ENVIRONMENTAL RISK MANAGEMENT AUTHORITY DECISION

Amended under section 67A of the HSNO Act on 23 June 2011

20 August 2007

Application code	GMD07031
Application type	To develop in containment genetically modified organisms under sections 40(1)(b) and 42A of the Hazardous Substances and New Organisms (HSNO) Act 1996.
Applicant	LanzaTech New Zealand Ltd.
Purpose	To develop a range of non-pathogenic bacteria modified for enhanced solvent production for potential use in the production of valuable resources from industrial by-products.
Date received	09 August 2007
Consideration date	17 August 2007
Considered by	Chief Executive, ERMA New Zealand

1 Summary of decision

1.1 Application GMD07031 to develop, as a project, genetically modified organisms (as described in Table 1 of this decision) in containment is **approved, with controls** (see Appendix 1 of this decision), having been considered in accordance with sections 40(1)(b) and 42A of the Hazardous Substances and New Organisms (HSNO) Act 1996 (the Act), the HSNO (Low-Risk Genetic Modification) Regulations 2003 (the Regulations), and the HSNO (Methodology) Order 1998 (the Methodology).

The organisms approved are:

1.2 The organisms approved for development are the genetically modified organisms described in Table 1:

Table 1: Organisms as recorded on ERMA New Zealand Register

Host organism	Category of host	Modified by	Category of modification/ containment
Escherichia coli (Migula 1895) Castellani and Chalmers 1919 non-pathogenic laboratory strains	1	Modified by standard non-conjugative cloning and expression plasmid vectors and standard non-conjugative suicide plasmid vectors used in the mutagenesis of the host organisms listed below containing DNA fragments derived from the genomic DNA of Risk Group 1[1] micro-organisms. The genetic material will consist of the coding, non-coding or regulatory regions, or antisense sequences of putative metabolic or regulatory genes or other DNA sequences that are involved in or will enhance solvent production from carbon sources. Vectors will include standard and commercially available promoters and other gene regulatory elements, reporter and selectable marker genes, protein purification tags and origins of replication. The following modifications are excluded: • Modifications that enhance the pathogenicity, virulence or infectivity of the host organism or enhance the ability to escape from containment. • Modifications that produce vertebrate toxins with LD_{50}< 100 µg/kg.	A/PC1

[1] Risk Group 1 micro-organisms are micro-organisms that are unlikely to cause disease in humans, animals, plants or fungi.

Table 1: Organisms as recorded on ERMA New Zealand Register continued

Host organism	Category of host	Modified by	Category of modification/ containment
Clostridium spp. Prazmowski 1880 non-pathogenic species (Risk Group 1) Except:	2	Modified by random transposon-mediated or site-directed mutagenesis to identify genes or DNA sequences that are involved in or will enhance solvent production from carbon sources.	B/PC2
Clostridium spp. Prazmowski 1880 non-pathogenic and non spore forming strains (Risk group 1)	1	Modified by standard non-conjugative expression plasmid vectors containing DNA fragments derived from the genomic DNA of Risk Group 1[1] micro-organisms. The genetic material will consist of the coding, non-coding or regulatory regions, or antisense sequences of putative metabolic or regulatory genes or other DNA sequences that are involved in or will enhance solvent production from carbon sources. Vectors will include standard and commercially available promoters and other gene regulatory elements, reporter and selectable marker genes, protein purification tags and origins of replication. The following modifications are excluded: • Modifications that enhance the pathogenicity, virulence or infectivity of the host organism or enhance the ability to escape from containment. • Modifications that produce vertebrate toxins with LD_{50}< 100 µg/kg.	A/PC1
Bacillus spp. Cohn 1872 non-pathogenic species (Risk Group 1)	2		B/PC2
Moorella thermoacetica (Fontaine et al 1942) Collins et al 1994 (Risk Group 1)	2		B/PC2
Geobacter spp. Lovley et al 1995 non-pathogenic species (Risk Group 1)	1		A/PC1
Acetobacterium woodii Balch et al 1977 (Risk Group 1)	1		A/PC1
Zymomonas mobilis (Lindner 1928) Kluyver and van Niel, 1936 (Risk Group 1)	1		A/PC1

