ERMA New Zealand Evaluation & Review Report

Application for Approval to Perform a Large Scale Fermentation in Containment of any Genetically Modified Organism

Application Number: GMF98010

Genetically Modified *Escherichia coli K12*

Key Issues

The application from the AgResearch seeks approval to perform large scale fermentations of genetically modified *Escherichia coli* K12 in containment. The Act places fermentations of a large scale in the same application group as field tests, which require public notification. The *E. coli* is modified by *Echinococcus granulosus* (hydatid disease) gene and an antibiotic ampicillin resistant gene. The actual fermentations of the bacteria are to take place at Industrial Research Limited, Gracefield, Lower Hutt.

Because this is a containment application, the key issues relate to the adequacy of the containment facility in conjunction with the proposed controls to prevent the unintended release of the modified bacteria

If the *E. coli* was released from containment it is unlikely that this would lead to the establishment of a self sustaining population of genetically modified *E. coli*. The *E. coli* K12 strain is a non-pathogenic strain of the bacteria and genetically crippled in that it does not contain a conjugated plasmid or bacteriophage and therefore cannot transduce its genetic material to other hosts. Taking in account the 'contained' nature of the inserted genetic material in the organism to be fermented, the risks remain low.

The risk of escape from containment appears to be negligible on considering that:

- the nature of the organism, as noted above, provides an initial containment of the genetic material,
- there is primary containment within the fermentation vessels, and
- the vessel is located within a containment facility.

The Authority may wish to consider the long term implications of this application that is seeking approval for an initial three fermentations and future fermentations of unspecified numbers and duration. Successful registration of the vaccine is expected to open up new markets for the vaccine, increasing the quantity required to be produced. The future commercial production of the vaccine would require ongoing monitoring of the large scale fermentation containment facility and internal audit procedures for compliance with the controls imposed.

The applicant has proposed a change regarding the method of killing the bacteria as detailed in Appendix 5. This information has been provided to the submitters with the distribution of the E & R Report. ERMA New Zealand considers that since the bacteria must go through the kill validation test and also a final heating prior to disposal that no additional risks should result from allowing an alternate method of killing the bacteria.

The government agencies and submitters have supported the approval of the application.

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Appendices

Attachment 1: Previous Approval

1. Introduction

Application Brief

1.1 The New Zealand Agriculture and Pastoral Research Institute Limited (AgResearch), a Crown Research Institute, seeks approval to perform large scale fermentations of a new organism in containment under *section 40(1)(c)* of the Hazardous Substances and New Organisms (HSNO) Act 1996.

The applicant proposes to ferment *Escherichia coli* genetically modified with DNA from *Echinococcus granulosus* (hydatids disease) and an ampicillin resistant marker gene. At the completion of each fermentation the modified *E. coli* is killed, validated as killed, and a protein from the *E. granulosus* DNA is extracted. The purified vaccine will be used in safety and efficacy trials against the cystic stage of hydatids disease. Initially three successful fermentations are required to enable commercial registration of the vaccine with the Animal Remedies Board and future fermentation will maintain vaccine supplies for potential overseas markets.

The purpose of this application falls under *section 39(1)(f)* of the HSNO Act: ***Maintaining a new organism in containment to produc****e antigens, biopesticides, biopharmaceuticals, enzymes, hormone, or* ***vaccines for release***.

The fermentations, if approved, would be conducted in a containment facility at Industrial Research Limited operated under a containment level of 'biosafety level 2 – large scale' (BL2-LS) under guidelines set out by the National Institute of Health in the United States. This standard is used due to the requirements of clients IRL's United States clients. The facility also meets, at least, the requirement for Physical Containment Level 2, Australian/New Zealand Standard 2243.3: 1995.

The development of the genetically modified *E. coli* was approved under the Advisory Committee on Novel Genetic Techniques (ACNGT) guidelines, by the local Supervisory Committee, and transferred to an approval under HSNO Act 1996 in the Gazette, Thursday 30 July 1998, Issue No. 101, item number 46. In addition three large scale fermentations were conducted in 1998 (field trial number 44 as gazetted) approved by the Minister for the Environment, on the recommendation of the Interim Assessment Group (IAG).

Project Team

1.2 The **project team** consists of the following ERMA New Zealand and external members:

Project Leader (Operations Group)	Denise McDonald
Scientific Advisor (Science & Research Group)	Dr Abdul Moeed
Policy Advisor (Policy & Analysis Group)	Bruce Chapman
External Scientific Advisor	Desmond G. Till (Consultant Microbiologist)

2. Process and Information Provided

2.1 The application was formally received in terms of statutory timing on **27 January 1999.**

Reports from other Government Agencies

2.2 Various government agencies were notified of the receipt of the application under *section 53(4)* of the Act.

Agencies consulted with

2.3 On the 10 February 1999 the **Ministry of Agriculture and Forestry** (MAF) wrote supporting the application and also requested clarification regarding the fermentation procedures. This further information was supplied on 4 March 1999 (*Appendix 2*). MAF then made a submission, dated 24 March 1998, taking into account the further information and supporting the application.

2.4 The **Department of Conservation** also provided comment on the application in a letter from Michael Cameron (New Organisms Officer) dated 24 March 1999. *(Appendix 3).*

DoC identified potential risks of the proposal to conservation:

'if the modified bacteria were to escape and disrupt ecological systems, acting as a pathogen to native or naturalised animals …(and) if the escaped bacteria gave rise to a new pathogenic agent through transduction of the inserted genes to existing bacteria in the new Zealand environment.'

