ENVIRONMENTAL RISK MANAGEMENT AUTHORITY DECISION

18 February 2011

Application code	ERMA200732
Application category	To develop in containment genetically modified organisms under section 40(1) of the Hazardous Substances and New Organisms (HSNO) Act 1996
Applicant	University of Otago University of Auckland
Application Purpose	To genetically modify *Escherichia coli*, yeast, viruses, cell lines and laboratory animals to study the effects of viral and cellular sequences and proteins
Date received	18 January 2011
Consideration date	11 February 2011
Considered by	A Committee of the Authority

1. Decision

1.1. The application to develop in containment the organisms listed in Table 1 is **approved with controls**, having been considered in accordance with the relevant provisions of the Hazardous Substances and New Organisms (HSNO) Act 1996 and the HSNO (Methodology) Order 1998.

2. Approved organism description

2.1. The approved organism description is listed in Table 1

Table 1: Organism description of the GMOs approved for development

For cloning and amplification of plasmids

Host organism:
Escherichia coli Migula (1895) Castellani and Chalmers 1919 Non pathogenic laboratory strains
Modified using:
Standard non-conjugative cloning plasmid vectors, recombinant mammalian expression plasmids and lentiviral, retroviral, adenoviral, poxviral, papillomaviral or baculoviral plasmids containing coding, non-coding and/or regulatory regions, antisense sequences or other RNA interference-inducing sequences of viral, eukaryotic and/or prokaryotic donors associated with viral cellular sequences and proteins.
Genetic material may be:
Eukaryotic, including human[1], viral, prokaryotic and/or synthetically produced genetic material (and deletion, substitution and chimeric mutants thereof) associated with viral and cellular sequences and proteins that regulate cellular and tissue physiology.
Regulatory elements, reporter and selectable marker genes and other features:
Vectors will include standard or fully characterised regulatory elements including promoters, regulatory element binding sites, transcriptional activators, enhancers, terminators, silencing elements, and multiple cloning sites and origins of replication. The vectors may also contain recombination sites, internal ribosomal entry sites (IRES), selectable marker genes, reporter genes, protein targeting, localisation and secretory signals, solubility enhancement tags, protein purification tags and affinity tags including epitope tags.
Characteristics of the genetically modified organisms:
No infectious viral particles will be produced.
Exclusions:
• Genetic material derived from Māori persons. • Genetic material derived from native flora or fauna. • Modifications that result in the GMO having a greater ability to escape from containment than the unmodified host organism.
Containment level/Category of Modification:
PC1/A

[1] derived from non-Māori persons only

For production of replication competent recombinant baculoviruses

Host organism:
Autographa californica multiple nucleopolyhedrovirus (laboratory non-pathogenic strains) Insect cell lines derived from *Spodoptera frugiperda* J. E. Smith, 1797 *Drosophila melanogaster* Meigen, 1830 *Trichoplusia ni* Hübner, 1803
Modified using:
For AcMNPV, recombinant polyhedrin-negative *AcMNPV* will be developed by homologous recombination using standard non-conjugative baculovirus plasmid transfer vectors as described below for insect cell lines. **For insect cell line,** non-conjugative baculoviral plasmid vectors, and recombinant polyhedrin-negative *AcMNPV* containing coding, non-coding and/or regulatory regions, antisense sequences or other RNA interference-inducing sequences of viral, eukaryotic and/or prokaryotic donors associated with viral cellular sequences and proteins
Genetic material may be:
Eukaryotic, including human[2], viral, prokaryotic and/or synthetically produced genetic material (and deletion, substitution and chimeric mutants thereof) associated with viral and cellular sequences and proteins that regulate cellular and tissue physiology.
Regulatory elements, reporter and selectable marker genes and other features:
Vectors will include standard or fully characterised regulatory elements including promoters, regulatory element binding sites, transcriptional activators, enhancers, terminators, silencing elements, and multiple cloning sites and origins of replication. The vectors may also contain recombination sites, internal ribosomal entry sites (IRES), selectable marker genes, reporter genes, protein targeting, localisation and secretory signals, solubility enhancement tags, protein purification tags and affinity tags including epitope tags.
Characteristics of the genetically modified organisms:
Recombinant baculoviruses carrying the genes of interest will be produced. These are able to replicate in insect cells, and also to deliver the genes of interest into mammalian cell lines, but cannot replicate in mammalian cells.
Exclusions :
• Genetic material derived from Māori persons. • Genetic material derived from native flora or fauna. • Modifications that result in the GMO having a greater ability to escape from containment than the unmodified host organism.
Containment level/Category of Modification:
PC1/A

[2] derived from non-Māori persons only

For production of replication competent recombinant papillomavirus:

Host organism:
Papillomaviridae (ICTVdB - The Universal Virus Database, version 4). *Saccharomyces cerevisiae* (Hansen, 1883) non-pathogenic laboratory strains Vaccinia virus (ICTVdB - The Universal Virus Database, version 4), Mammalian cell lines derived from • *Homo sapiens* Linnaeus, 1758 • *Mus musculus* Linnaeus, 1758 • *Mus spretus* Lataste, 1883 • *Rattus rattus* Linnaeus, 1758 • *Rattus norvegicus* Berkenhout, 1759 • *Chlorocebus aethiops* Linnaeus, 1758 • *Ovis aries* Linnaeus, 1758 • *Bos taurus* Linnaeus, 1758 • *Canis familiaris* Linnaeus, 1758 • *Oryctolagus cuniculus* Linnaeus, 1758 • *Sylvilagus* sp Gray, 1867 • *Cricetulus griseus* Milne-Edwards, 1867 • *Cricetus cricetus* Linnaeus, 1758 • *Cavia porcellus* Linnaeus, 1758
Modified using:
For yeast: Yeast plasmid vectors containing the full-length genome of the target papillomavirus (including mutants and derivatives thereof), papillomaviral genes encoding the early viral protein (E2) and capsid proteins (L1 and L2) carried on three separate plasmids. ***For mammalian cell lines containing papillomavirus genomic DNA, using recombinant vaccinia virus system***: Recombinant vaccinia virus containing genes coding for the papillomavirus L1 and/or L2 capsid proteins and a helper vaccinia virus encoding T7 RNA polymerase. ***For mammalian cell lines in organotypic rafts:*** Non-conjugative plasmid vectors or retroviral vectors containing papillomavirus genomic DNA. ***For mammalian cell transfection system:*** Non-conjugative plasmids containing papillomavirus genomic DNA with SV40 origin of replication, and genes encoding SV40 large T antigen, and papillomaviral capsid proteins L1 and/or L2. Genetic material may include coding, non-coding and/or regulatory regions, antisense sequences or other RNA interference-inducing sequences of viral, eukaryotic and/or prokaryotic donors associated with viral cellular sequences and proteins.
Genetic material may be:
Eukaryotic, including human[3], viral, prokaryotic and/or synthetically produced genetic material (and deletion, substitution and chimeric mutants thereof) associated with viral and cellular sequences and proteins that regulate cellular and tissue physiology.
Regulatory elements, reporter and selectable marker genes and other features:
Vectors will include standard or fully characterised regulatory elements including promoters, regulatory element binding sites, transcriptional activators, enhancers, terminators silencing elements, and multiple cloning sites and origins of replication. The vectors may also contain

