Import risk analysis: Non-Viable Biological Products, Microorganisms and other Viable Cells into New Zealand

Biosecurity New Zealand
Ministry of Agriculture and Forestry
Wellington
New Zealand

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Ministry of Agriculture and Forestry
Te Manatu Ahuwhenua, Ngaherehere
ASB Bank House
101-103 The Terrace
P O Box 2526
Wellington
New Zealand

Telephone: +64 4 474 4100
Facsimile: +64 4 474 4133
Internet: http://www.maf.govt.nz

Pre-Clearance
Biosecurity New Zealand

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Approved for general release

Debbie Pearson
Director Preclearance
Biosecurity New Zealand

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EXECUTIVE SUMMARY

The intention of this risk analysis is to analyse the risks involved in the importation of those biological products that are not already under formal control by other mechanisms. Risk Goods that would generally fall under a broader definition of biological products that are not included in this risk analysis include:

- Human medicinal products (Ministry of Health)
- Human vaccines (Ministry of Health)
- Animal medicines (MAF/ACVM)
- Animal vaccines (MAF/ACVM)
- Food and food supplements (NZFSA)
- Agricultural products pesticides, herbicides etc (ACVM)
- Live animals and germplasm (MAF)
- Plants including seeds and cuttings (MAF)
- New organisms Including GMOs (ERMA/ MAF)
- Importation of human cells or organs (MoH)

Risk goods not covered in the above list that are covered in the three parts of this risk analysis are:

1. Non-viable products that have been derived from living organisms or are identical to products produced from living organisms.
2. Viable microorganisms
3. Viable cells

Part 1 (non-viable products or biological products): The risk goods that fall into Category 1 will predominantly be used by laboratories. The laboratories into which biological products that are considered to be risk goods will be imported, may be registered as transitional facilities (MAF Standard 154.02.17: *Standard for transitional facilities for biological products*) and are subject to the restrictions imposed by the standard.

Applications may also be received to import products that are to be used directly in animals. These are considered to be high risk and special recommendations have been made concerning them (see below).

It is recommended that products that have been classified in this risk analysis as being non-risk goods should be imported without restrictions.

The numbers of products that are offered for sale by suppliers of biological products are too great for individual consideration of each product. Therefore generic groups of products have been considered and ways to provide control for individual products that are considered risk goods have been suggested.

The following products which were included in the preliminary hazards list were found to be of negligible concern and were not considered to be potential hazards requiring further investigation:

- Amino acids
- Antimicrobials (antibiotics)
- Small molecular weight fermentation products.

Products that were submitted to more detailed examination as potential hazards but were then found to be of negligible concern included.

- Culture media.
- Proteins derived from microorganisms by fermentation.
- Test kits that contain no live organisms.

Products that are of concern are included in the categories:

- Products derived from animal and plant tissues.
- Products derived from blood.
- Products derived from microorganisms.
- Products derived from eggs.

Most biological products will be purchased from recognised suppliers of biological products and only a few of the products in these categories are of concern. Therefore the following method of controlling the importation of potentially hazardous biological products has been recommended:

i) The importation of the products should be controlled by a permit system operated by MAF.

ii) Suppliers of biological products should submit their catalogues of products for sale to MAF. Working in collaboration with suppliers of biological products MAF should identify products of concern. Products that are not purified or certified free of microorganisms and viruses and are derived from animals or plants of unknown disease status, or from microorganisms that are unwanted or notifiable pathogens (Ministry of Agriculture and Forestry 2004), or new organisms should be classed as products of concern i.e. risk goods. All other products should be classed as being of no concern i.e. non-risk goods.

iii) A general permit for all non-risk goods should be issued and these products should be imported and traded without restriction.

iv) A restricted permit should be issued for products of concern. Under the restrictions defined in this permit suppliers of biological products should be able to import these products into a transitional facility. These products should only be sold to laboratories that are registered by MAF as transitional facilities. When selling the products to laboratories they should include a notice that warns the laboratory that under their terms of

registration as transitional facilities they must not inject animals or plants with, or otherwise expose them to the products and must retain the products in the transitional facility.

v) Laboratories that wish to import products independently of recognised importer/suppliers should apply for a "permit to import". The application should include details of the origin of the product, its method of manufacture, the source from which it will or has been derived and relevant details about its purity and the reason for importation. MAF may then issue a permit, which may be either a general or a restricted permit. Restricted permits should require that on arrival in New Zealand the products are directed to a transitional facility with relevant restrictions defined on the permit.

vi) Alternatively IHSs should be written to cover the contents of individual catalogues. The risk products from these catalogues should be identified and a permit system to control their importation into transitional facilities should be specified in the IHSs.

vii) Products for use in animals are considered to be high risk cases and should be imported on a restricted permit basis for each batch of products imported. The restrictions should include MAF's requirements for pre-importation testing and certification of the products.

viii) Laboratories that wish to import products independently of recognised supplier/importers should apply for a "permit to import". The applications should include details of the origin of the product, its method of manufacture, the source from which it has been derived and relevant details about its purity and the reason for importation, MAF may then issue a permit with relevant restrictions defined on the permit.

All vector organisms including any plasmids, cosmids, phage, viruses or transmissible sequences contained in them should be classified as microorganisms and imported under the restrictions pertaining to microorganisms in the relevant IHS. All organisms containing cloned polynucleotide sequences should be classed as new organisms requiring ERMA approval for importation.

Parts 2 and 3, Viable microorganisms and cells: Microorganisms, cell cultures and other live cells from plants and animals have been analysed separately. It has been recommended that they should be imported subject to the issue of a "Permit to Import" issued by MAF. It has also been suggested that a decision tree should be used to formalise the process of deciding whether microorganisms and cell cultures should be given a "Permit to Import".

Novel products that do not fall into any existing category, should be imported only on the basis of a restricted "Permit to import" after consultation about the appropriate restrictions with DoC, ERMA, and MAF.

1.0 BACKGROUND

Large numbers of different biological products are imported into New Zealand. These include non-viable biological products, microorganisms and cell cultures or living cells. Some of these products may be considered to be risk goods under the Biosecurity Act (1993). At present "authority to import" is provided by the issuing of a permit, on a case by case basis, by the Ministry of Agriculture and Forestry (MAF). This practice is contrary to Section 22 of the Biosecurity Act which requires import health standards (IHSs) to be in place for these products. It is MAF's policy that IHSs should be based on a risk analysis. No risk analysis has been done for biological products. The Group Director of MAF Biosecurity described the present situation as being "a less than ideal practice". A proposed solution of writing an IHS without a risk analysis was strenuously criticised by groups invited to comment on the suitability of a proposed draft IHS (January 2004). In particular the Department of Conservation (DoC) and MAF's Indigenous Flora and Fauna Group were strongly opposed to having an IHS that was not based on a formal risk analysis. This analysis represents an attempt to provide a suitable risk analysis.