Michael Cameron *(New Organisms Officer)*

However, DoC noted that it has no objections to approval provided ERMA can verify the information and analysis in the application that assesses such risks to conservation as being small.

2.5 Wairoa District Council made a submission supporting the application and Northland Regional Council noted that it was not within their mandate but gave support, in principle to the aims of the work. Landcare Research New Zealand Ltd posed some questions based on the application summary information, querying the nature of the risks and the consequences should the live modified *E. coli* escaped into the environment. These questions were covered off in the application itself and Andrew Patrick (IRL) also provided a response (see *Appendix 4* on behalf of the applicant).

2.7 Refer also to *paragraph 4.12* (*page 15*) of this report for further discussion on issues raised in by the Department of Conservation.

2.8 The application was publicly notified on **10 February 1999** in *The Dominion*, *The New Zealand Herald*, *The Press* and *The Otago Daily Times*.

Public submissions closed on **24 March 1999.**

Submissions

2.9 As outlined above two submissions were received and three agencies made comments on the application. No submitter indicated that they wish to be heard in support of their written submission.

Submissions and other responses were received from the following parties:

Table 1: Summary of Submissions/

Submitter No.	Submitter Name	Organisation / Contact	Contact Details	Wish to be Heard?
1	Mr Kevin Corrin	Ministry of Agriculture and Forestry	P O Box 10 240 WELLINGTON	No
2	Mr Victor Minter	Wairoa District Council	P O Box 54 WAIROA	No

Table 2: Agency comment

Submitter No.	Name	Organisation / Contact	Contact Details
3.	Ms Caroline Pratt	Science Planner Landcare Research New Zealand Ltd	P O Box 69 LINCOLN
4.	Mr Bob Cathcart	Land Operations Manager Northland Regional Council	P O Box 9021 WHANGAREI
5.	Mr Michael Cameron	Biodiversity Recovery Unit Department of Conservation	P O Box 10 420 WELLINGTON

Copies of all submissions and responses received are included in this report as *Appendix 3*. Since the submissions did not raise any issues resulting from the application, other than to support the approval of the application, no *Summary of Submissions* document or table listing issues raised was considered necessary.

Ngä Kaihautu Tikanga Taiao Report

2.10 Ngā Kaihautu does not consider that this application poses a significant risk to the relationship between Māori and their taonga, and therefore have not provided a full report to the Authority.

Supporting Documentation for the E&R Report

2.11 No confidential information was provided as part of the application or supporting material.

2.12 The evaluation and review of this application is based on the information supplied by the applicant, internal reviews undertaken by ERMA New Zealand, external scientific review, information from other government agencies and submissions received on the application, and specifically included:

1. **Application Form 4:** (ER-AF-NO4-3 9/98) Field Test (Including Large Scale Fermentation) in Containment any Genetically Modified Organism.
2. Appendices to the application (Appendices 1-10)
3. External Scientific Review undertaken by Dr Desmond G. Till (*Appendix 1* of this report).
4. Further information supplied by the Applicant in response to queries by MAF and a request under Section 58 (1) (*Appendix 2* of this report)
5. Submissions/Agency comments received (*Appendix 3* of this report) including comment from the Ministry of Agriculture and Forestry and Department of Conservation (DoC).
6. Further information received from the applicant in response to the queries by Landcare Research New Zealand Ltd (*Appendix 4* of this report).
7. Further information supplied by the Applicant (*Appendix 5* of this report) requesting a change in the application from heat killing of the bacteria to investigating and trialling the use of chemical agents.

3. Consideration of the Application

3.1 This application is to be considered via the decision path, *Figure 3* in the ERMA New Zealand Protocol 7*, Decision Paths*. This decision path relates to applications to develop/import/field test any new organism in containment. A copy of the relevant decision path is included as **Figure 3** and a fold out version is included as *Appendix 7*.

3.2 Following the initial steps in the decision path, the application was received and deemed appropriate and valid. An initial review of the identification and assessment of risks by the applicant was undertaken by ERMA New Zealand.

Figure 3 Decision path for applications to develop/import/field test any new organism in containment (section 40)

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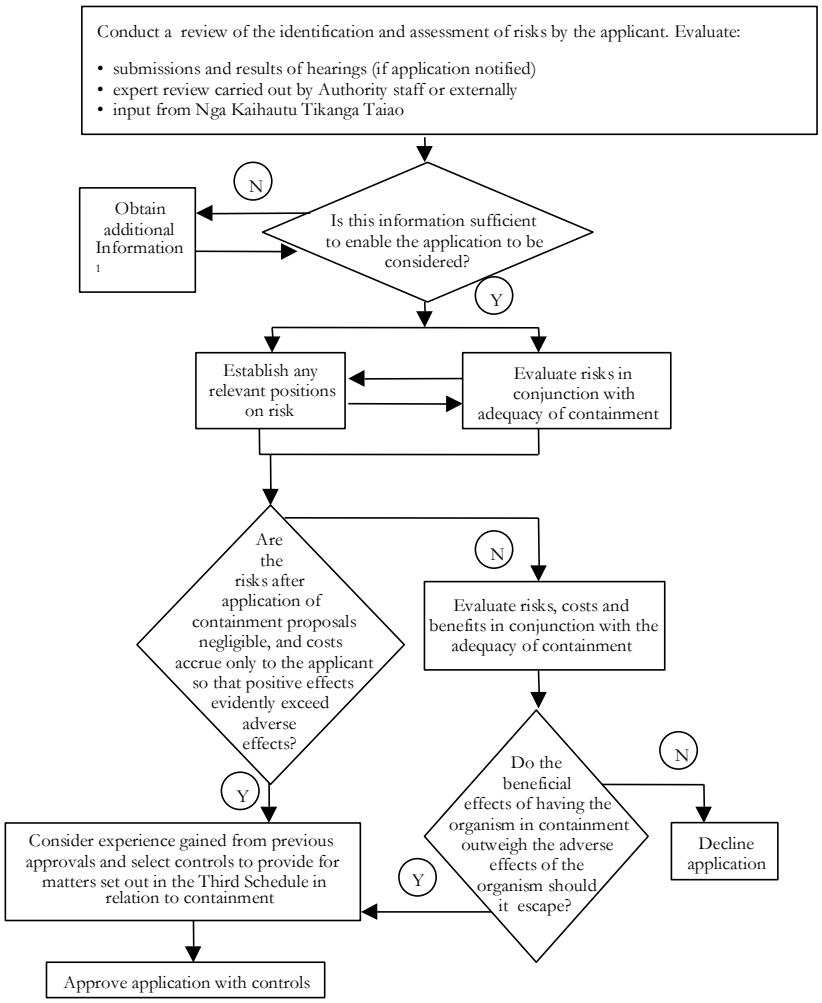
Note [1]: The Authority may decline an application if insufficient information is available to enable it to determine the adverse effects.