[3] derived from non-Māori persons only

recombination sites, internal ribosomal entry sites (IRES), selectable marker genes, reporter genes, protein targeting, localisation and secretory signals, solubility enhancement tags, protein purification tags and affinity tags including epitope tags.
Characteristics of the genetically modified organisms:
Papillomaviruses, which may have altered genomes, will be produced. These are capable of infection but are animal species-specific.
Exclusions:
• Genetic material derived from Māori persons. • Cells derived from Māori persons • Genetic material derived from native flora or fauna. • Modifications that result in the GMO having a greater ability to escape from containment than the unmodified host organism.
Containment level/Category of Modification:
PC2/B

For production of replication competent recombinant poxvirus

Host organism:
Poxviridae (ICTVdB - The Universal Virus Database, version 4), includes the Vaccinia virus (ICTVdB - The Universal Virus Database, version 4), and orf viruses (ICTVdB - The Universal Virus Database, version 4)
Modified using:
Homologous recombination of poxvirus with plasmid vectors containing coding sequence of viral, eukaryotic and/or prokaryotic donors associated with viral cellular sequences and proteins flanked by an intergenic insertion site of poxvirus.
Genetic material may be:
Eukaryotic, including human[4], viral, prokaryotic and/or synthetically produced genetic material (and deletion, substitution and chimeric mutants thereof) associated with viral and cellular sequences and proteins that regulate cellular and tissue physiology.
Regulatory elements, reporter and selectable marker genes and other features:
Vectors will include standard or fully characterised regulatory elements including promoters, regulatory element binding sites, transcriptional activators, enhancers, terminators, silencing elements, and multiple cloning sites and origins of replication. The vectors may also contain recombination sites, internal ribosomal entry sites (IRES), selectable marker genes, reporter genes, protein targeting, localisation and secretory signals, solubility enhancement tags, protein purification tags and affinity tags including epitope tags.
Characteristics of the genetically modified organisms:
Recombinant, infectious orf viruses will be produced.
Exclusions:
• Genetic material derived from Māori persons. • Genetic material derived from native flora or fauna. • Modifications that result in the GMO having a greater ability to escape from containment than the unmodified host organism.
Containment level/Category of Modification:
PC2/B

[4] derived from non-Māori persons only

For production of replication competent recombinant adenovirus

Host organism:
Adenoviridae (ICTVdB - The Universal Virus Database, version 4) isolate OAdV287 (OvAd7) Cell lines derived from: *Ovis aries* Linnaeus, 1758
Modified using:
A linearised DNA genome containing the coding sequence of viral, eukaryotic and/or prokaryotic donors associated with viral cellular sequences and proteins within the viral inverted terminal repeats (ITRs).
Genetic material may be:
Eukaryotic, including human[5], viral, prokaryotic and/or synthetically produced genetic material (and deletion, substitution and chimeric mutants thereof) associated with viral and cellular sequences and proteins that regulate cellular and tissue physiology.
Regulatory elements, reporter and selectable marker genes and other features:
Vectors will include standard or fully characterised regulatory elements including promoters, regulatory element binding sites, transcriptional activators, enhancers, terminators, silencing elements, and multiple cloning sites and origins of replication. The vectors may also contain recombination sites, internal ribosomal entry sites (IRES), selectable marker genes, reporter genes, protein targeting, localisation and secretory signals, solubility enhancement tags, protein purification tags and affinity tags including epitope tags.
Characteristics of the genetically modified organisms:
Recombinant viruses that are replication competent within sheep cell lines but which are replication defective viral vectors when used to transducer cells derived from other animals.
Exclusions:
• Genetic material derived from Māori persons. • Genetic material derived from native flora or fauna. • Modifications that result in the GMO having a greater ability to escape from containment than the unmodified host organism.
Containment level/Category of Modification:
PC2/B

For production of replication competent recombinant Hepatitis B virus

Host organism:
Hepadnaviridae (ICTVdB - The Universal Virus Database, version 4),Hepatitis B virus Cell lines derived from: *Homo sapiens* Linnaeus, 1758
Modified using:
Recombination of wild type HBV genomes and genomes with specific deletions and point mutations with plasmid vectors.
Genetic material may be:

[5] derived from non-Māori persons only

Eukaryotic, including human[6], viral, prokaryotic and/or synthetically produced genetic material (and deletion, substitution and chimeric mutants thereof) associated with viral and cellular sequences and proteins that regulate cellular and tissue physiology.
Regulatory elements, reporter and selectable marker genes and other features:
Vectors will include standard or fully characterised regulatory elements including promoters, regulatory element binding sites, transcriptional activators, enhancers, terminators, silencing elements, and multiple cloning sites and origins of replication. The vectors may also contain recombination sites, internal ribosomal entry sites (IRES), selectable marker genes, reporter genes, protein targeting, localisation and secretory signals, solubility enhancement tags, protein purification tags and affinity tags including epitope tags.
Characteristics of the genetically modified organisms:
Infection of hepatoma cell lines will result in the transient expression of viral genes and secretion of infectious HBV.
Exclusions:
• Genetic material derived from Māori persons. • Genetic material derived from native flora or fauna. • Modifications that result in the GMO having a greater ability to escape from containment than the unmodified host organism.
Containment level/Category of Modification:
PC2/B

For production of replication defective viral particles from retroviral, adenoviral, adenoviral-associated viral, and papillomaviral vectors