2 Consideration

Sequence of the consideration

2.1 The application was formally received and verified as containing sufficient information on 09 August 2007.

2.2 The decision was based on the information supplied by the applicant in the application form: *Develop in containment a project of low risk genetically modified organisms by rapid assessment* (NO3P).

2.3 The application was considered by Rob Forlong, the Chief Executive of ERMA New Zealand. Relevant staff within ERMA New Zealand, including the Senior Advisor, Māori, were involved in providing advice on the consideration of the application.

2.4 In reaching my decision I have considered matters relevant to the purpose of the Act, as specified in Part II, and followed the relevant provisions of the Methodology.

2.5 In accordance with section 42A of the Act for rapid assessment, the approach adopted was to identify the circumstances of the genetic modification, to evaluate these against the criteria specified in the Regulations established under section 41 of the Act, and to consider whether there are any residual risks that require further consideration. This approach covered the following issues:

- Purpose of the application (section 39 of the Act);
- Assessment against the criteria of the Regulations;
- Identification and assessment of the risks and other impacts of the organism;
- Precedents; and
- Containment controls.

Purpose of the application

2.6 The purpose of this research is to genetically modify various non-pathogenic species of *Clostridium*, *Bacillus* and *Geobacter*, and *Moorella thermoacetica*, *Zymomonas mobilis* and *Acetobacterium woodii* to enhance the ability of these bacteria to produce specific solvents (such as ethanol) from carbon sources and to increase the variety of carbon sources able to be used by these bacteria. The ultimate goal of this research is to produce bacteria which could be used at commercial manufacturing plants to convert harmful waste by-products into valued products.

2.7 I have determined that this application is for a valid purpose being *the development of any [new] organism* as provided for in section 39(1)(a) of the Act.

Assessment against the criteria for low-risk genetic modification

Category of host organism

2.8 The non-pathogenic laboratory strains of *Escherichia coli (E. col*i), non-pathogenic species of *Geobacter, Zymomonas mobilis* and *Acetobacterium woodii* and the non pathogenic, non spore forming strains of *Clostridium* spp. to be used by the applicant are not capable of causing disease in humans, animals, plants or fungi or normally infect, colonise, or establish in humans. These organisms do not produce desiccation-

resistant structures, such as spores or cysts and their main biological characteristics are known. As such, non-pathogenic laboratory strains of *E. coli,* non-pathogenic species of *Geobacter*, *Zymomonas mobilis* and *Acetobacterium woodii* and the non pathogenic, non spore forming strains of *Clostridium* spp. are considered Category 1 host organisms as defined in clause 7(1) of the Regulations.

2.9 The non-pathogenic species of *Clostridium* (other than the non-pathogenic, non-spore forming *Clostridium* spp.) and *Bacillus*, and *Moorella thermoacetica* are Risk Group 1 micro-organisms that can produce desiccation-resistant structures such as spores that can normally be disseminated in the air. As such, the non-pathogenic species of *Clostridium* and *Bacillus*, and *Moorella thermoacetica* are considered Category 2 host organisms as defined in clause 7(2) of the Regulations.

Category of genetic modification

2.10 The genetic modifications to non-pathogenic laboratory strains of *E. coli*, non-pathogenic species of *Geobacter*, *Zymomonas mobilis* and *Acetobacterium woodii* and the non pathogenic, non spore forming strains of *Clostridium* spp. (described in Table 1) are not expected to increase the pathogenicity, virulence or infectivity of the organisms to laboratory personnel, the community, or the environment. In addition, the developments will not result in the organisms having a greater ability to escape from containment than the unmodified organisms. Therefore, the genetic modifications to the non-pathogenic laboratory strains of *E. coli*, non-pathogenic species of *Geobacter*, *Zymomonas mobilis* and *Acetobacterium woodii* and the non pathogenic, non spore forming strains of *Clostridium* spp. as described in Table 1 of this decision are Category A genetic modifications as defined in clause 5(1) of the Regulations and shall be contained at a minimum of Physical Containment Level 1 (PC1).