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2.0 DEFINITIONS AND ABBREVIATIONS.

2.1 Acronyms

ACVM Agricultural Compounds and Veterinary Medicines group.

DoC The Department of Conservation.

ERMA Environmental Risk Management Authority.

MAF Ministry of Agriculture and Forestry.

MoH Ministry of Health

2.2 Definitions

Biological products. Non-viable products that have been derived from living organisms, or are identical to products derived from living organisms.

Cell culture. A defined population of cells propagated *in vitro* and derived from a single common ancestor tissue.

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Import Health Standard (IHS). A document issued by the Director-General under Section 22 of the Biosecurity Act 1993 specifying the requirements to be met for the effective management of risks associated with the importation of risk goods.

Microorganism. A microscopic organism including protozoa, fungi, bacteria, viruses and unicellular algae.

New organism. A new organism is-
(a) An organism belonging to a species that was not present in New Zealand immediately before 29 July 1998:
(b) An organism belonging to a species, subspecies, infrasubspecies, variety, strain, or cultivar prescribed as a risk species, where that organism was not present in New Zealand at the time of promulgation of the relevant regulation:
(c) An organism for which a containment approval has been given under this Act:
(ca) An organism for which a conditional release approval has been given:
(cb) A qualifying organism approved for release with controls:
(d) A genetically modified organism:
(e) An organism that belongs to a species, subspecies, infrasubspecies, variety, strain, or cultivar that has been eradicated from New Zealand.
(Explanatory Note: For a complete definition refer to HSNO, Section 2)

Polynucleotide. A polynucleotide is a piece of DNA or RNA. It is made up a string of the nucleotides that are the building blocks of DNA and RNA.

Risk goods. Any organism, organic material, or other thing, or substance,, that (by reason of its nature, origin, or other relevant factors) it is reasonable to suspect constitutes, harbours, or contains, an organism that may –
(a) Cause unwanted harm to natural and physical resources or human health in New Zealand: or
(b) Interfere with the diagnosis, management, or treatment, in New Zealand of pests or unwanted organisms.

Transitional facility.
(a) Any place approved as a transitional facility in accordance with + 39 [of the Biosecurity Act 1993] for the purpose of inspection, storage, treatment, quarantine, holding, or destruction of uncleared goods; or
(b) A part of a port declared to be a transitional facility in accordance with Section 39.

3.0 SCOPE

The scope of this risk analysis is limited to those biological products that are not already under formal control by other mechanisms. Risk Goods that would generally fall under a broader definition of biological products that are not included in this risk analysis include:

- Human medicinal products (Ministry of Health)
- Human vaccines (Ministry of Health)
- Animal medicines (MAF/ACVM)

- Animal vaccines (MAF/ACVM)
- Food and food supplements (NZFSA)
- Agricultural products pesticides, herbicides etc (ACVM)
- Live animals and germplasm (MAF)
- Plants including seeds and cuttings (MAF)
- New organisms Including GMOs (ERMA/ MAF)
- Importation of human cells or organs (MoH)

This risk analysis does not include any consideration of the social and cultural implications of importing biological products, microorganisms or living cells. Implications for the environment and human health are included. However, implications for the environment are generally limited to possible deleterious effects that the importation of biological products, microorganisms or living cells could have on plants and animals in the environment. Broader environment issues such as climate change, water conservation etc are not affected by the importation of the products discussed in this risk analysis and are not included in the scope. Consideration of the economic effects is only discussed in a general manner. No detailed analysis of costs is attempted because the data to do this in a reasonable manner is not available, or it is not appropriate for, or relevant to this risk analysis

This risk analysis has been divided into three parts:

Part 1: Biological products as defined above.

Part 2: Microorganisms.

Part 3: Living cells derived from higher animals or plants.

3.1 Scope: Biological products (Part 1)

The products that fall into Part 1 will generally be used by laboratories. The laboratories into which biological products will be imported, may be registered as transitional facilities (MAF Standard 154.02.17: *Standard for transitional facilities for biological products*) and in these cases will be subject to the restrictions imposed by the standard. However, in some cases applications may be received to import biological products into unregistered laboratories or for use in animals e.g. media for diluting semen or processing embryos for transplantation. These cases are also included in this risk analysis.

The biological products of concern are given in a preliminary hazard list for biological products in Section 6.3.

3.2 Scope: Microorganisms (Part2)

All live microorganisms as defined in Section 2 are included in this risk analysis. Microorganisms will in most cases be imported for use in laboratories that are registered transitional facilities. However, applications for importation from non-registered laboratories or non-laboratory based users of the products are also included in this risk analysis.

Importation of new organisms including microorganisms that are not known to already occur in New Zealand are subject to approval for the importation of a new species by the Environmental Management Resource Authority (ERMA) under the Hazardous Substances and New Organisms (HSNO) Act of 1996 and its subsequent amendments.

For clarification the following examples are given:

- A particular species of microorganism may be cultured and an enzyme extracted from either the excreted products or from the biomass of the microorganism. The extracted enzyme is a biological product. A quantity of killed whole organisms is also a biological product. However, the living organism itself is not a biological and importation of a viable organism for the purpose of producing the enzyme in New Zealand must be imported as a microorganism.

- A diagnostic test-kit that contains no living organisms is a biological product but diagnostic test kits that contains viable organisms (probably as test antigen or positive control), should be classified as living organisms and imported under the conditions specified for microorganisms.

- DNA and RNA may be imported as purified linear polynucleotides or incorporated into plasmids, phages, cosmids, viruses, transposable elements etc. that are considered to be parts of the vector organisms in which they are contained.. Vectors used to host genetic material are living organisms and must therefore, be imported as living organisms not as biological products. Importation of a polynucleotide sequence cloned in a plasmid that is contained in a bacterium will require clearance from ERMA because it is a new organism. Polynucleotide sequences that are in the form of purified linear sequences are not living organisms and are therefore biological products.