Host organism:
Homo sapiens (Linnaeus, 1758) cell lines
Modified using:
Standard non-conjugative cloning plasmid vectors, recombinant mammalian expression plasmids and retroviral, adenoviral, adenoviral associated viral and papilloma viral plasmids containing coding, non-coding and/or regulatory regions, antisense sequences or other RNA interference-inducing sequences of Viral, eukaryotic and/or prokaryotic donors associated with cellular sequences and proteins. The viral sequences required for virion packaging and/or replication are carried on separate plasmids to ensure that replication defective particles are produced.
Genetic material may be:
Eukaryotic, including human[7], viral, prokaryotic and/or synthetically produced genetic material (and deletion, substitution and chimeric mutants thereof) associated with viral and cellular sequences and proteins that regulate cellular and tissue physiology.
Regulatory elements, reporter and selectable marker genes and other features:
Vectors will include standard or fully characterised regulatory elements including promoters, regulatory element binding sites, transcriptional activators, enhancers, terminators, silencing elements, and multiple cloning sites and origins of replication. The vectors may also contain recombination sites, internal ribosomal entry sites (IRES), selectable marker genes, reporter genes, protein targeting, localisation and secretory signals, solubility enhancement tags, protein purification tags and affinity tags including epitope tags.
Characteristics of the genetically modified organisms:
Cells express viral proteins and nucleic acids to produce viral particles. These cannot

[6] derived from non-Māori persons only
[7] derived from non-Māori persons only

replicate.
Exclusions:
• Genetic material derived from Māori persons. • Cells derived from Māori persons • Genetic material derived from native flora or fauna. • Modifications that result in the GMO having a greater ability to escape from containment than the unmodified host organism.
Containment level/Category of Modification:
PC2/B

For transduction and infection of mammalian cell lines with recombinant replication defective viral vectors and viruses:

Host organism: Mammalian cell lines and primary cultures to include epithelial or fibroblasts originating from:
Mus musculus Linnaeus, 1758 *Mus spretus* (Lataste, 1883) *Rattus rattus* Linnaeus, 1758 *Rattus norvegicus* Berkenhout, 1759 *Homo sapiens* Linnaeus, 1758 *Chlorocebus aethiops* Linnaeus, 1758 *Ovis aries* Linnaeus, 1758 *Bos taurus* Linnaeus, 1758 *Canis familiaris* Linnaeus, 1758 *Oryctolagus cuniculus* Linnaeus, 1758 *Sylvilagus* sp Gray, 1867 *Cricetulus griseus* Milne-Edwards, 1867 *Cricetulus migratorius* Pallas, 1773 *Cricetus cricetus* Linnaeus, 1758 *Cavia porcellus* Linnaeus, 1758
Modified using:
Recombinant replication defective retroviral, adenoviral, adenoviral associated vectors or recombinant baculoviruses, papillomaviruses, poxviruses and hepadnaviruses containing coding, non-coding and/or regulatory regions, antisense sequences or other RNA interference-inducing sequences of viral, eukaryotic and/or prokaryotic donors associated with cellular sequences and proteins.
Genetic material may be:
Eukaryotic, including human[8], viral, prokaryotic and/or synthetically produced genetic material (and deletion, substitution and chimeric mutants thereof) associated with viral and cellular sequences and proteins that regulate cellular and tissue physiology.
Regulatory elements, reporter and selectable marker genes and other features:
Vectors will include standard or fully characterised regulatory elements including promoters, regulatory element binding sites, transcriptional activators, enhancers, terminators, silencing elements, and multiple cloning sites and origins of replication. The vectors may also contain recombination sites, internal ribosomal entry sites (IRES), selectable marker genes, reporter genes, protein targeting, localisation and secretory signals, solubility enhancement tags, protein purification tags and affinity tags including epitope tags.

[8] derived from non-Māori persons only

Characteristics of the genetically modified organisms:
Infectious particles may be produced when cell lines are transfected with recombinant papillomaviruses, adenoviruses, hepadnaviruses, orf and vaccinia viruses.
Exclusions:
• Genetic material derived from Māori persons. • Cells derived from Māori persons • Genetic material derived from native flora or fauna. • Modifications that result in the GMO having a greater ability to escape from containment than the unmodified host organism.
Containment level/Category of Modification:
PC1/A when using recombinant baculoviruses PC1/A + biological safety cabinet when using recombinant replication defective viral particles. PC2/B when using recombinant replication competent viruses other than baculovirus.

For transduction and infection of whole animal tissue:

Host organism: Animals, including transgenic animals of the following:
Mus musculus Linnaeus, 1758 *Rattus rattus* Linnaeus, 1758 *Oryctolagus cuniculus* Linnaeus, 1758
Modified using:
Recombinant, retroviral, adenoviral, adenoviral associated vectors or recombinant papillomaviruses and poxviruses and hepadnaviruses containing coding, non-coding and/or regulatory regions, antisense sequences or other RNA interference-inducing sequences of viral, eukaryotic and/or prokaryotic donors associated with cellular sequences and proteins.
Genetic material may be:
Eukaryotic, including human[9], viral, prokaryotic and/or synthetically produced genetic material (and deletion, substitution and chimeric mutants thereof) associated with viral and cellular sequences and proteins that regulate cellular and tissue physiology.
Regulatory elements, reporter and selectable marker genes and other features:
Vectors will include standard or fully characterised regulatory elements including promoters, regulatory element binding sites, transcriptional activators, enhancers, terminators silencing elements, and multiple cloning sites and origins of replication. The vectors may also contain recombination sites, internal ribosomal entry sites (IRES), selectable marker genes, reporter genes, protein targeting, localisation and secretory signals, solubility enhancement tags, protein purification tags and affinity tags including epitope tags.
Characteristics of the genetically modified organisms:
Infectious particles may be produced when animals are infected with recombinant papillomaviruses, orf and vaccinia viruses
Exclusions:
• Genetic material derived from Māori persons. • Genetic material derived from native flora or fauna. • Modifications that result in the GMO having a greater ability to escape from containment than the unmodified host organism.
Containment level/Category of Modification:
PC2/B

[9] derived from non-Māori persons only

3. Purpose of the application

3.1. The purpose of the application is to genetically modify *Escherichia coli,* yeast, viruses, cell lines and laboratory animals to study the effects of viral and cellular sequences and proteins.

3.2. This research is designed to provide new knowledge about the biology of virus infection and the link between infection and disease pertinent to a range of different viruses.

4. Legislative Criteria for Application

4.1. The application was lodged pursuant to section 40(1) of the HSNO Act. The decision was determined in accordance with section 45, taking into account the matters relevant to the purpose of the HSNO Act, as specified under Part 2 of that Act. Unless otherwise stated, references to section numbers in this decision refer to sections of the HSNO Act.

4.2. Consideration of the application followed the relevant provisions of the Methodology, with particular regard to clauses 12 (dealing with assessment of risks) and 13 (dealing with assessment of costs and benefits). Unless otherwise stated, references to clause numbers in this decision refer to clauses of the Methodology.