2.11 The genetic modifications to non-pathogenic species of *Clostridium* (other than the non-pathogenic, non-spore forming *Clostridium* spp.) and *Bacillus*, and *Moorella thermoacetica* (described in Table 1) are not expected to increase the pathogenicity, virulence or infectivity of the organisms to laboratory personnel, the community, or the environment. In addition, the developments will not result in the organisms having a greater ability to escape from containment than the unmodified organisms. Therefore, the genetic modifications to non-pathogenic species of *Clostridium* (other than the non-pathogenic, non-spore forming *Clostridium* spp.) and *Bacillus*, and *Moorella thermoacetica* as described in Table 1 of this decision are Category B genetic modifications as defined in clause 5(2) of the Regulations and shall be contained at a minimum of Physical Containment Level 2 (PC2).

2.12 I am satisfied that the developments meet the criteria for low-risk genetic modification specified in the Regulations. The developments involving non-pathogenic laboratory strains of *E. coli*, non-pathogenic species of *Geobacter*, *Zymomonas mobilis* and *Acetobacterium woodii* and the non pathogenic, non spore forming strains of *Clostridium* spp. meet the requirements of Category A modifications as defined in clause 5(1) of the Regulations in that the modifications involve Category 1 host organisms and are to be contained at a minimum of PC1 containment. The developments involving non-pathogenic species of *Clostridium* (other than the non-pathogenic, non-spore forming *Clostridium* spp) and *Bacillus*, and *Moorella thermoacetica* meet the requirements of Category B modifications as defined in clause

5(2) of the Regulations in that the modifications involve Category 2 host organisms and are to be contained at a minimum of PC2 containment.

Identification and assessment of the risks, costs and other impacts of the organism

2.13 I consider that the information provided by the applicant is relevant and appropriate to the scale and significance of the risks, costs, and benefits associated with the application (as required by clause 8 of the Methodology). In accordance with clauses 9, 10 and 12 of the Methodology (which incorporate sections 5, 6, and 8 of the Act) the information supplied by the applicant has been evaluated as follows:

2.14 I consider that, given the biological characteristics of the organisms, the containment system and the controls attached to this approval (see Appendix 1 of this decision), there is no evidence for, nor any reason to expect, any non-negligible adverse effects of the proposed genetically modified organisms on humans, animals, plants, other organisms or the environment.

2.15 I have considered the potential Māori cultural effects in accordance with sections 6(d) and 8 of the Act and clauses 9(b)(i), 9(c)(iv) of the Methodology, in consultation with the Senior Advisor, Māori. As this application does not involve the use of genetic material from native or valued flora and fauna or from Māori, and as this application is for a development in containment, there is no requirement for the applicant to consult with Māori.

2.16 Although recognising that iwi/Māori maintain an ongoing interest and concern in the potential long term cultural implications of genetic modification generally, I consider that this application poses negligible risk of adverse effects to the relationship of Māori culture and traditions with their ancestral lands, water, sites, waahi tapu, valued flora and fauna, and other taonga.

2.17 This assessment is made with the understanding that all associated containment regulations, controls and conditions are met by the applicant.

Precedents

2.18 I must consider each application on its merits, and am therefore not bound by the stance taken in previous decisions. However, in reflecting on previous decisions that involved similar genetic modifications to those proposed by this application, I note that genetic modifications to a range of bacteria conforming to the Regulations, have been considered and approved on several occasions by both Institutional Biological Safety Committees (IBSCs) and the Chief Executive of ERMA New Zealand, under delegated authority. For example, in application GMD06004, a proposal to modify *Pseudomonas aeruginosa* and *Pseudomonas syringae* to investigate genes involved in virulence was approved.