Identification, Assessment and Evaluation of Risks, Costs and Benefits

4.1 This section contains an identification, assessment and evaluation of risks, costs, benefits associated with this application. This section also identifies any significant omissions, conflicts or gaps in the information provided.

4.2 It is noted that the only perceived environmental risk relates to the large scale fermentation process, as the vaccine product resulting is a protein derived from the destruction of the fermented *E. coli*. The trialling of the vaccine product in sheep does not involve any new or genetically modified organisms.

4.3 In identifying and assessing the significance of the risks associated with this application the applicant has applied a procedure adapted from Australia New Zealand Standard 4360:1995, Appendix D.

4.4 The risks identified, in the application event tree analysis on pages 16-17, relate to the potential for a breach in the primary containment, being a fermentation vessel and secondary containment, being a containment laboratory. However these 'risks' could be more clearly thought of as sources of risk.

4.5 Although these sources such as minor spills and leakage could lead to potential breach of containment and potential risks to the environment others such as 'contamination of seed' appear to only be a risk to the success of any fermentation rather than a risk to the environment and the health and safety of people and communities.

4.6 Following the identification of the sources of risks limited to the actual fermentation the applicant proceeds to explain why a release of the organism would not have negative effects on the environment and in doing so highlights potential risks which have been included in Table 3 Identification of Risks on *page 13*.

4.7 The external reviewer considers adequate information is provided on the potential risks from the production of hydatid vaccine by the large-scale fermentation of a genetically modified strain of *E.coli*.

"The information provided is well validated and scientifically robust".

Desmond Till, External Consultant (Appendix 1)

4.8 There were no issues identified for which a variance of views has been expressed between the applicant and submitters. However, the comments provided by the Department of Conservation do require further consideration *(see section 4.12 page 15)*.

Identification of Risks

4.9 The following table presents an identification of risks associated with this application, following the format laid out in the *Methodology*. This includes risks identified in the application, submissions, in the external scientific review and by ERMA New Zealand.

For a number of the matters identified in the *Methodology* no specific risks have been identified, as the **applicant** has made no explicit references to these issues in the application.

These include:

- The life-supporting capacity of air, water, soil and ecosystems [*clause 9(a)* of the *Methodology*].
- The maintenance and enhancement of the capacity of people and communities to provide for their economic, social, and cultural well being; and the reasonably foreseeable needs of future generations [*clause 9(b)* of the *Methodology*].
- Significant displacement of any native species within its natural habitat [*clause 10(a)*].
- Significant deterioration of natural habitats [*clause 10(b)*].
- Significant adverse effects on human health and safety [*clause 10(c)*]. Refer to risks identified under (*Public Health*).
- Significant adverse effects on New Zealand's inherent genetic diversity [*clause 10(d)*].

Table 3 Identification of Risks

(Source: *Annotated Methodology for the consideration of applications for Hazardous Substances and New Organisms under the HSNO Act 1996, August 1998)*

Name of the Risk	**Statutory Reference** (refers to relevant clauses of the *Methodology)*	**Reference Note**
1. *E. coli* escapes and acts as pathogen to native or naturalised animals. 2. The escaped *E. coli* gives rise to a new pathogenic agent through transduction of the inserted genes into existing bacteria in New Zealand. 3. The *E. coli* escapes, survives and acts as an aggressive competitor to the detriment of the existing microorganism flora and fauna.	9(c)(i) The sustainability of all native and valued introduced flora and fauna. 9(c)(ii) The intrinsic value of ecosystems.	Refer to DoC Comments dated 24 March 1999 (Appendix 3 of report) Refer to application page 18. Refer to section 9 and conclusions of external review, Appendix 1 of this report.
4. Potential harm to humans resulting from contact with the genetically modified *E. coli.* 5. The *E. coli* escapes and forms a dominant strain of ampicillin resistant bacteria.	9(c)(iii) Public health.	Refer to Landcare Research New Zealand Ltd Comments 18 March 1999 (Appendix 3 of report) Refer to Brenner assessment of risk to individuals in application section 12 page 19.
6. Risk to relationship of Māori and their culture and traditions with taonga.	9(c)(iv) The relationship of Māori and their culture and traditions with their ancestral lands, water, sites, waahi tapu, valued flora and fauna, and other taonga.	The applicant has not identified any risks posed by this application to the relationship of Māori culture and traditions with their ancestral lands, water, sites, waahi tapu, valued flora and fauna, and other taonga. ERMA New Zealand has not identified any risks posed by this application to the relationship of Māori culture and traditions with their ancestral lands, water, sites, waahi tapu, valued flora and fauna, and other taonga. Refer to application page 13 and appendix