4.3. This application was required to be considered by the Authority as it included developments that were considered “not low-risk” genetic modifications (clause 1(e) of the Schedule of the HSNO (Low-Risk Genetic Modification) Regulations 2003) involving “viral vectors whose host range includes human cells and that contain one or more inserted nucleic acid sequence(s) coding for a product that can lead to uncontrolled mammalian cellular proliferation or be toxic to mammalian cells, or both”.

5. Application process

5.1. The application was formally received on 18 January 2011. In accordance with section 19(2)(b) of the Act, the Authority appointed a decision making Committee to consider the application (the Committee). The Committee comprised Dr Kieran Elborough (Chair), Dr Shaun Ogilvie, and Dr Valarie Orchard.

5.2. In accordance with section 45(1)(a)(i) of the HSNO Act 1996, the Committee determined that the application was for an appropriate purpose under 39(1)(a) of the HSNO Act 1996; namely the development of any genetically modified organism.

5.3. The following information was available to the Committee for the consideration of the application:

- The completed Authority approved application form (Form NO3)
- Evaluation and Review (E&R) Report from the Agency
- Update memorandum from the Agency

5.4. In accordance with section 58(1)(c) and clause 5 the Department of Conservation (DOC) and Ministry of Agriculture and Forestry (MAF) were invited to comment on this application. There were no comments from DOC. The response from MAF was included in Appendix 4 of the E&R report.

5.5. It was considered there was not likely to be significant public interest in this application. Therefore, the application was not publicly notified, in accordance with ERMA New Zealand policy.

6. Sequence of Consideration

6.1. In accordance with clause 24 of the Methodology, the Committee looked sequentially at identification, assessment and evaluation of risks, costs and benefits. Interposed with this was the consideration of the adequacy of the proposed containment regime, and the ability of the organism to escape and to form a self-sustaining population. Management techniques were considered in relation to the identified risks (clause 24) and those risks identified as significant were assessed (clause 12). Costs and benefits were assessed in accordance with clause 13 of the Methodology.

6.2. Finally, taking account of the risk characteristics established in accordance with clause 33 of the Methodology, the combined impact of risks, costs and benefits was evaluated in accordance with clause 34.

7. Identification of potentially significant adverse effects (risks and costs)

7.1. The Committee identified the adverse effects (risks and costs) related to the application in accordance with clauses 9 and 10 of the Methodology.

7.2. The Committee identified the following potentially significant adverse effects:

- effects on human health and safety due to occupational exposure (further assessed in section 9.4-9.14)
- effects on the environment due to the uncontrolled spread of recombinant-competent viruses (further assessed in section 9.15-9.17)

7.3. The Committee did not identify any potentially significant adverse effects on society and the community, or for the market economy.

7.4. The Committee considered the potential adverse Māori cultural effects and noted sections 6.6 of the E&R Report. The Committee noted that University of Otago had undertaken consultation with Ngai Tahu representative on Otago's Institutional Biological Safety Committee (IBSC). The Committee also noted that the University of Auckland had discussed this application with representatives of Ngati Ehatua and iwi of the greater Auckland region who are members of the Auckland Biological Safety Committee. The Committee concurred with the E&R Report (section 5.21) and considered that the nature and level of the consultation process was adequate, and did not identify any significant effects on Maori culture.

8. Adequacy of the Proposed Containment Regime

8.1. In assessing risks and costs, the Committee considered issues affecting the adequacy of the containment regime (in accordance with section 45(1)(a) of the HSNO Act); the potential for population establishment and population eradication (sections 37 and 44 of the HSNO Act and clauses 10(e) and 10(f) of the Methodology); and other matters in order to give effect to the purpose of the HSNO Act (section 45(2)(b)). Risk management techniques were used in relation to the identified risks and costs (clauses

12(d) and 24 of the Methodology). As such, the assessment of risks and costs was taken into account in setting the containment requirements that are discussed in this section.

Ability to Escape from Containment

8.2. In considering the ability of the organism to escape from containment, the Committee considered the:

- biological characteristics of the organism
- potential pathways for escape of the organism from the containment facility
- proposed containment regime
- ability of the organism to establish a self-sustaining population

8.3. The controls imposed by this approval (as specified in **Appendix 1**) address the matters detailed in the Third Schedule Part I of the HSNO Act: Matters to be addressed by containment controls for importing, developing or field testing of genetically modified organisms under the Act, plus other controls to give effect to the purpose of the Act. These controls incorporate a requirement for the management of risks and costs (under clauses 12(d) and 24 of the Methodology) posed by the genetically modified viral vectors, viruses, cell lines, rodents and rabbits subject to this approval. The controls have been imposed to ensure that exposure of laboratory workers and other persons, and the outside environment, to risks and costs posed by the organisms is negligible.

Ability of organism(s) to establish a self-sustaining population and the ease of eradication

8.4. In accordance with section 44 and 37 of the HSNO Act the Committee considered the ability of the organism to establish undesirable self-sustaining populations, should it escape from containment, and the ease with which such populations could be eradicated.

8.5. The Committee considered that with the containment controls it has imposed (refer to Appendix 1 of this decision) it is highly improbable that the modified viral vectors, viruses, cell lines, yeast, rodents or rabbits will escape or be removed inadvertently from containment and establish self-sustaining populations.

8.6. In the highly improbable event that a virus, cell line or yeast escaped it would be **highly improbable** that a self-sustaining population would be able to form. This is due to the to the specialised growth conditions required for their survival.

8.7. The Committee considered that in the **highly improbable** event that mice or rabbits did escape from containment, and were able to breed, the genetically modified traits would confer no selective advantage (which would be required for a trait to persist in a population). Therefore it would be highly improbable that an undesirable self-sustaining population of animals expressing the genetically modified trait would form.

9. Assessment of adverse effects

9.1. In assessing risks and costs, issues affecting the adequacy of the containment regime and the potential for population establishment and population eradication were considered (as required by sections 37 and 44 of the HSNO Act and clause 10(e) of the Methodology). The containment regime was considered in the context of a risk management regime for controlling the identified risks and costs (clauses 12(d) and 24). In doing so, the Committee set controls to satisfactorily provide for the matters in the Third Schedule (Part I) of the HSNO Act. It was then considered whether or not there were any residual risks that required further consideration.

9.2. The potential risks and costs assessed here are those identified as significant, having regard for those matters set out in clauses 9 and 10 of the Methodology. Risks were considered in terms of the requirements of clause 12 of the Methodology, including the assessment of consequences and probabilities, the impact of uncertainty and the impact of risk management. Costs were considered in terms of clause 13 of the Methodology.

9.3. Two potentially significant adverse effects were identified by the Committee - the infection of laboratory workers with a viral vector or virus via accidental injection; and adverse effects on the environment due to the uncontrolled spread of recombinant-competent viruses. These adverse effects are assessed below.