2.19 I consider that this current application does not raise any novel issues and there are no residual risks that require further consideration.

Containment

2.20 The experiments proposed in this application, to develop genetically modified non-pathogenic laboratory strains of *E. coli*, non-pathogenic species of *Geobacter*, *Zymomonas mobilis* and *Acetobacterium woodii* and the non pathogenic, non spore forming strains of *Clostridium* spp. meet the requirements of Category A genetic modifications as defined in clause 5(1) of the Regulations. Category A experiments are required to be contained within a Physical Containment level 1 facility (PC1).

2.21 The experiments proposed in this application, to develop genetically modified non-pathogenic species of *Clostridium* (other than the non-pathogenic, non-spore forming *Clostridium* spp.) and *Bacillus*, and *Moorella thermoacetica* meet the requirements of Category B genetic modifications as defined in clause 5(2) of the Regulations. Category B experiments are required to be contained within a Physical Containment level 2 facility (PC2). I note that the applicant has operating procedures in place to prevent spore dispersal such as the use of sealed vessels and biological safety cabinets.

2.22 The facility to be used shall be approved as a containment facility under section 39 of the Biosecurity Act, in accordance with the MAF Biosecurity New Zealand/ERMA New Zealand Standard *Facilities for Microorganisms and Cell Cultures: 2007*. This containment regime contains clear guidelines for the safe handling and disposal of cultures.

3 Decision

3.1 I am satisfied that this application is for one of the purposes specified in section 39(1) of the Act, being section 39(1)(a): *the development of any [new] organism*.

3.2 Based on consideration and analysis of the information provided, and having considered the characteristics of the organisms that are the subject of this approval, the modifications and the criteria for low-risk genetic modification detailed in the Regulations, I am of the view that the organisms meet the criteria for rapid assessment under section 42A of the Act.

3.3 I have considered all the matters to be addressed by the containment controls for Importing, Developing or Field testing of Genetically Modified Organisms detailed in the Third Schedule Part I, of the Act, and in accordance with section 42A(3)(b), this approval is subject to the controls specified in Appendix 1.

3.4 I consider that this current application does not raise any novel issues and there are no residual risks that require further consideration.

3.5 Pursuant to section 42A(3)(a) of the Act, and acting under delegation from the Authority provided for in section 19 of the Act, I have approved this project application for genetically modified non-pathogenic laboratory strains of *E. coli*, non-pathogenic species of *Clostridium*, *Bacillus* and *Geobacter*, *Moorella thermoacetica*, *Zymomonas mobilis* and *Acetobacterium woodii* described in Table 1 of this decision, subject to the controls specified in Appendix 1 of this decision.

APPENDIX 1: CONTROLS REQUIRED BY THIS APPROVAL

In order to provide for the matters detailed in Part I of the Third Schedule of the Act[2], *Containment Controls for Importation, Development and Field Testing of Genetically Modified Organisms,* and other matters in order to give effect to the purpose of the Act, the approved organisms are subject to the following controls:

1 To limit the likelihood of any accidental release of any organism or any viable genetic material[3].

1.1 The approved organism shall be developed and maintained within a containment facility which complies with these controls.

1.2 The person responsible for a particular research area and/or the person responsible for the operation of the containment facility shall inform all personnel involved in the handling of the organism of the Authority's controls.

1.3 The facility shall be approved and registered by MAF as a containment facility under section 39 of the Biosecurity Act, in accordance with the MAF/ERMA New Zealand Standard (below), and controls imposed by the Authority (as follows):

[2] Bold headings in the following text refer to Matters to be Addressed by Containment Controls for Import, Development and Field Testing of Genetically Modified Organisms, specified in the Third Schedule of the Act.