3.3 Scope: Living cells derived from animals or plants (Part 3)

Cell cultures consist of living cells that are derived from higher animals or plants. Cell cultures are most commonly imported as specific cell lines that are used for the cultivation of viruses. Other uses of cell lines include studies of cell metabolism and identification of toxic substances. Cell cultures may be knowingly infected with viruses when imported or may be free from contaminating viruses. If infected with a virus a cell

cultures will be subject to the regulations contained in IHSs for both microorganisms and cell cultures.

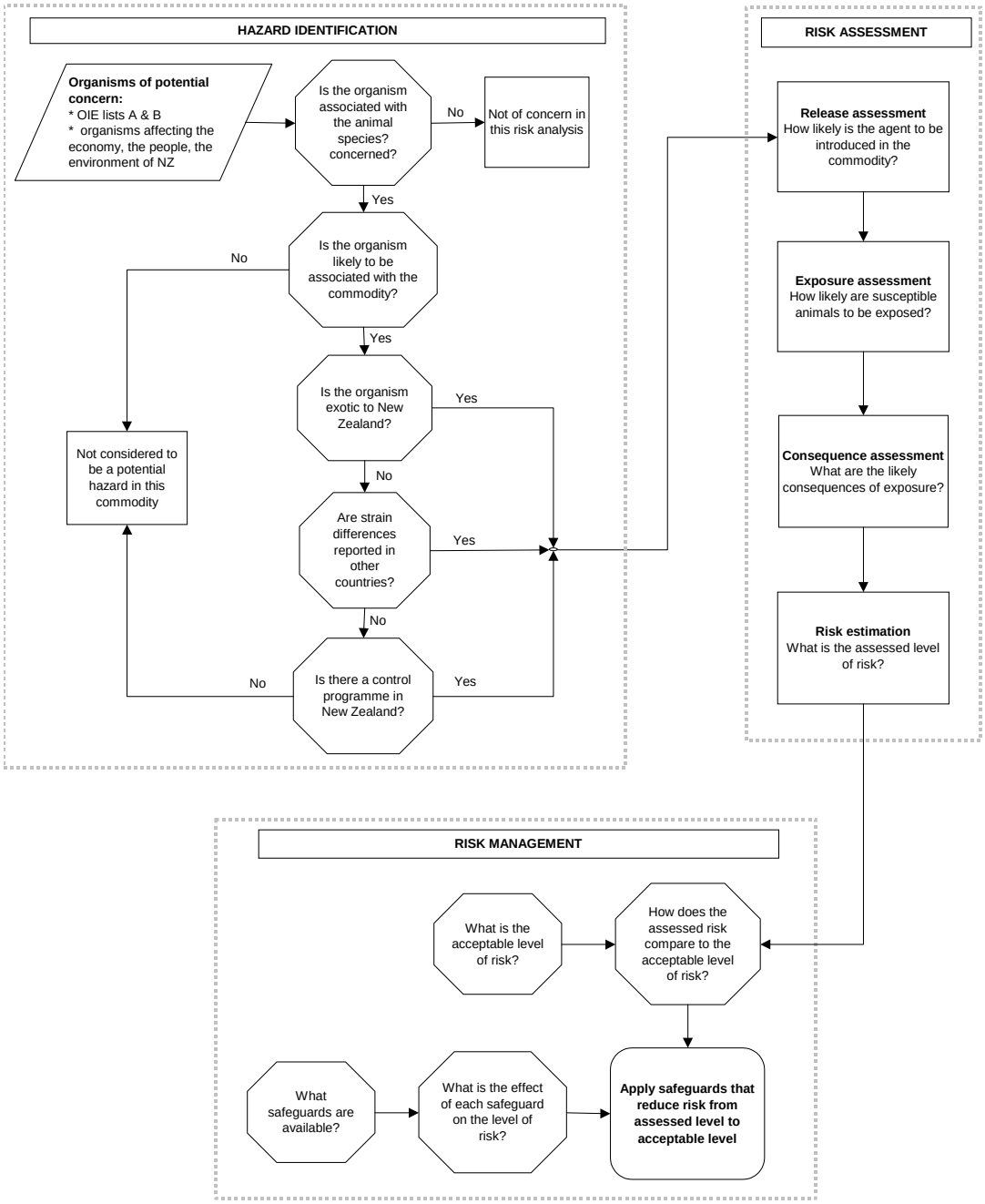
Applications to import living cells will generally be confined to requests to import established cell lines which are cells that have been adapted to grow in the laboratory and will continue to grow indefinitely in artificial media. Occasional requests could also be received to import primary cell cultures. In addition rare requests may be received to import cells for the purpose of cloning animals. Although this type of request is likely to be exceptional, MAF has already had to deal with such a request and this circumstance will also be covered in this risk analysis. Requests to import cells for other uses such as treatment of human diseases or new technologies not known at the time of writing this risk analysis may also occur.

This risk analysis does not include the importation of *Plants in tissue culture* which are defined as: plants *in vitro* that have been prepared as tissue culture from one parent by asexual reproduction (clonal techniques) under sterile conditions.

4.0 METHODOLOGY OF RISK ANALYSIS

The methodology used in this risk analysis follows the guidelines in Section 1.3 of the Office International des Epizooties (OIE) *Terrestrial Animal Health Code* (Anonymous 2004a). In New Zealand, the OIE risk analysis framework is applied as described in *Import Risk Analysis Animals and Animal Products* (Murray 2002). The risk analysis process used by the MAF is summarised in Figure 1.

Figure 1. The risk analysis process.



4.1 Hazard List

The first step in a risk analysis is the drawing up of a hazard list. This risk analysis is presented in three parts (See Section 3). A full hazard list is only necessary for Part 1. For the other parts the hazards are discussed in the text relating to those parts.

4.2 Risk Assessment

Under the OIE methodology, Risk Assessment consists of:

a) *Release assessment*: The likelihood of a pathogenic organism being imported in the commodity.

b) *Exposure assessment*: The likelihood of animals or humans in New Zealand being exposed to an organism in an imported commodity.

c) *Consequence assessment*: The consequences of entry, establishment or spread of an imported organism.

d) Risk *estimation*: An estimation of the risk posed by the imported products based on the release, exposure and consequence assessments. If the risk estimate is non-negligible, then the organism is a potential threat and risk management measures are required to reduce the level of risk to an acceptable level.