Assessment of adverse effects – Infection of laboratory workers

9.4. Adverse health effects on human health and safety due to occupational exposure was identified as a potentially significant adverse effect. The Committee considered the likelihood and the magnitude of an adverse effect occurring in the event that a researcher did inadvertently inject themselves with a retroviral vector or virus.

9.5. The Committee concurred with the conclusion reached in the E&R report (section 6.3) that the accidental transduction/infection of laboratory workers with viral vectors or viruses is **highly improbable**, as the accidental transduction/infection of laboratory workers with viral vectors or viruses would be reduced by the requirement to comply with the Standards outlined in this decision. Furthermore, additional controls (**Appendix 1**) have also been imposed to further reduce the risk to users.

9.6. The Committee also noted that the viral vectors will be replication defective. In the event that a person did become infected, the vectors will only be able to express the transgene and cannot establish an ongoing viral infection. Therefore, it is highly improbable that the viral vector will spread to other cells within the individual or from the individual to other people.

9.7. The Committee also note that while some replication competent viruses will used, theses viruses have both species and tissue specificity. In the event that a person did become infected with one of these viruses it is highly improbable that it will result in an ongoing viral infection.

9.8. The Committee also noted, in accordance with clause 33, that an element of the risk posed with an accidental needle stick injury in the use of viral vectors and viruses is voluntary in the sense that people choose to be involved in this sort of research. In accordance with clause 33(c), the Committee noted the viral vectors are not capable of uncontrollable spread as the vector would only transduce a cell once. Therefore,

infection would be limited to exposed workers and could not be passed to other people. It is noted that while the potential adverse effects are irreversible (i.e. may cause disease), they general public is not exposed to the risk and the risks are well understood by the researchers who work with the viral vectors. The viruses also have high species and tissue specific infection properties which would limit the probability of exposure leading to an ongoing viral infection.

9.9. The Committee noted the degree of scientific uncertainty (clauses 25(1), 29, 30, 32 and 33) relating to the adverse effects in the event of a researcher inadvertently injecting themselves with recombinant replication defective viral vectors. The worst case scenario is that inadvertent infection with a recombinant retroviral vector may induce insertional mutagenesis and oncogenic dysregulation within affected cells.

9.10. The Committee noted the degree of scientific uncertainty (clauses 25(1), 29, 30, 32 and 33) relating to the adverse effects in the event of a researcher inadvertently injecting themselves with viruses. The worst case scenario is that inadvertent infection depends on the recombinant virus and vary from skin lesions and warts to Hepatitis B.

9.11. Therefore, in order to minimise the potential occupational risks associated with the use of the viral vectors and viruses the Committee has imposed additional controls to the Standards which state that:

When using replication competent viruses or replication deficient viral vectors approval users must be trained in their safe production and use. **(Additional control 6).**

and;

The approval holder must ensure that any new information identified pertaining to the risks associated with the viral vectors and viruses under this approval be forwarded to ERMA New Zealand immediately. **(Additional control 7).**

9.12. These additional controls will ensure that the staff using the approval maintain up-to-date knowledge of the risks of using the viral vectors and viruses to users and the environment, incorporate this knowledge into training of new users and inform ERMA New Zealand if any newly risks are reported.

9.13. To reduce the possibility that an approval user could inadvertently expose themselves when handling viral vectors and viruses the Committee has made a requirement that a class II biological safety cabinet is used (**Additional control 8**). To minimise the potential risk of accidental colonisation of workers with infected cell lines, laboratory workers should not infect cultures of their own cells, nor, as a general rule, those of their immediate family or other members of the laboratory (**Additional control 9**).

9.14. To limit the chance of inadvertently producing a replication competent viral particle, when working with replication defective viral vectors the Committee has imposed the following additional controls:

To mitigate the risk of producing replication competent viral particles:

(a) *No genes are to be cloned into a replication defective viral vector that could result in the production of products functionally similar to those produced by the replication competent virus, and*

(b) *Packaging or production of replication defective viral vectors that are capable of infecting human cells must be conducted in incubators dedicated to the use of such vectors.* **(Additional control 10).**

and;

Additional control 11 *Large-scale production of adenoviral particles must include tests for the production of replication competent viral vectors using recognised and validated procedures. If replication competent viral vectors are detected, the entire production batch must be destroyed immediately.*

9.15. To remove the possibility of unwanted interaction between AAV and adenoviral replication defective vectors the production and packaging of AAV and adenoviral replication defective vectors should be separated either spatially or temporally (**Additional control 12**).

9.16. In summary, the Committee agreed that the use of viral vectors and viruses, as described in this application, poses a low risk to the researchers, and that the containment regime and the applicant's experience in working with those viral vectors mean that it is **highly improbable** that those working with the vectors will become infected (clause 12(c)). While there is some uncertainty about the nature of the effects of any particular infection incident (because of the range of genes potentially involved), the experimental laboratory procedures to be used are well established and the Committee is confident that the risks are **negligible** and can be adequately managed (clause 12(e)).

Assessment of adverse effects – Uncontrolled spread of a recombinant replication competent virus in the environment

9.17. The Committee noted, in accordance with clause 33, that there is an element of risk posed with the escape of recombinant viruses which may come into contact with susceptible host species, where the virus can replicate and establish a self-sustaining population in that host. The virus can be transmitted by viral particles shed into the environment, then come into contact with a susceptible host and establish infection. In accordance with clause 33(c), the Committee noted that the viruses will only be used on animals within an appropriate physical containment facility as designated in **Appendix 1**.

9.18. The Committee concurred with the conclusion reached in the E&R report (section 6.4) that the uncontrolled spread of a recombinant replication competent virus in the environment is improbable, as any risk is sufficiently mitigated by the requirement to comply with the Standards outlined in this decision. Furthermore, additional controls (**Appendix 1**) have also been imposed to further reduce the risk.

9.19. In order to reduce the potential risk to the environment the Committee has imposed additional controls. **Additional control 8** requires that a Class II biological safety cabinet should be used for all experiments involving handling viral vectors and viruses that may result in the production aerosols, in order to reduce the possibility that a researcher could inadvertently tranduce/infect themselves. **Additional control 13** restricts the maximum titre of viral infectious particles per volume that can be stored, while recommending when using viruses or viral particles that volumes and titres be as small as practical when used in animals for personal safety. **Additional control 14** requires strict handling and destruction of all shed scab material from viral work in animals as this is potentially infectious.