Name of the Risk	**Statutory Reference** (refers to relevant clauses of the *Methodology)*	**Reference Note**
		4. Refer to section 2.10 page 8.
	(v) The economic and related benefits to be derived from the use of a particular hazardous substance or new organism.	Refer to section 4.15, page 15 of this report. Refer to application section 12 page 20.
	(vi) New Zealand's international obligations.	ERMA New Zealand notes that there appear to be no implications in the context of this application.
7. The *E. coli* escapes, replicates and establishes an undesirable population.	10(e) The ability of the organism to establish an undesirable self-sustaining population anywhere in New Zealand.	Refer to application section 12 page 18. Refer to section 9 and conclusions of external review, Appendix 1 of this report.
8. The *E. coli*, following an escape is unable to be eradicated (detected) and is undesirable.	10(f) The ease with which the organism could be eradicated if it established an undesirable self-sustaining population.	Refer to application section 12 page 18. Refer to conclusions of external review, Appendix 1 of this report.
Refer to risks 4 and 5.	10(g) The ability to cause disease, be parasitic, or become a vector for human, animal or plant disease.	Refer to application section 12 page 18.

Assessment and Evaluation of Risks

4.10 Of the risks identified in **Table 1** above, ERMA New Zealand considers that **no** risks are significant. The following discussion is the basis for this view of insignificance.

4.11 The applicant has assessed the implications of risks arising from escape from containment to be negligible. Based on comments of the external reviewer, ERMA New Zealand has no reason to dispute the assessment, provided the containment regime as proposed in the application is adhered to ie.

- initial containment of genetic material within a laboratory strain of the bacteria,
- primary containment in the fermentation vessels,
- the operation being performed in a containment facility of the required standard

and that containment controls are complied with.

4.12 The Department of Conservation queried whether there is potential for the modified bacteria, should they escape, to disrupt ecological systems or to give rise to a new pathogenic agent and transduce its genetic material. In response ERMA New Zealand accepts the external reviewers consideration that the *E. coli* K12 strain cannot transduce its genetic material to other hosts and that should the organism escape it would not be likely to survive in the environment due to its physiological characteristics.

4.13 The proposed containment regime is outlined in the application. In addition *section 5* of this report outlines proposed controls which are designed to manage the risks associated with the large scale fermentation of the bacteria.

4.14 The controls have been designed with the aim of reducing the probability of any escape from containment, and reducing the likelihood that a self-sustaining population may form in the event of an escape. However, such controls cannot totally eliminate the possibility that escape will occur, as complete compliance cannot be guaranteed.

Assessment of Costs

4.15 In the costs section of application the risks of any adverse effects is assessed to be low, and therefore no costs have been presented.

4.16 There are likely to be certain direct monetary costs associated with this application that are borne by the applicant.

Assessment of Benefits

4.17 The benefits identified in the application are those resulting from the knowledge gained from the commercial production of the vaccine, overseas sales and use of the vaccine product, for which the fermentation process produces the protein.

5 Application of Controls

5.1 The Third Schedule of the HSNO Act 1996, Part I, identifies *Matters to be Addressed by Containment Controls for Development and Field Testing of Genetically Modified Organisms* (GMOs).

5.2 Controls are designed with the aim of reducing the probability of any escape from containment, and reducing the likelihood that a self-sustaining population may form in the event of an escape. However, such controls cannot totally eliminate the possibility that escape will occur, as complete compliance cannot be guaranteed.

5.3 The controls set out below are designed to manage the risks associated with the large scale fermentation of genetically modified *E. coli* in containment. Specifically, they relate to the maintenance of *E. coli* in secure containment. These controls are put forward as the basis for assessing the risks associated with approving the *large scale fermentation in containment* of *E. coli.* The Authority may wish to amend these controls (in order to make them more or less 'strict') to achieve a level of probability of escape that is appropriate for the application.

5.4 The auditing of the facility against the proposed containment standards is highlighted by the external reviewer as an essential practice to establish and maintain the integrity of the containment system. ERMA New Zealand notes that compliance audits for the lab and processes are required under the Standard identified in Control 1a) Ministry of Agriculture and Forestry (MAF) and ERMA New Zealand Standard 154.03.02:1999 Containment Facilities for Microorganisms.

5.5 If approval is granted, operation of the containment facilities must specifically ensure the following (in respect to the matters identified in the Third Schedule of the HSNO Act):

Table 6 Proposed Controls according to the Third Schedule of the HSNO Act 1996

*(*Source: *Hazardous Substances and New Organisms Act 1996 Third Schedule Part I Matters to be Addressed by Containment Controls for Development and Field Testing of Genetically Modified Organisms)*

1. To limit the likelihood of any accidental release of any organism or any viable genetic material:

Matters to be addressed	Proposed Controls or Options for Controls
1(a) Requirements for treatment and decontamination to prevent escape by way of expelled air, discharge of water or liquid waste, removal of solid waste or goods, or breaches in facility boundary.	1 The containment facility shall be operated and constructed in accordance with the: a) Ministry of Agriculture and Forestry (MAF) and ERMA New Zealand Standard 154.03.02:1999 Containment Facilities for Microorganisms; b) Australia New Zealand Standard 2243.3:1995 Physical Containment Level 2 (PC2); c) Containment System for Industrial Research's Fermentation facility. (Appendix 3 of the application); d) Biosafety Level 2-large scale (National Institute of Health U.S. Guidelines).