It is important to understand that not all of the above steps may be necessary in all risk assessments. The OIE methodology makes it clear that if the likelihood of release is negligible for a certain potential hazard, then the risk estimate is automatically negligible and the remaining steps of the risk assessment need not be carried out. The same situation arises where the likelihood of release is non-negligible but the exposure assessment concludes that the likelihood of exposure to susceptible species in the importing country is negligible, or where both release and exposure are non-negligible but the consequences of introduction are concluded to be negligible.

4.3 Risk Management

Risk management consists of

a) *Risk evaluation*: A determination is made as to whether sanitary measures are necessary.

b) *Risk management objectives*: The objectives of the risk management process are defined.

c) *Risk management options*: The options available for managing the risk are identified, and risk reduction effects are considered

d) *Recommendations*: The options available for managing the risk are identified, and risk reduction effects are considered

4.4 Risk Communication

Risk Communication, involves the communication of the results of the risk analysis to all the parties that are affected by its findings and would be involved in the implementation of the recommendations. Risk communication is not part of this risk analysis. It will be undertaken by MAF using their standard procedures for consultation with interested parties and public consultation.

5.0 CLEARANCE TO ENTER NEW ZEALAND

To import a biological product an importer must have a valid import permit issued by MAF. The import permit may document specific restrictions that apply to the particular importation. According to the conditions specified on their import permits, biological products, microorganisms or viable cells will be eligible for biosecurity clearance or directed to a registered transitional facility operating to MAF Standard 154.02.17: *Standard for Transitional Facilities for Biological Products.*

6.0 PART 1: BIOLOGICAL PRODUCTS

6.1 General considerations

The number of products that can be classified as biological products is vast. A single company (Sigma- Aldrich) manufactures over 40,000 products. Several other companies also produce vast numbers of products. These companies do not provide detailed information about their manufacturing processes in their catalogues. Therefore, individual consideration of products is not possible. Auditing of the companies and their manufacturing methods is also not possible. In this risk analysis and in subsequent writing of IHSs and issuing of import permits judgements may have to be made on the basis of general principles. The following general principles should be considered:

- No biological product is in itself a risk factor because they are not living organisms capable of reproducing themselves or continuously producing a harmful product.
- Biological products only become risks to biosecurity if they are contaminated with a viable agent. The likelihood that this will occur is negligible unless the product is produced from a pathogenic organism or from an animal or plant that is infected with a pathogenic organism or an organism that would be classified as a risk good in New Zealand.
- Products derived from non-pathogenic organisms or organisms that are not harmful to the environment are of no concern, even if contaminated by viable source organisms. This generally also applies to products derived from harmless vector organisms expressing proteins coded for by cloned genes of other organisms. An exception would be a case where the vector contains a cloned gene for a harmful product such as a toxin or a virulence factor that could render the harmless vector virulent.
- Except in rare cases only healthy animals and plants that are not showing any signs of disease and are kept in a hygienic manner would be used for production of biological products. This cannot be individually verified for products listed in extensive product catalogues. However, in developed countries manufacturer's operations are audited by agencies that are concerned with biosecurity and animal welfare. It is also in the company's best interests to ensure that donor animals remain healthy and plants are not diseased.
- In infectious diseases of animals, other than a minority of diseases in which a carrier state occurs, animals are infectious for only short periods of time during the course of the disease. After recovery their tissues are no longer infected with the disease causing organism. The likelihood of an apparently healthy animal being used for production of biological products during the critical period when it is infectious is very low. Therefore, for many of the diseases of concern the likelihood of biological products being contaminated with pathogens derived from the donor animal is very low. However, in

some diseases such as bovine viral diarrhoea, apparently healthy animals can be chronic carriers of infectious agents.

- Many biological products sold by reputable companies are highly purified products. The purification procedures usually involve steps that will eliminate contaminating organisms see Section 6.2. The likelihood that a highly purified product sold in small quantities will be infected with a pathogenic organism is low.
- Highly purified and expensive products will be used carefully and sparingly by the importers. They will be safely disposed of after use and the risks involved in their use are negligible.
- Ultimately the reputation of the manufacturer of the products and their descriptions of the products is a valuable indicator of quality and safety

6.2 Product purification processes

Many biological products are purified from such source material as animal or plant tissues (including blood), eggs or products derived from microorganisms. The most common products derived from these sources are proteins (particularly enzymes).

An initial extraction process in which the product is extracted into a solution that is clarified by filtration or centrifugation will generally remove most or all contaminating organisms. Many subsequent procedures used to purify molecules of interest are highly specific and will result in the separation of these molecules from any contaminating microorganisms. Papers on the internet from Pierce Biotechnology Incorporated (Sections on Technical Information and Pathway) describe some of the common methods used to purify proteins (Pierce- Biotechnology 2002). Common methods used for purification include:

- Solvent precipitation by strong solvents, particularly by high concentrations of alcohol and acetone, inactivates most microorganisms and viruses e.g. alcohol at 75% or acetone at 10% inactivates SARS virus and smallpox virus is inactivated by acetone fixation (CDC. 2004).
- Salt precipitation especially by ammonium sulphate is also commonly used in the purification of proteins but is less likely to destroy microbes than solvents, and microorganisms could co-precipitate with the proteins of interest at these steps.
- Gel filtration and ultrafiltration procedures separate molecules based on their molecular size. Since even viruses are many times larger than biological molecules such as protein molecules they are likely to be separated from them. Filtration through membranes, with pores of 0.22µm will remove bacteria and other microorganisms other than viruses and mollicutes. Some filters are available that remove many viruses (Pall-Corporation 2004; Oshima et al 1996), but these are only used in the manufacture of a few specific products.

- Ion exchange chromatography separations are based on the charge characteristics of the molecules at different pH and ion concentrations. Since the charge characteristics of microorganisms are unlikely to closely match those of most biological molecules that are being purified, separation from microbes may occur at these steps.
- High pressure liquid chromatography (HPLC) methods are also based on molecular charge characteristics, non-specific affinity characteristics or molecular size.
- Affinity chromatography is based on highly specific interactions between the molecule being purified and a specific ligand such as an antibody or a specific acceptor molecule. These separations are likely to be highly specific for the molecule being purified and separation from microorganisms is likely.
- Hydrophobic molecules such as lipids are often extracted in solvents such as chloroform, ether, acetone etc. The extracted products are unlikely to contain viable microorganisms and viruses.
- Small molecular weight products (MW<1000) are usually efficiently separated from microorganisms and purified small molecules can be assumed to be safe.
- Molecules that have been chemically synthesised or purified by crystallisation can be assumed to be free from extraneous organisms.
- Some proteins are produced by expression of the gene in a cloned vector such as *E coli* e.g. "Platelet derived growth factor – BB human" (Sigma catalogue). These products are safe because the *E coli* strain is non-pathogenic and is the only possible source of microbial contamination.