9.20. In summary, the Committee agreed that the use of viruses and viral vectors, as described in this application, poses a low risk to the environment, and the containment regime and the applicant's experience in working with those viruses mean that it is **highly improbable** that animals infected with those viruses would escape, giving rise to the possibility of uncontrolled spread of the recombinant replication competent virus in the environment if handling and containment procedures are followed (clause 12(c)). While there is some uncertainty about the nature of the effects of any particular infection incident (because of the range of genes potentially involved), the experimental laboratory procedures to be used are well established and the Committee is confident that the risks are **negligible** and can be adequately managed (clause 12(e)).

10. Identification and assessment of potentially significant beneficial effects

10.1. The Committee agreed with the E&R Report and identified the following benefits associated with the application, in accordance with the Methodology clauses 9, 10, 13 and 14, and section 6(e) of the HSNO Act.

10.2. The Committee concurred with section 7 of the E&R Report and considered that there are benefits to scientific knowledge by maintaining New Zealand's standing in the international science community, the applicant's ability to attract research funding, and their publications in top-tier peer-reviewed scientific journals. Furthermore, advancing the understanding of how viruses modulate their host may lead to the development of therapeutic strategies for the treatment of diseases such as cancer.

10.3. The Committee identified that these benefits are **likely** to occur, their magnitude may range from **minimal** to **moderate** therefore depending on the success of the research and the scientific value of the research results, resulting in a **low** to **medium** benefit.

10.4. The Committee concurred with section 7 of the E&R Report and noted that though the applicant has noted there are no obvious monetary benefits in the short term the research may lead to the development of new and novel therapeutic strategies in the future. The magnitude of the expected monetary benefit is difficult to gauge, but could potentially be large if the research was successful (i.e. proceeded to clinical trials and the market). However, there is a high degree of uncertainty surrounding the expected monetary benefits, as it depends on how successful the research is and a number of other factors. It was also difficult to gauge the distributional effects of the benefits over time as there are too many uncertainties.

11. Other Matters

11.1. The Committee did not identify any international obligations relevant to this application.

11.2. The Committee considered that no other matters were relevant in setting controls outside the Third Schedule, in order to give effect to the purpose of the HSNO Act (in accordance with section 45(2)(b)).

11.3. The Committee noted that the development and use of variola viruses is not permitted in New Zealand as these are considered "unwanted organisms" under the Biosecurity Act 1993. The possession and use of variola viruses (one type of Pox virus) is restricted to the World Health Organization Collaborating Centre for Smallpox and Other Poxvirus Infections, located at the Centres for Disease Control and Prevention, Atlanta, Georgia.

12. Overall Evaluation and weighing up of Adverse and Beneficial Effects, and the Overall Adequacy of Containment

12.1. In reaching its decision on this application, the Committee records that the following criteria in the HSNO Act and Methodology have been particularly relied on (in accordance with clauses 21 and 36(2)(b) of the Methodology):

12.2. The application has been considered in the context of the purpose and principles of the HSNO Act (section 4-8 inclusive).

12.3. Pursuant to section 45(1)(a)(i) of the HSNO Act, the Committee is satisfied that the purpose of the application falls under section 39(1)(a) the development of any genetically modified organism.

12.4. In accordance with section 45 of the HSNO Act, and clauses 9, 10 and 12 of the Methodology, the Committee concluded that after taking account of the organism description and the impact of containment and other controls set out in **Appendix 1,** each of the risks and costs were **negligible**. Thus, the Committee considered the application under clause 26 of the Methodology.

12.5. As assessed in section 10 of the decision the benefits are largely scientific. Depending on the success of the research and the scientific value of the research results, resulting in a **low** to **medium** benefit.

12.6. The Committee then considered whether, given the organism description and the containment and controls proposed, the benefits outweigh the non-negligible risks and costs. The Committee concluded that the benefits do outweigh the risks and costs.

12.7. The Committee was satisfied that the cell lines and rodents transduced with recombinant retroviral vectors can be adequately contained (sections 45(1)(a)(iii) and 44(b) of the HSNO Act), by the controls required in this decision (refer to **Appendix 1**).

12.8. The Committee recommends that the approval holder identifies in their contingency plan procedures for the treatment of an approval user's accidental exposure to viral vectors or viruses.

12.9. In accordance with clause 36(2)(b) of the Methodology, the Committee records that in reaching this conclusion, it has applied the balancing tests in section 45 of the Act.

12.10. The Committee noted all risks are **negligible** and that after considering the impact of the combined controls, the organisms can be adequately contained. Consequently, the Committee determined that the beneficial effects outweigh the adverse effects of the application.

13. Decision

13.1. Pursuant to section 45(1)(a)(i) of the Hazardous Substances and New Organisms Act 1996, the Committee is 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 genetically modified organism.

13.2. Having considered all the possible effects in accordance with sections 45(1)(a)(ii) and 44 and pursuant to clause 26 of the Methodology, and based on consideration and analysis of the information provided and taking into account the application of risk management controls specified in this decision, the view of the Committee is that the risks (or costs) of adverse effects associated with the development in containment of the organisms listed in Table 1 are outweighed by the benefits.

13.3. The Committee is satisfied that the proposed containment regime, as set out in **Appendix 1**, will adequately contain the organism as required by section 45(1)(a)(iii) of the Hazardous Substances and New Organisms Act 1996.

13.4. In accordance with clause 36(2)(b) of the Methodology the Committee records that, in reaching this conclusion, it has applied the balancing tests in section 45 of the Hazardous Substances and New Organisms Act 1996 and clause 26 of the Methodology and has relied in particular on the criteria set out in the following sections of the Act:

- section 44 additional matters to be considered;
- section 45 determination of application;
- section 37 additional matters to be considered;
- The Third Schedule-Part I matters to be addressed by containment controls for importing, developing or field testing of genetically modified organisms.

13.5. The Committee has also applied the following criteria in the Methodology:

clause 9 – equivalent of sections 5 and 6;

clause 10 – equivalent of sections 36 and 37;

clause 12 – evaluation of assessment of risks;

clause 13 – evaluation of assessment of costs and benefits;

clause 21 – the decision accords with the requirements of the Hazardous Substances and New Organisms Act 1996 and Regulations;

clause 22 – the evaluation of risks, costs and benefits – relevant considerations;

clause 24 – the use of recognised risk identification, assessment, evaluation and management techniques;

clause 25 – the evaluation of risks;

clause 26 – the risks and costs are negligible and outweighed by the benefits;

clause 33 – the risk characteristics; and

clause 34 – the aggregation and comparison of risks, costs and benefits.

13.6. The application for development in containment of organisms described in Table 1 is thus **approved**, ***with*** **controls**, as set out in **Appendix 1**.