1(b) Equipment and requirements for facility construction to enable the requirements for treatment and decontamination to be readily met.	Refer to Control 1.
1(c) Requirements to be complied with for the access of persons to the facility.	2 Only authorised persons shall have access to the containment facility.
1(d) Procedures and requirements for transport, identification, and packaging for all biological material to and from the facility and within the facility.	3 All biological material in the containment facility shall be unambiguously identified at all times. 4 The *E. coli* shall be transported in secure containment to the IRL containment facility. The containers shall provide comparable security to that of the containment facility for Microorganisms (MAF/ERMA New Zealand Standard 154.03.02: 1999)and shall be sealed before transfer.
1(e) Requirements for the disposal of any biological material.	5 *E. coli* shall be validated killed prior to cell harvest and prior to disposal the effluent shall be autoclaved at 121 degrees Celsius for 30 minutes to ensure no live organisms exist and that any remaining DNA is denatured.
1(f) Requirements for facility construction.	Refer to Control 1.
1(g) Requirements to secure the facility and openings, including securing against failure in the event of foreseeable hazards.	Refer to Control 1.

2. To exclude unauthorised people from the facility:

Matters to be addressed	Proposed Controls or Options for Controls
2(a) Means of identification of all entrances to the facility.	Refer to Control 1.
2(b) The numbers of entrances and access to the facility.	Refer to Control 1.
2(c) Security requirements for the entrances and the facility.	Refer to Control 1.

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

Matters to be addressed	Proposed Controls or Options for Controls
3(a) Monitoring requirements to establish the presence of other organisms.	6 The applicant shall confirm and record in the log book that the modified *E. coli* remains the dominant organism at the completion of each fermentation.

3(b) Phytosanitary requirements.	Refer to Control 1.
3(c) Requirements to secure the facility and openings against likely unwanted organisms.	Refer to Control 1.

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

Matters to be addressed	Proposed Controls or Options for Controls
4(a) Requirements to prevent the contamination of work surfaces, equipment, clothing and the facility generally.	Refer to Control 1.
4(b) Requirements for laboratory practice to control infection by ingestion or breaks in skin cover.	Refer to Control 1.
4(c) Means to control infection by inhalation.	Refer to Control 1.

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

Matters to be addressed	Proposed Controls or Options for Controls
5(a) Eradication plan for escaped organisms.	7 A contingency plan for the eradication of escaped material shall prepared, a copy provided to ERMA New Zealand at least one week prior to the first large scale fermentation taking place, and immediately implemented should a spill from the primary or secondary containment facility occur.
5(b) Requirements to limit the likelihood of an escaped organism spreading, surviving and breeding, including, but not limited to:	Refer to Control 7.
(i) Exclusion zones (spatial or temporal); and	N/A
(ii) Location of the facility outside the usual habitat range of the organism.	N/A

6. Inspection and monitoring requirements:

Matters to be addressed	Proposed Controls or Options for Controls
6 Inspection and monitoring	8 The Authority or its authorised agent or properly authorised

requirements for containment facilities (including any inspection required before commencement of the development or field testing).	enforcement officers, may inspect the facility at any reasonable time. 9 The applicant shall provide reports to the Authority annually detailing the fermentations performed in that period and outlining the proposed fermentation programme for the coming year. 10 The containment facility shall be inspected by the AgResearch and IRL Institutional Biological Safety Committees (IBSCs) at least once a year. The inspection shall be timed to coincide with a fermentation where possible. The AgResearch IBSC's shall present a report on each inspections and copy to MAF and the Authority.

7. Implementation of controls:

Matters to be addressed	**Proposed Controls or Options for Controls.**
7(a) The qualifications required of the person responsible for implementing the controls imposed by an approval.	11 The applicant shall inform all staff involved in the operation and management of the field test of the conditions and controls applicable to the large scale fermentation. 12 The applicant shall ensure adequate training of all personnel involved in the large scale fermentation of the bacteria.
7(b) The provision of a management plan specifying procedures for implementing controls imposed by an approval.	Refer to Control 1 and 7.

Additional Controls.	13 If for any reason a breach of containment occurs the applicant is required to notify the facility Supervisor[1], the Ministry of Agriculture and Forestry and the Authority immediately the event is noticed. 14 The applicant shall maintain a logbook recording the fermentation processes, including kill validation results. 15 For this application, controls 1-14 above, constitute the standard applicable to the approval of a place as a containment facility for the purposes of *section 39* of the Biosecurity Act 1993.

[1] An inspector appointed under the Biosecurity Act.

6. Commentary on Other Issues

Previous Considerations

6.1 The development of the genetically modified *E. coli* was approved under the ACNGT by the local Supervisory Committee and transferred to an approval under HSNO Act 1996 in the Gazette, Thursday 30 July 1998, Issue No. 101, item number 46. In addition three large scale fermentations were conducted in 1998 (trial number 44 as gazetted) approved by the Minister for the Environment, on the recommendation of the Interim Assessment Group (IAG)). The fermentations were performed in 1998, however further fermentations are required to develop a more efficient process, hence the need for the current application.

A copy of the IAG recommendation, the deemed approval from the gazette and the completion report from AgResearch for the 1998 fermentations are included in *Appendix 5* of this report.

Consideration of the organism by another Country

6.2 The applicant has indicated that the genetically modified *E. coli* for production of the hydatid vaccine antigen has not been previously considered by the government of any prescribed state of country (*section 16* of the application).