Some typical examples of protein purification procedures are given for the purification of antibodies (Stec et al 2004), enzymes (Wang and Ng 2004), antifungal peptides (Wong 2003) and virus particles (Kramberger et al 2004).

If a biological molecule is present in a tissue at a concentration of 0.01%, it must be purified 9,000 times to produce a product of 90% purity. To achieve this degree of purification 99.99% of the contaminating material must be removed. Since in practice purification of a desired molecule never results in 100 % recovery of the molecule more than 99.99% of the unwanted material in the original preparations will have been removed in the purification procedure. These figures are only indicative of the minimal likely removal of contaminating organisms. In most procedures all or the bulk of the contaminating organisms are likely to be removed in the initial extraction steps or at other steps in the process.

The likelihood of contamination of highly purified products is generally low. However, viral contamination of purified human blood products is known to occur in products produced by older methods of purification and where efficient methods for the clearance of viruses have not been used (Franchini et al 2002; Franchini et al 2004; Hayashi et al 2003). Contamination of porcine derived commercial pepsin with circovirus 2 viral DNA, but not viable organism, has been described (Fenaux et al 2004).

6.3 Preliminary hazard list for biological products

The first step in this risk analysis is the identification of potential hazards. A preliminary hazard list was constructed and each class of product in the preliminary hazard list was considered individually. Products considered to be potential hazards were then examined using the subsequent steps in the risk analysis process.

The scope of what should be investigated in a risk analysis for biological products was previously decided by MAF (*MAF scope list*). It was as follows:

- Microorganisms for laboratory use
- Cell lines
- Culture media (with animal tissue extracts and additives)
- Molecular biological products
  - nucleic acids
  - plasmids
  - restriction enzymes
- Tissues and tissue extracts
  - crude tissue extracts
  - biochemicals
  - other proteins
- Egg extracts
  - proteins
  - lecithins
  - others
- Blood and blood products
  - sera
  - antisera
  - plasma
  - hormones
  - albumens
  - globulins
  - antibodies
- Test kits
- Animo acids

- Products derived from microbial fermentation
  - mostly proteins
  - antimicrobials for media
  - alcohols, esters

To produce a preliminary hazard list for biological products the "MAF scope list" was modified as follows:

- "Microorganisms for laboratory use" was removed from the list as they are covered in Part 2 (microorganisms) of this risk analysis.
- Cell lines were removed from the list because they are covered in Part 3 (Living cells derived from higher animals or plants) of this risk analysis.
- The culture media category was broadened to include culture media used for culture of plant microorganisms.
- "Molecular biological products" was changed to "products used for genetic modification of organisms" to more specifically reflect the products of concern. The sub-category "Nucleic acids" was changed to "Polynucleotide sequences". Plasmids, cosmids, phages, transposable elements and viruses that contain contained in any organisms or cultured cells are considered to be living organisms and are therefore not included in the preliminary hazard list for biological products. However, some discussion on these elements is necessary and is included in the document.
- The category "Tissues and tissue extracts" has been simplified to contain no sub-categories.
- "Egg extracts" was simplified to a single category with no specified sub-categories
- Blood products has been simplified to a single category since all the risks relate to the starting product which is blood and are similar for all biological products derived from blood.

The Preliminary Hazard list for biological products is given below:

- Culture media
- Products used for genetic modification of organisms.
  - Polynucleotides.
  - Restriction endonucleases.
- Products derived from animal or plant tissues.
- Products derived from eggs.
- Products derived from blood.
- Test kits

- Amino acids

- Products derived from microbes (fermentation and culture)

    - Proteins
    - Antimicrobials
    - Small molecular weight products - alcohols, esters etc

Each of the products in the preliminary hazard list was then analysed (Hazard Identification Sections) and potential hazards were subjected to a full risk analysis.

6.4 Culture media

6.4.1 Hazard identification

6.4.1.1 Agents of concern

All exotic animal and plant pathogens that could contaminate imported microbiological or cell culture medium or imported components of culture media that are used to make media.

6.4.1.2 General considerations

Culture media may contain amino acids or small peptides derived from animal or plant tissues. However, for the purposes of this risk analysis culture media that contain whole serum, blood, or animal or plant tissues that have not been sterilised are not classified as culture media but according to the risk goods they contain e.g. a compounded medium containing whole serum is considered in the section relating to blood products.

Vast numbers of culture media are used by microbiologists, so that individual consideration of media is not possible. Some simple and easily accessible references describing the nutritional requirements of microorganisms and preparation of media include (Eddleman 1999; Lindquist 2004; Renfroe 1998; Reynolds 2004).

The main ingredients found in microbiological media are:

- **Carbon sources.** The most common carbon sources are simple sugars especially glucose but can be complex carbohydrates including starch and other polysaccharides. A great number of other carbon sources are included particularly in special media used to test the fermentation capabilities for the identification of species of organisms. Carbon sources such as glucose are derived from plant sources and are extracted and purified in a manner that would exclude pathogenic microorganisms. Sucrose and glucose are commonly imported in large quantities as human foodstuffs. The processes of extraction and purification are likely to have eliminated contaminating microorganisms and viruses. In addition the ingredients used in media are always sterilised before or during the process of manufacturing the medium. The likelihood of contamination of carbon sources used in media production is remote.

- **Nitrogen sources.** Nitrogen sources vary according to the type of microorganism being cultured, from simple nitrogen containing chemicals

such as nitrates and ammonia to amino acids, peptides and complex proteins. Compounds commonly imported as constituents of complete media or as products used for formulating media are digests of proteins called peptones. Peptones contain a variety of peptides and amino acids made by acid hydrolysis or enzymatic digestion of proteins sourced from animal or plant proteins such as meat, or milk proteins, soy proteins, gelatine or yeast. Enzymes most commonly used for digestion are trypsin or pancreatic extracts. Similar products are used for food flavouring. These products are widely used and easily sterilised. The likelihood that they will be contaminated with pathogens is remote.