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Dr Kieran Elborough
Chair of the Decision-making Committee

18 February 2011
Date:

Approval codes: GMD100887 - 911

Approval numbers for Organisms in Application ERMA200732

Approval Code	Organism	BCH number*
GMD100887	*Escherichia coli* (Migula 1895) Castellani & Chalmers 1919	
GMD100888	*Autographa californica* multiple nucleopolyhedrovirus	
GMD100889	*Spodoptera frugiperda* (Smith, 1797)	
GMD100890	*Drosophila melanogaster* (Meigen, 1830)	
GMD100891	*Trichoplusia ni* (Huebner, 1803)	
GMD100892	Papillomaviridae	
GMD100893	*Saccharomyces cerevisiae* (Meyen ex E. C. Hansen, 1883)	
GMD100894	Vaccinia virus	
GMD100895	*Homo sapiens* (Linnaeus, 1758)	
GMD100896	*Mus musculus* Linnaeus, 1758	
GMD100897	*Mus spretus* (Lataste,1883)	
GMD100898	*Rattus rattus* (Linnaeus, 1758)	
GMD100899	*Rattus norvegicus* (Berkenhout, 1769)	
GMD100900	*Chlorocebus aethiops* (Linnaeus,1758)	
GMD100901	*Ovis aries* Linnaeus, 1758	
GMD100902	*Bos taurus* Linnaeus, 1758	
GMD100903	*Canis familiaris* (Linnaeus, 1758)	
GMD100904	*Oryctolagus cuniculus* (Linnaeus, 1758)	
GMD100905	*Sylvilagus* sp Gray, 1867	
GMD100906	*Cricetulus griseus* (Milne Edwards,1867)	
GMD100907	*Cricetus cricetus* Linnaeus, 1758	
GMD100908	*Cavia porcellus* (Linnaeus,1758)	
GMD100909	Poxviridae	
GMD100910	*Atadenovirus Ovine adenovirus* ICTV isolate OAdV287 (OvAd7)	
GMD100911	*Orthohepadnavirus Hepatitis B virus* ICTV	

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

Appendix 1: Controls required by this approval

In order to provide for the matters detailed in Part I of the Third Schedule to the Act[10], *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:

The controls imposed on the approval

1	The approval holders (University of Otago and University of Auckland) must ensure compliance with the following controls.
2	This approval is limited to the importation of the organisms described in **Table 1**, for the purpose of genetically modify *Escherichia coli*, yeast, viruses, cell lines and laboratory animals to study the effects of viral and cellular sequences and proteins
3	Unless otherwise specified by the following controls, the approval holder must ensure that the location and nature of the development and the disposal of the approved organisms are in accordance with the activities described in the application.
4	The approved organism must be maintained within a containment facility in accordance with following MAF/ERMA New Zealand Standard and the additional controls (as follows): • MAF/ERMA New Zealand Standard: *Facilities for Microorganisms and Cell Cultures: 2007a* and MAF/ERMA New Zealand Standard: *Containment Facilities for Vertebrate Laboratory Animals*[11]; • Australian/New Zealand Standard AS/NZS 2243.3:2002[1] Safety in laboratories: Part 3: Microbiological aspects and containment facilities; and • A minimum of Physical Containment level 1 (PC1) requirements of the above Standards for developments involving the use of non pathogenic laboratory strains of *Escherichia coli*, use and production of baculoviruses and the transduction of mammalian cell lines with replication defective viral vectors (PC1 in combination with a biological safety cabinet). • A minimum of Physical Containment level 1 (PC1) requirements of the above Standards including a biological safety cabinet for developments involving the transduction of mammalian cell lines with replication defective viral vectors. • A minimum of Physical Containment level 2 (PC2) requirements of the above Standards for developments using yeast and mammalian cell lines for the production of replication defective viral vectors or production of and infection with replication competent viruses (excluding baculoviruses) and the use of transgenic and non-transgenic rodents and rabbits.
5	The approval holder must ensure that within 24 hours of the discovery of any breach of containment[12] the MAF Inspector responsible for supervision of the facility, has

[10] 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.

[11] Any reference to MAF/ERMA New Zealand or AS/NZS Standards in these controls refers to any subsequent version approved or endorsed by ERMA New Zealand.

	received notification of the breach, and the details of any action taken by the facility since the breach occurred.
Controls additional to the Standards	
6	When using replication competent viruses or replication deficient viral vectors, approval users must be trained in their safe production and use.
7	The approval holder must ensure that any new information identified pertaining to the risks associated with the viral vectors and viruses under this approval be forwarded to ERMA New Zealand immediately.
8	The handling of viruses and viral vectors must be done within a Class II Biological Safety Cabinet to reduce exposure from aerosols and spillage. In the event that animal inoculation within a Class II Biological Safety Cabinet is not practical, all viral preparation must be done in a Class II Biological Safety Cabinet before transport to the animal within containment facility approved to the MAF/ERMA Standard *Containment Facilities for Vertebrate Laboratory Animals*. Transportation packaging must be secure. The Class II Biological Safety Cabinet must be decontaminated with a suitable disinfectant immediately following work. Tissue culture plates containing viral particles must be within a larger secondary container to capture any spills.
9	Cells sourced from approval users or working in, or associated with, the facility must not be used for viral work.
10	To mitigate the risk of producing replication competent viral particles: (a) No genes are to be cloned into a replication defective viral vector that could result in the production of products functionally similar to those produced by the replication competent virus, and (b) Packaging or production of replication defective viral vectors that are capable of infecting human cells must be conducted in incubators dedicated to the use of such vectors.
11	Large-scale production of adenoviral particles must include tests for the production of replication competent viral vectors using recognised and validated procedures. If replication competent viral vectors are detected, the entire production batch must be destroyed immediately.
12	To limit the likelihood of unwanted interaction between AAV and adenoviral replication defective vectors the production and packaging of AAV and adenoviral replication defective vectors should be separated either spatially or temporally.
13	To reduce exposure of approval users to virus, high titre viral cultures (concentrations of greater than 2 X 10^{14} infectious particles per ml) must be safely and securely stored in volumes of no greater than 5 mL. The volume and titre of virus cultures used in animals must be kept as small as practically possible.

[12] Breach of containment includes: the escape of an organism(s), unauthorised entry and/or structural integrity of facility compromised.

14	All shed scab material from viral work in animals shall be considered potentially infectious and must be destroyed as described for bedding material and waste in the MAF/ERMA Standard *Containment Facilities for Vertebrate Laboratory Animals*.