7. Status of Associated Approvals

7.1 AgResearch wishes to perform the fermentations in order to complete testing of the resulting vaccine and gain approval from the Animal Remedies Board.

8. Precedents

8.1 Not Applicable

9. Overall Evaluation

9.1 Having considered the risks identified in *section 4* in conjunction with the adequacy of containment, as described in *section 5*, the decision path obliges the Authority to form a view as to whether the risks after the application of containment controls are *negligible*[2] and costs accrue only to the applicant.

9.2 In forming a view, the Authority should take into account:

- the impact of controls to reduce the possibility of escape from containment;

[2] The *Annotated Methodology* defines *negligible risks* as *risks which are of such little significance in terms of their likelihood and effect that they do not require active management and/or after the application of risk management do not need to be justified by counter-balancing benefits.*

- the adverse effects that would occur if the event of escape from containment; and

- the character of the risks (eg whether they involve long or short term effects).

9.3 None of the potential adverse effects, should the organism escape, have been identified as being significant. In particular, the evidence presented indicates that it is extremely unlikely that an undesirable self sustaining population would be established. Further, the applicant has provided evidence of benefit, at least to the applicant.

Provided that the Authority can be satisfied with the surety of containment, there appears to be a case for determining that the risks associated with this application are negligible.

9.5 If the Authority is of the view that the risks are not negligible it must weigh risks and costs against benefits. The risks, costs and benefits as assessed by the applicant are outlined in *section 4* of this report. If the Authority deems that a full risk-cost-benefit assessment is appropriate it may wish to consider whether there is sufficient information to proceed.

Appendix 1: External Scientific Reviewer

EXTERNAL REVIEW: ERMA File *Ref.* GMF98010

COMMENTS BY: DESMOND G. TILL

APPLICATION TO PERFORM LARGE SCALE FERMENTATION OF GENETICALLY MODIFIED *ESCHERICHIA COLI K-12*

Comments for incorporation into the review of the application to perform large-scale fermentation of genetically modified *Escherichia coli* K-12 for hydatids vaccine production.

- Section 4.
  Detailed and explicit narrative information has been supplied, sufficient to assess at face value any potential risk from the production of hydatid vaccine by the large-scale fermentation of a genetically modified strain of *E. coli.*
  It should be noted that the only perceived environmental risk relates to the large scale fermentation process, as the product resulting is a protein derived from the heat destruction of the genetically modified organism. The design of the process as described should ensure that no live genetically modified organism would impact on the environment. The large-scale fermentation process was approved by the IAG for "Field Testing and Release of Genetically Modified Organisms", subject to conditions on the 21st.July 1997.
  From the documentation provided these conditions appear to have been fulfilled.
  The information provided is well validated and scientifically robust.

- Section 6.
  As the only perceived risk is during the fermentation process, identification of risks is confined to the fermentation process only. The risks have been assessed qualitatively using procedures and tables adapted from AS/NZS 4360:195 Appendix D.
  The Containment System for Industrial Research's Fermentation Facility is described in Appendix 3 of the documentation provided. It is described as operating under a containment level designated biosafety level 2-large scale (BL2-LS). For this type of operation, the risks appropriately identified in Table 1 are from spills.

- Section 7
  The applicant has provided systematically detail of the process where the genetically modified *E. coli* is used for the manufacture of hydatids vaccine. Table 2 clearly identifies where these steps pose a potential for spills and control measures automatically in place. This is further supported by information in Appendix 3: Industrial Research Containment Pilot Facility.

- Section 8
  The analysis of associated level of risk in Table 2 for spills and secondary containment (primary containment is in the fermentation vessel) are identified and controls appropriate. If a major spill (conservatively described as <1-L) has occurred

from primary containment Aerosols, Material Entry and Exit and Personnel Flow would be considered High or Significant risk depending on the size of the spill. Risk Management is further covered in Appendix 3: Industrial Research Containment Pilot Facility.

- Section 9
  The summary of risk assessment for the proposed large-scale fermentation of a genetically modified strain of *E. coli-K12,* is a realistic presentation of the comprehensive information provided.
  Although there is no known data to assess the potential risk from this organism if it was accidentally released into the environment, nor is there any known data to assess the potential for this organism to develop virulence factors, the data presented suggests a minimal risk should this occur. The procedures for containment at the IRL facility support the likelihood of such an event as minimal. In addition, the physiological characteristics of this particular strain of *E. coli* suggest that it would not be able to compete and survive in the environment.
  The conclusion that the use of the Containment Facility at IRL for large scale fermentation of this organism in the production of hydatid vaccine is of low risk is considered justified, and the documentation provided supports this in accordance with the requirements of section 40 of the Hazardous Substances and New Organisms Act 1996.

Note: Additional Matters and Controls Required

Narrative information only has been provided to support the claims for this procedure's containment integrity and quality assurance. Examples are:

- Fermentation of the genetically modified *E. coli* is performed at Industrial Research Ltd.(IRL), Gracefield, Lwr. Hutt, under a containment level designated 'biosafety level2 – large scale' (BL2-LS)
- The facility at IRL is a class 2 laboratory as defined in AS/NZS 2243:3
- Both primary and secondary containment is employed for large-scale fermentations as described in Appendix 3 Containment System for IRL Fermentation Facility.
- After fermentation the organism will be heat killed
- All effluent and spillage's are disposed of via the 2000-L kill tank facility at IRL.The kill tank operates using qualified procedures to ensure that autoclaving times are guaranteed to inactivate microorganisms.
- Appendix 3, 3.2 Air Handling – The fermenters are contained within a class 10,000 clean room environment. The room is validated on an annual basis by a NZ accredited tester.
- Appendix 3, 3.3 Environmental Monitoring – Swabbing of the environment over four years of operation has never yet detected any breach of primary containment.