- **Vitamins and minerals.** Some media contain added vitamins and minerals. A common source of vitamins for culture media is yeast extracts. As these are derived from cultivated yeast they are in principle free from animal and plant pathogens. For some media more exact supplementation with purified vitamins is required. Sources of purified vitamins are similar to those imported as human food supplements and pharmaceuticals and are safe to import. Minerals are added as required for a particular medium. They are added to media in the form of simple salts of minerals such as iron, magnesium, cobalt etc. Vitamins and minerals (with the exception of vitamin C) are easily heat sterilisable. They are not a biosecurity risk.

- **Solidifying agents.** Agar is the most commonly used agent for solidifying medium. It is an inert polysaccharide substance that is extracted from kelp, and can therefore be assumed to be free from animal and plant pathogens. Gelatin is also sometimes used as a solidifying agent. It is prepared by heat extraction from animal tissues that contain collagen (traditionally hides and hooves). The acid or alkaline partial hydrolysis method of production and subsequent sterilisation involved in the production of gelatin (Anonymous. undated) renders the product safe for oral consumption and cosmetic use.

- **Blood and serum.** Blood and serum is added to some media. However, Blood is usually added as a fresh sterilely collected, unprocessed product and is not imported but sourced from local animals. Serum may be from local animals or purchased from manufacturers. Serum products such as foetal calf serum constitute a distinct risk of being contaminated with viruses. Foetal calf serum or other blood products are classified as blood products not media ingredients. For the purposes of this analysis they have been considered in Section 6.9.

- **Other ingredients.** Other ingredients required for specialised media include a large variety of growth factors and nutrients or inhibitors such as antibiotics or bile salts to prevent growth of contaminants. These products are usually added as carefully measured amounts of the purified ingredient and are sterile or sterilisable and purified and do not constitute a biosecurity hazard.

6.4.1.3 Conclusion

Although it is considered to be unlikely, some ingredients of imported culture media could be contaminated with infectious pathogens. Culture media are therefore considered to be potential hazards for this analysis.

6.4.2 Risk Assessment

6.4.2.1 Release assessment

Formulated media and media ingredients that do not contain whole blood or serum or animal or plant proteins will be free from infectious pathogens. Media that contain potentially harmful animal or plant products are considered under the appropriate sections of this risk analysis. In addition when imported medium or medium ingredients are used in a laboratory they are sterilized by autoclaving or filtration before or after being constituted into a complete medium, by the addition of water and possibly other components. Therefore the likelihood of release of infectious agents in microbiological media is negligible.

6.4.2.2 Risk estimation

Since the release assessment is estimated to be negligible, under the method used for this risk analysis (Section 4.2) risk is considered to be negligible.

6.4.3 Risk management

6.4.3.1 Risk Evaluation

Because risk has been estimated to be negligible, according to the methodology used in this analysis (Section 4.2), no risk management measures are necessary. Therefore media products are eligible for biosecurity clearance and do not have to be kept in a transitional facility.

6.5 Polynucleotides

6.5.1 Hazard identification:

6.5.1.1 Agents of concern:

Imported polynucleotide material.

6.5.1.2 General considerations:

Modern technology allows DNA and RNA that specify the sequence of particular proteins (gene product) to be ligated into the genome of many (theoretically all) organisms. Insertion into a host genome can be achieved in such a way that it is followed by expression of the gene product. This technology is commonly used by scientists for a very wide range of applications. However, in New Zealand the HSNO Act makes the ligation of any imported DNA or RNA into a host organism illegal unless the specific application has been approved by ERMA.

Gene sequences can be imported as purified DNA or RNA or as polynucleotide sequences that have been ligated into vector organisms or their plasmids, phages etc. Gene sequences that have been cloned into live hosts are considered to be part of those live organisms. Therefore host organisms such as, viruses, phages and cell cultures and any cloned gene sequences they contain are living organisms, not biological products and are considered in Part 2 of this risk analysis. Purified DNA or RNA not contained in any host is not a self replicating organism and is therefore a biological product. Since it is not capable of self replication it is not in itself a risk good under the Biosecurity Act. The methods used to purify DNA including such methods as extraction in phenol are sufficient to ensure that the purified polynucleotide material will not be contaminated with living source organisms.

6.5.1.3 Conclusions

Purified DNA is not a risk good and the likelihood that it will be contaminated with viable source organisms is negligible. Therefore it is not a potential hazard in this risk analysis.

DNA or RNA contained in living organisms is considered under Part 2 of this risk analysis.

6.6 Restriction enzymes

6.6.1 Hazard identification:

6.6.1.1 Agents of concern:

Harmful organisms that could contaminate preparations of restriction enzymes.

6.6.1.2 General considerations

Restriction enzymes (endonucleases) are used to cleave DNA sequences at specific sites to generate fragments of DNA and the generation of insertion sites, during gene manipulation experiments. Fragments of DNA from such digestions are also used to for analysis of DNA structure and exact identification of species and strains of organisms and for forensic analysis of DNA.

Restriction enzymes are produced by microorganisms as defence tools. They operate by destroying foreign DNA such as the DNA of invading viruses by cutting it at specific sites. The sites, in an organism's own DNA that could be attacked by the enzymes it produces, are protected by methylation of nucleotide residues at the specific cleavage sites. Since their discovery in the 1970's over 3,000 different restriction endonucleases have been purified and characterised (Anonymous 2005b). New enzymes are still being discovered. Restriction enzymes are widely used by workers active in the many fields of biology. An internet site gives lists some 380 types of restriction endonucleases and suppliers (Anonymous 2005c).This listing covers only the products of a few of the producers and suppliers of restriction endonucleases. Restriction enzymes, like all enzymes, are proteins that are unable to replicate spontaneously and are therefore, not in themselves biosecurity hazards. The only concern about their use is that they could be contaminated with pathogenic organisms.

6.6.1.3 Conclusions

Restriction enzymes could be contaminated with pathogenic organisms and for the purposes of this risk analysis are considered to be potential hazards.

6.6.2 Risk analysis

6.6.2.1 Release assessment

Restriction enzymes are produced from cultures of microorganisms. Therefore the source organism for their production could contaminate for the product. The likelihood that contamination of product would occur with organisms other than the source organism is

no more likely than for any other biological or food product that is exposed to a non-sterilised environment, and is therefore, negligible.