Appendix 2: Qualitative scales for describing effects

Qualitative descriptors for risk/benefit assessment

This section describes how we and the Authority address the qualitative assessment of risks, costs and benefits. Risks and benefits are assessed by estimating the magnitude and nature of the possible effects and the likelihood of their occurrence. For each effect, the combination of these two components determines the level of the risk associated with that effect, which is a two dimensional concept. Because of lack of data, risks are often presented as singular results. In reality, they are better represented by 'families' of data which link probability with different levels of outcome (magnitude).

The magnitude of effect is described in terms of the element that might be affected. The qualitative descriptors for magnitude of effect are surrogate measures that should be used to gauge the end effect or the 'what if' element. Tables 1 and 2 contain generic descriptors for magnitude of adverse and beneficial effect. These descriptors are examples only, and their generic nature means that it may be difficult to use them in some particular circumstances. They are included here to illustrate how qualitative tables may be used to represent levels of adverse and beneficial effect.

Table 1 Magnitude of adverse effect (risks and costs)

Descriptor	Examples of descriptions - ADVERSE
Minimal	Mild reversible short term adverse health effects to individuals in highly localised area Highly localised and contained environmental impact, affecting a few (less than ten) individuals members of communities of flora or fauna, no discernible ecosystem impact Local/regional short-term adverse economic effects on small organisations (businesses, individuals), temporary job losses No social disruption
Minor	Mild reversible short term adverse health effects to identified and isolated groups Localised and contained reversible environmental impact, some local plant or animal communities temporarily damaged, no discernible ecosystem impact or species damage Regional adverse economic effects on small organisations (businesses, individuals) lasting less than six months, temporary job losses Potential social disruption (community placed on alert)
Moderate	Minor irreversible health effects to individuals and/or reversible medium term adverse health effects to larger (but surrounding) community (requiring hospitalisation) Measurable long term damage to local plant and animal communities, but no obvious spread beyond defined boundaries, medium term individual ecosystem damage, no species damage Medium term (one to five years) regional adverse economic effects with some national implications, medium term job losses Some social disruption (e.g. people delayed)
Major	Significant irreversible adverse health effects affecting individuals and requiring hospitalisation and/or reversible adverse health effects reaching beyond the immediate community Long term/irreversible damage to localised ecosystem but no species loss Measurable adverse effect on GDP, some long term (more than five years) job losses Social disruption to surrounding community, including some evacuations
Massive	Significant irreversible adverse health effects reaching beyond the immediate community and/or deaths Extensive irreversible ecosystem damage, including species loss Significant on-going adverse effect on GDP, long term job losses on a national basis Major social disruption with entire surrounding area evacuated and impacts on wider community

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Table 2 Magnitude of beneficial effect (benefits)

Descriptor	Examples of descriptions -BENEFICIAL
Minimal	Mild short term positive health effects to individuals in highly localised area Highly localised and contained environmental impact, affecting a few (less than ten) individuals members of communities of flora or fauna, no discernible ecosystem impact Local/regional short-term beneficial economic effects on small organisations (businesses, individuals), temporary job creation No social effect
Minor	Mild short term beneficial health effects to identified and isolated groups Localised and contained beneficial environmental impact, no discernible ecosystem impact Regional beneficial economic effects on small organisations (businesses, individuals) lasting less than six months, temporary job creation Minor localised community benefit
Moderate	Minor health benefits to individuals and/or medium term health impacts on larger (but surrounding) community and health status groups Measurable benefit to localised plant and animal communities expected to pertain to medium term. Medium term (one to five years) regional beneficial economic effects with some national implications, medium term job creation Local community and some individuals beyond immediate community receive social benefit.
Major	Significant beneficial health effects to localised community and specific groups in wider community Long term benefit to localised ecosystem(s) Measurable beneficial effect on GDP, some long term (more than five years) job creation Substantial social benefit to surrounding community, and individuals in wider community.
Massive	Significant long term beneficial health effects to the wider community Long term, wide spread benefits to species and/or ecosystems Significant on-going effect beneficial on GDP, long term job creation on a national basis Major social benefit affecting wider community

The likelihood applies to the composite likelihood of the end effect, and not either to the initiating event, or any one of the intermediary events. It includes:

the concept of an initiating event (triggering the hazard), and

the exposure pathway that links the source (hazard) and the area of impact (public health, environment, economy, or community).

Thus, the likelihood is not the likelihood of an organism escaping, or the frequency of accidents for trucks containing hazardous substances, but the likelihood of the specified adverse effect[13] resulting from that initiating event. It will be a combination of the likelihood of the initiating event and several intermediary likelihoods[14]. The best way to determine the likelihood is to specify and analyse the complete pathway from source to impact.

Likelihood may be expressed as a frequency or a probability. While frequency is often expressed as a number of events within a given time period, it may also be expressed as the number of events per head of (exposed) population. As a probability, the likelihood is

[13] The specified effect refers to scenarios established in order to establish the representative risk, and may be as specific as x people suffering adverse health effects, or y% of a bird population being adversely affected. The risks included in the analysis may be those related to a single scenario, or may be defined as a combination of several scenarios.

[14] Qualitative event tree analysis may be a useful way of ensuring that all aspects are included.

dimensionless and refers to the number of events of interest divided by the total number of events (range 0-1).

Table 3 Likelihood

Descriptor	Description
Highly improbable	Almost certainly not occurring but cannot be totally ruled out
Very unlikely	Considered only to occur in very unusual circumstances
Unlikely (occasional)	Could occur, but is not expected to occur under normal operating conditions.
Likely	A good chance that it may occur under normal operating conditions.
Highly likely	Almost certain, or expected to occur if all conditions met

Using the magnitude and likelihood tables a matrix representing a level of risk/benefit can be constructed.

In the example shown in Table 4, four levels of risk/benefit are allocated: A (negligible), B (low), C (medium), and D (high). These terms have been used to avoid confusion with the descriptions used for likelihood and magnitude, and to emphasise that the matrix is a tool to help decide which risks/benefits require further analysis to determine their significance in the decision making process.

For negative effects, the levels are used to show how risks can be reduced by the application of additional controls. Where the table is used for positive effects it may also be possible for controls to be applied to ensure that a particular level of benefit is achieved, but this is not a common approach. The purpose of developing the tables for both risk and benefit is so that the risks and benefits can be compared.

Table 4 Level of risk

	Magnitude of effect				
Likelihood	**Minimal**	**Minor**	**Moderate**	**Major**	**Massive**
Highly improbable	A	A	A	B	B
Very unlikely	A	A	B	B	C
Unlikely	A	B	B	C	C
Likely	B	B	C	C	D
Highly likely	B	C	C	D	D