None of these examples are endorsed by such as, ISO 9002 accredited management systems, ISO Guide 25 Laboratory Accreditation or results and validation records. These are examples of where auditing for compliance is essential to establishing the integrity of the containment procedures, for compliance with the requirements of ERMA under the HSNO ACT.

Potential for Escape of GMO:

Provided the laboratory is audited to establish that it's methods are as stated, and it's quality assurance and validation of containment conditions are as specified, it is my considered opinion that the potential for escape of the GMO is very minimal.

Possibility of Eradication of the GMO if Escape Occurs:
This would depend on where the organism escaped to, but in essence, attempts at eradication would most likely be unachievable. [I concur however with the applicants hypothesis that it may not survive in the environment because of its physiological characteristics – but also the ability of microorganisms to adapt to an environment cannot be underestimated.

Consequences of Escape on People and the Environment:
Given the characteristics of this organism and the fact that it is just another *E.coli,* even if it does have an Ampicillin resistance gene in it's plasmid, I cannot see that it is likely to be of any great concern to people or the environment.

Finally, I would like to again stress my comments on the need for the laboratory to be audited to ensure all it's claims pertaining to containment are as stated. That is the crux of it all.

Desmond G Till
Consultant Microbiologist
8 April 1999

Appendix 2: Applicant Response to MAF request for further information

(a) Are the risks and possible effects of escape (particularly any harmful ones) adequately covered?

The risks are adequately covered in the document attached to the application entitled "Industrial Research's containment pilot plant fermentation facility. Along with each organism grown, IRL will perform a trial where the organism is grown on dirt from the environment and its survivability determined.

(b) Are there any organisms which could be inseparable from the genetically modified E. coli which could escape and have harmful effects?

See answer to (3)

(c) Are there any potential problems with identification of the genetically modified organism?

See answer to (3)

MAF Queries

(1) I had some initial concerns re- the comment on P6 that the amount of air flowing into the fermenter would be changed to control growth of the organism. If increased, could the outlet air filters become blocked earlier than expected. I note that dual filters are used, and that in the case of one locking, it can be isolated for unblocking and the outflow diverted to the second filter. I think that this procedure needs to be clarified given possible increased outflow.

The fermenter runs with an antifoam control system. This means that even if the air is increased, the system will still be able to sense the foam. The system is run with 2 sterile filters, one in operation and one closed. If the foam did get into the filter, this can be isolated and sterilised. The other filter is then opened. Over pressure problems are further covered in the attached publication as noted above.

(2) In the description of the containment facility under 3.1 there is a comment that indicates that the facility is frequently switched between containment levels BL2 and BL1. This use of the facility at different levels, and possibility of accidental use at an inappropriate lower level is of concern. Systems must be in place to prevent this, or a ruling must be made that a BL-2 facility shall always be operated only at BL-2.

There are strict procedures in place to ensure that the facility is operating at the correct level prior to conducting a fermentation. Both BL2 and BL1 operate under -ve pressure and the difference is what the staff are required to wear (labcoats versus full-cover suits). BL1 is used during maintenance of the facility and not for production fermentations.

(3) The final concern is regarding the microbiological monitoring program summarized on pages 7 & 8. The document does not describe what happens if the pre-heat micro shows the culture to be mixed, especially with another gram negative, heamolytic, bacteria. The presence of VTEC E. coli in the environment especially O157:H7 is of concern both domestically and internationally. The micro summary does not indicate how a mixed culture would be handled to verify the absence of these organisms. This is not so much a problem if the culture is successfully heat killed, although enterotoxins are produced by O157 and it is phage encoded, hence transferable.

1. **The E. coli culture is derived from a single colony and the original plates autoclaved. Therefore a monoxenic culture is established.**

2. **Any phage would quickly be identified on the original plates or as the plates used to confirm monoxenity during the fermentation.**

3. **The strain carries an ampicillin resistance marker. It is a non-conjugative strain and therefore ampicillin resistant cultures are assumed to carry the plasmid and hence be the correct strain.**

4. **Ampicillin is used in all growth medium and so any associated ampicillin sensitive organisms would be inactivated.**

5. **During monoxenity checks, cultures are plated onto ampicillin positive and negative medium. By comparing the amount of growth, contaminants would quickly be identified.**

In addition, the document does not describe what happens if the post-heat kill test is positive. Perhaps another IRL procedures document explains this monitoring programme in more detail.

After inactivation cultures are checked. If any viability is detected it is heat inactivated again, irrelevant of what type of organism.

Fermenter contents are held in containment until the results of purity checks have been confirmed. Contaminated cultures are heat sterilised which will inactive any phages.

Andrew Patrick
Industrial Research Ltd
4 March 1998

Appendix 3: Submissions and Agency Comments

Appendix 4: Applicant's response to Landcare Research NZ Ltd. comments

-----Original Message-----
From: Andrew Patrick [SMTP:A.Patrick@irl.cri.nz]
Sent: Tuesday, 6 April 1999 13:18
To: Denise McDonald
Subject: Response to Peter Mcgregor

Dear Denise,

The following is the response to Peter McGregors comments on ERMA application GMF98010.

What are the consequences if live, modified E.coli escape into the environment interms of the health risks and GMO survivability?