The product catalogues of the following 5 major producers of restriction endonucleases were examined:

{http://www.fermentas.com/catalog/re/index.html#REases (Fermentas);

http://www.jenabioscience.com/index.php/367021933cc8eca381e0ef8a20b259e1/1/page/112/1129/- (Jena Bioscience);

http://www.neb.com/nebecomm/products/category1.asp (New England Biolabs);

http://www.sigmaaldrich.com/Area_of_Interest/Life_Science/Molecular_Biology/Cloning_and_Expression/Product_Lines.html#Restriction%20Endonucleases (Sigma-Aldrich):

https://catalog.invitrogen.com/index.cfm?fuseaction=viewCatalog.viewCategories&npc=92&pc=108&nc=108 (Invitrogen)}

It was found that the vast majority of the organisms used for production of the enzymes were non-pathogenic organisms and even if they did contaminate the final product would not represent a biosecurity threat unless they were organisms that are not found in New Zealand and could damage the environment. A few "pathogenic organisms" are used and these are organisms that have a widespread distribution and are often only opportunistic pathogens (e.g. *Proteus vulgaris, Haemophilus influenzae*) or cause common endemic diseases of minor economic importance (e.g. *Moraxella bovis*). None were unwanted or notifiable organisms (Ministry of Agriculture and Forestry 2004). Some companies use non-pathogenic host organisms into which the gene for the production of the enzyme has been cloned. These genetically modified donor organisms (usually strains of *E coli*) are not pathogens and are therefore not a biosecurity threat e.g. of 240 products produced by New England Biologicals, 160 are produced in cloned host organisms (Anonymous 2005b). However, it is not possible to obtain information about all companies and all products therefore the likelihood that a pathogen of concern would be used for production of a restriction enzyme is considered to be very low but non-negligible.

The products sold by various companies are all represented as having been purified from particular donor organisms. Specifics of how they are purified are not given. However, correspondence with several companies (see Appendix 1) elicited some general information that indicated that procedures such as gel chromatography, ion exchange chromatography and affinity chromatography were used. These procedures would ensure that whole live microorganisms were eliminated from the end products. Some companies indicated that the products were free from contaminating DNA (and therefore also from organisms). Even when freedom from DNA was not indicated directly, it can be inferred because if there was contaminating DNA it would interfere with the results of specificity tests for which data is given in suppliers catalogues. The specificity tests are designed to detect minute amounts of contaminating restriction endonucleases. It can be concluded

that the likelihood that restriction endonucleases offered for sale by reputable commercial companies will be contaminated with microorganisms is negligible.

6.6.2.2 Risk estimation

Since release and exposure assessments are both considered to be negligible, under the methods used in this analysis (Section 4 .2), the risk is negligible.

6.6.3 Risk management

6.6.3.1 Risk evaluation

Because risk is considered to be negligible the implementation of risk management measures is not justified.

6.7 Biological products derived from animal or plant tissues

6.7.1 Hazard Identification

6.7.1.1 Agents of concern

Infectious, pathogenic agents and agents capable of damaging the environment that could contaminate products extracted from tissues of animals or plants.

6.7.1.2 General considerations

Products covered in this section may be produced from animal or plant tissues or cultures of animal or plant cells.

The range of products that could be classified as tissue extracts is so wide that it is not possible to consider single products or even to make a listing of products that are of concern .For this reason general principles about the safety of such products must be applied. Important principles that should be considered have been discussed in Sections 6.1and 6.2. Particular note should be taken of whether the product has been sterilised by one of the following processes.

- *Filtration*: Sterilisation by filtration (membranes with pore sizes 0.2 μm) will remove microorganism other than viruses. The European pharmacological convention classified as adequate a filtration method for bacteria moulds and yeasts the delivers a sterility assurance level (SAL) of 10^{-6} i.e. a probability of not more than one viable microorganims in 10^{6} sterilised items of the final product (Committee-for-Proprietary-Medicinal-Products 1996).Methods are also available for the removal of viruses and even for prions. However these methods seldom result in the removal of all virus or prion particles and their efficacy is judged by the reduction in viral titres they achieve (Committee-for-Proprietary-Medicinal-Products 1996).
- *Irradiation*: Gamma γ irradiation treatment will destroy viruses as well as other organisms (Committee-for-Proprietary-Medicinal-Products 2003). However, it is rarely used on biological products offered for sale.
- *Heat treatment*: Heat treatment to sufficient temperature and time will destroy all organisms of concern but since it denaturises many biological products it is not commonly used.
- *Chemical treatments*: Treatments such as solvent/detergent treatment (Korneyeva et al 2002; Remington et al 2004) and treatment with caprylate (octanoic acid) (Dichtelmuller et al 2002; Korneyeva et al 2002; Remington et al 2004) have been used to destroy viruses especially in blood products for medicinal use in humans. However, reference to the use of such methods was not found in catalogues of biological products.

More details on methods used to remove viruses from blood products are given in Section 6.9. The technical problems involved in removing viruses and prions from biological products have not been completely solved. Inactivation by heat or harsh chemicals may inactivate the biological product. Removal by filtration may remove large molecular weight proteins. Clearance data for removal of viruses is reported in log reductions of the numbers of viruses and this implies that reduction to nil may not have been achieved (Aranha-Creado et al 2005; Cameron-Smith et al 2000; Nader 2005; Oshima et al 1996); (Johnston et al 2000b). Although complete removal of all viruses cannot be guaranteed the good record of the safe use of biological products in human patients in recent times indicates that modern methods have achieved a high degree of efficiency. Guidelines for the clearance of viruses and the validation steps required are given by both USA (Committee-for-Proprietary-Medicinal-Products 1996; FDA 1998) and European authorities (Committee-for-Proprietary-Medicinal-Products, 1996), for biologicals for use in humans. However, these methods are usually applied to products for medicinal use but data on sterilisation is seldom given in catalogues of biological products for laboratory use.

The source of the product is also important. If the donor animal or plant from which the product has been derived is free from infectious pathogens the product will be free from pathogens. Unfortunately detailed information is seldom available but it can be assumed that if the product was produced in a developed country under accepted systems of quality assurance it will be free from those disease agents that are not known to occur in that country.

The extent of purification of the product is an important factor (Section 6.2).

A modern method of producing safe animal proteins is to clone the animal or plant gene into a suitable host system. Since the host is not a pathogen the product produced from it is safe and free from contaminating animal pathogens. An example is the cloning of the gene for trypsin into corn and the production of a safe purified product from the corn (Kao and Caple Undated).