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

1.4 The construction, operation and management of the containment facility shall be in accordance with the:

1.4.1 MAF Biosecurity New Zealand/ERMA New Zealand Standard *Facilities for Microorganisms and Cell Cultures: 2007*[4];

1.4.2 Australian/New Zealand Standard AS/NZS 2243.3:2002[4]: *Safety in laboratories: Part 3: Microbiological aspects and containment facilities*;

1.4.3 Physical Containment level 1 (PC1) requirements of the above Standards for developments involving non-pathogenic laboratory strains of *E. coli*, non-pathogenic species of *Geobacter*, *Zymomonas mobilis* and *Acetobacterium woodii* and the non pathogenic, non spore forming strains of *Clostridium* spp. and

1.4.4 Physical Containment level 2 (PC2) requirements of the above Standards for developments involving non-pathogenic species of *Clostridium* other than the non-spore forming species) and *Bacillus,* and *Moorella thermoacetica*.

2 To exclude unauthorised people from the facility.

2.1 Construction and operation of the containment facility shall comply with the requirements of the standards listed in control 1.4 relating to the identification of entrances, numbers of and access to entrances and security requirements for the entrances and the facility.

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

3.1 Construction and operation of the containment facility shall comply with the requirements of the standards listed in control 1.4 relating to the exclusion of other organisms from the facility and the control of undesirable and unwanted organisms within the facility.

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

4.1 Construction and operation of the containment facility shall comply with the requirements of the standards listed in control 1.4 relating to the prevention of unintended release of the organism by experimenters working with the organism.

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

5.1 Construction and operation of the containment facility shall comply with the requirements of the standards listed in control 1.4 relating to controlling the effects of any accidental release or escape of an organism.

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

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

5.3 In the event of any breach of containment of the organism, the contingency plan for the attempted retrieval or destruction of any viable material of the organism that has escaped shall be implemented immediately. The contingency plan shall be included in the containment manual in accordance with the requirements of standards listed in control 1.4.

6 Inspection and monitoring requirements for containment facilities.

6.1 The operation of the containment facilities shall comply with the requirements contained in the standards listed in control 1.4 relating to the inspection and monitoring requirements for containment facilities.

6.2 The containment manual shall be updated, as necessary, to address the implementation of the controls imposed by this approval, in accordance with the standards listed in control 1.4.

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

7.1 The training of personnel working in the facility shall be in compliance with the standards listed in control 1.4.

_____ 20 August 2007

Rob Forlong Date

Chief Executive, ERMA New Zealand

Approval codes: GMD004693 – 99

Amended in June 2011:

Non pathogenic, non spore forming strains of *Clostridium* have been specified as Category 1 host organisms.

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Rob Forlong Date

Chief Executive, ERMA New Zealand

Approval numbers for Organisms in Application GMD07031

Approval Code	Organism
GMD004696	*Escherichia coli* (Migula 1895) Castellani and Chalmers 1919 (GMD07031)
GMD004695	*Clostridium* spp. Prazmowski 1880 non-pathogenic strains (Risk Group 1) and non pathogenic, non spore forming strains (GMD07031)
GMD004694	*Bacillus* spp. Cohn 1872 non-pathogenic strains (Risk Group 1) (GMD07031)
GMD004697	*Geobacter* spp. Lovley et al. 1995 non-pathogenic strains (Risk Group 1) (GMD07031)
GMD004698	*Moorella thermoacetica* (Fontaine et al. 1942) Collins et al. 1994 (GMD07031)
GMD004693	*Acetobacterium woodii* Balch et al. 1977 (GMD07031)
GMD004699	*Zymomonas mobilis* (Lindner 1928) Kluyver and van Niel, 1936 (GMD07031)

As of 15 September 2009, new BCH numbers cannot be provided. Please use the appropriate Approval number in lieu of the BCH number.