1. The E.coli strain used in this application is a non-pathogenic strain. It is a non-conjugative strain i.e it is unable to pass on genetic material from one strain to another.

2. With each organism grown, IRL will perform a trial where the organism is grown on soil from the environment and its survivability determined. Thus far from experiments carried out, there has been no detected survival of the E.coli. This means that the likely hood for survival if the E.coli reached the environment is vertically nil.

3. The strain carries an ampicillin resistance marker. Ampicillin resistant cultures are assumed to carry the plasmid . In the environment, there is no ampicillin, so the E.coli would rapidly lose the plasmid.

4. The expression of the protein from the gene is induced by IPTG, which is a lactose inducer. In the environment, there is no lactose or IPTG, so the gene would not be expressed.

I hope this answers the questions raised. If you require any further information, do not hesitate to contact me.

Andrew Patrick

Appendix 5: Further information to the application regarding method of killing the bacteria

-----Original Message-----
From: Heath, David [SMTP:heathdd@agresearch.cri.nz]
Sent: Wednesday, 28 April 1999 17:50
To: Denise McDonald
Subject:

Denise McDonald
Applications Officer
ERMA New Zealand
04 496 4831
28th April, 1999

Dear Denise,

When the Committee reviews our application to ferment more than 10 litres of genetically-modified E.coli, would you please ask them to consider the following:

We have stated that the bacteria will be killed by 65C for 30 minutes, and we give the procedure for validating that all bacteria are killed.
We have found that this degree of heat denatures our proteins, and makes inclusion bodies very difficult to solubilise. In scale-up, the procedure has not turned out to be satisfactory.

We would like to change the document (bottom of Page 7)from" Once fermentation is complete, the fermenter is inactivated by heating to 65C+-1C and held at that temperature for 30 minutes. The fermenter is then cooled to 25C. Samples will be taken before and after heat killing, and will be tested in parallel as soon as possible after sampling to validate heat treatment has killed the bacteria" to:-

"At the end of fermentation the bacteria will be killed before processing. Killing will be validated as described below"

We wish to investigate methods of killing bacteria that do not denature inclusion bodies. As an example, chemical killing using 3M urea has been suggested to us by Australian biotechnologists.

Please contact me if you have any queries.

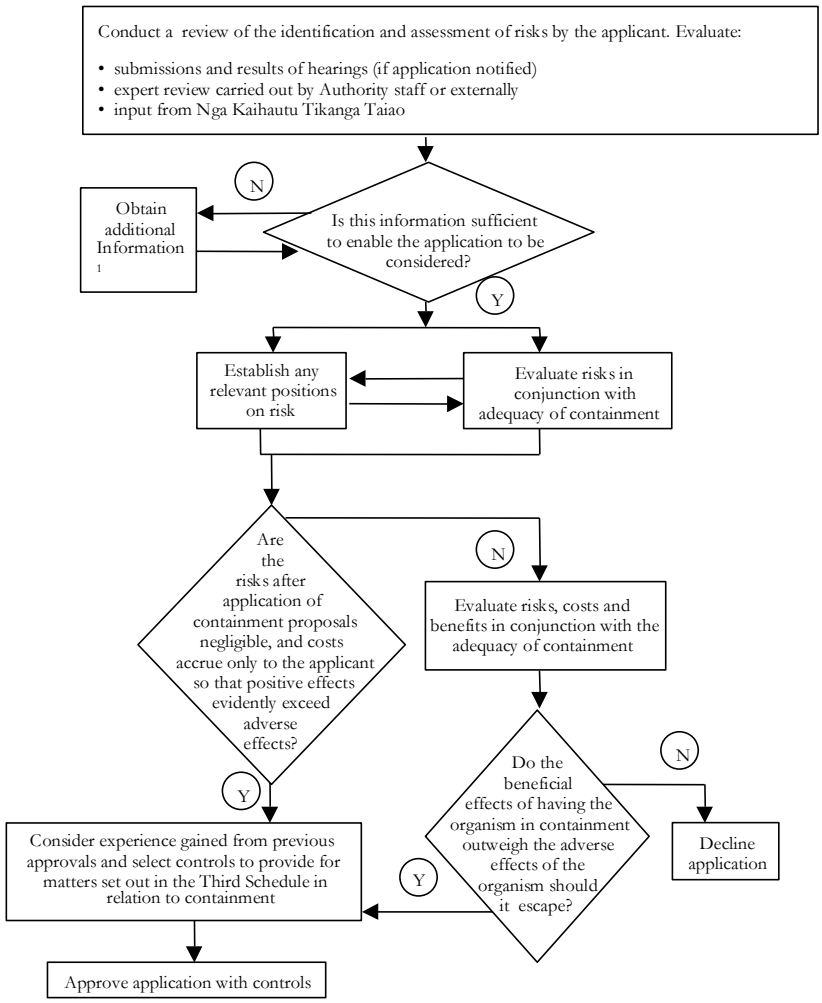
Kindest regards

David Heath
Dr David D Heath
Programme Leader: Parasite Biological Control
Agresearch, Wallaceville Animal Research Centre
P O Box 40063, Upper Hutt, New Zealand
Tel. +64 4 5286089 Fax +64 4 5281380
heathdd@agresearch.cri.nz

Appendix 6:

Figure 3 Decision path for applications to develop/import/field test any new organism in containment (section 40)

Note [1]: The Authority may decline an application if insufficient information is available to enable it to determine the adverse effects.



Attachment 1: Previous Approval

5a IAG recommendation
5b Deemed approval controls
5c AgResearch Final Report (Field Trial 44)