (type strain MH96) (Proteobacteria:Enterobacteriaceae) as a bio-insecticide against a range of insect pests found in productive ecosystems. This bacterium was isolated from a diseased larva of the New Zealand grass grub, Costelytra zealandica (Coleoptera: Scarabaeidae), found in 1996 in New Zealand and has been classified by ERMA as being “not a new organism to New Zealand”.

If effective, the bacterium will be formulated and commercialised as an environmentally-sustainable pest control option. Because the potential product would be marketed as an alternative to chemical pesticides it would be formulated to standards that would enable organic certification. Therefore the only potentially hazardous properties of the organism and any formulation components are ecotoxic properties.

As there is currently no information about aquatic ecotoxicity (subclass 9.1), all containment trials will be located away from open water and there is little likelihood of the organism entering. The laboratory trials that Yersinia entomophaga does not survive for long periods in the soil (subclass 9.2) and does not affect development of two earthworm species (Aporrectodea caliginosa and Eisenia fetida). Effects on soil microbial communities are unknown but due to the short survival period of Yersinia entomophaga, soil is not considered to be at significant risk from application of the organism. No adverse side effects have been found from intraperitoneal injection of Yersinia entomophaga into mice. No further terrestrial vertebrate toxicity testing has been carried out to date, but research to date indicates that risk of ecotoxicity in subclass 9.3 is low.

Terrestrial invertebrates (subclass 9.4) are the target for the ecotoxic effects of Yersinia entomophaga. The organism has a broad-spectrum but not universal effect on insects. In particular, beneficial organisms such as honey bees are not affected by oral ingestion of dose rates of Yersinia entomophaga that will be applied in the containment field trials. A feature that ensures a low risk of Yersinia entomophaga causing non-target effects in containment field trials is the low persistence of the organism in soil and on plant surfaces.

During containment field trials, sites will be not readily accessible by the public and will be fenced to prevent unwanted vertebrates entering the trial site. Cages or netting will be applied to treated areas (e.g. branches of trees) to prevent access by birds and to minimise movement of invertebrates. All trial sites will be located well away from ponds or waterways. Yersinia entomophaga formulations will be produced in a PC2 facility and transported to the field site in double sealed containers. The treatments will be applied as spray, broadcast, drill or bait formulations according to the target pest and production system. Treatments will be applied by skilled researchers using standard practices for application of pesticides. Equipment used to apply Yersinia entomophaga formulations will be cleaned and sterilised on site or taken back to a PC2 laboratory for cleaning and sterilisation. Measurements of residual Yersinia entomophaga activity will be made at the end of the trial and appropriate action taken to maintain containment until the Yersinia entomophaga is no longer detectable or remove the bacterium from the site. A detailed trial protocol and management plan will be submitted to ERMA before the start of each trial.

(See comments under “Section 8.4 of Form” in the User Guide)
## CHECKLIST

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<th>Status</th>
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</tr>
<tr>
<td>Appendices enclosed</td>
<td>Yes/ NA</td>
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<tr>
<td>Initial fee enclosed</td>
<td>Yes</td>
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<tr>
<td>Application signed and dated</td>
<td>Yes</td>
</tr>
<tr>
<td>Electronic copy of application e-mailed to ERMA NZ</td>
<td>Yes</td>
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</tbody>
</table>

Signed: ___________________________  Date: ___________________________