Biological products produced for use as medicinal products are more stringently controlled than products for laboratory use and more information is available about these products. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) set up by the regulatory authorities of the USA, Japan and Europe has produced a guideline on “Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin” (ICH. 1998). Although the principles in this paper could be usefully used to ensure the safety of biological products for laboratory use there is no indication in the catalogues of biological products that similar principles are used by them in the production of their products. To follow such stringent methods would probably not be cost effective for the production of biological products for laboratory use.

6.7.1.3 Conclusions

The substances included in the category "Tissues and tissue extracts" include a large and variable number of products. Therefore, the general principles of the risks involved in importing the substances should be considered rather than attempting to identify individual products. Since some of the products included in the general category could be contaminated with pathogenic organisms the general category is considered to be a potential hazard in this analysis.

6.7.2 Risk analysis

6.7.2.1 Release assessment

An inspection of catalogues of biological products for laboratories reveals many products that have been extracted from animal or plant sources. Examples are:

Enzymes: Pepsin, trypsin, rennin etc that are produced from tissues of a variety of animals.

Hormones: Insulin, glucagon etc produced from animal and human tissues.

Crude tissue extracts: Liver acetone powder, liver concentrate, lung acetone powder (from a variety of animals), keratin from human skin etc.

Monoclonal antibodies: These antibodies are commonly in the form of mouse ascitic fluid.

Starch: From potatoes or rice

Molecules extracted from tissues: Preparations of cytochromes extracted from heart muscle of various animals.

Actin and myosin: Extracted from muscle of various animals etc.

Fatty acids: Oleic, stearic, linoleic acid etc extracted from plant sources.

Cholic acid: Extracted from animal bile

The above are only a few examples of the many (thousands?) of products that are available.

Catalogues were examined to identify some of the available products. Few products of concern were identified. The vast majority of the products can be considered safe because they:

- are highly purified (>= 90% purity) and/or,
- are produced by harsh methods that will destroy all microorganisms e.g. liver powder is desiccated in acetone, solvent extraction of lipids, harsh chemical methods such as hydrogenation, distillation etc. and/or.
- are produced from animals or plants that do not harbour organisms that are serious biosecurity risks e.g. monoclonal antibodies in the form of ascites fluid from laboratory mice and/or
- the starting source is a product produced by the expression of a cloned gene in a harmless vector.

Products such as lyophilized pituitary extract from bovines are of concern whereas pituitary acetone powder from fish is not. Acetone is commonly used to fix and inactivate smears and tissue sections containing virus (CDC. 2004), it has been shown to be efficient in the inactivation of viruses even at comparatively low concentrations e.g. inactivation of SARS virus by 10% acetone (Anonymous-University-of-Canberra 2005). Therefore tissues dried in acetone will contain no viable microorganisms other than possibly bacterial spores which are of minimal concern in this risk analysis. Lipids including fatty acids are not of concern because the are extracted by solvents and/or, modified by harsh chemical methods involving high pressures and temperatures and are generally not suitable substrates for the growth and preservation of pathogenic organisms

It is not possible to examine all available products and some products cannot be assumed to be safe without knowledge of the details of manufacture. Therefore, it is concluded that the likelihood that biological products sold commercially will contain pathogenic microorganisms is very low but not negligible.

6.7.2.2 Exposure assessment

The transmission of a pathogenic organism from a facility where the contaminated product is being used would only be possible if:

- The product is transferred from the laboratory to somewhere outside the laboratory and that animals or plants are then exposed to it. For example if it was disposed of as unsterilised general waste, found its way to a municipal landfill and was consumed by rats, seagulls or other animals or contaminated plants.

- It is injected into or administered to a live animal or used to infect plants, outside of the facility where it is being held and it is then was transmitted to "in contact animals or plants". This could be the case if an imported product is used for the manufacture of a product that is intended to be used directly in animals e.g. a medium containing serum might be used in the preparation of embryos intended for transplanting into New Zealand animals.

- The product is injected into or otherwise used in animals or plants in non-secure facilities and is then transmitted in waste water, urine, faeces etc or by aerosol to animals or plants outside the laboratory.

- The contaminating organism is a zoonotic organism and is transmitted to a laboratory worker inside the laboratory.

The likelihood of any of these events happening and resulting in contamination of animals plants, people or the environment, is negligible if the products are being used in a laboratory that is a transitional facility, but non-negligible if it is not. It is of particular concern if the imported product is intended to be used in animals or plants.

6.7.2.3 Consequence assessment

The consequences of a pathogenic organism being transmitted to animals, humans or plants would depend on the organism concerned and could vary from negligible to catastrophic. In view of the nature of the products sold for laboratory use and unlikelihood that they would contain agents of serious animal, human or plant diseases the consequences are likely to be low but are non-negligible.

6.7.2.4 Risk evaluation

Since release, exposure and consequence assessments are all assessed to be non-negligible risk is non-negligible

6.7.3 Risk management

6.7.3.1 Risk evaluation

Risk is non-negligible and therefore risk management procedures should be implemented to reduce the risk to an acceptable level.

6.7.3.2 Risk management objective

The objective is to reduce to the lowest possible level the likelihood of introducing unwanted or notifiable pathogens (Ministry of Agriculture and Forestry 2004) and to prevent the use of unsafe products in animals or plants.

6.7.3.3 Risk management options

The number of products involved is too great to consider drawing up guidelines for each individual product.

For the vast majority of the products intended for use in laboratories risk is negligible. Control of risk goods could be achieved by co-operation between MAF and companies that supply and manufacture biological products. Products could be classified as risk or non-risk goods. Risk goods could be defined as non-purified products derived from animals or plant tissues that are manufactured in a manner that does not inactivate or remove contaminating microorganisms. Non-risk goods could be "the rest" and could include highly purified, sterilised and chemically synthesized products produced from animal or plant tissues. MAF could then issue a permit for all non-risk goods in catalogues of suppliers of biological products and these could be imported and sold by the importer without restrictions. A permit for the importation of those products considered to be risk goods could specify that the supplier of biological products could only import the identified risk goods into a transitional facility. These products could then only be sold to laboratories that are registered transitional facilities. Products sold to these laboratories could contain a warning that under the terms of their registration as transitional facilities they are not permitted to inject or otherwise use risk goods in animals or plants unless special clearance has been obtained from MAF.

Alternatively separate IHSs could be written for specified catalogues. These IHSs could specify those products in the catalogues that are considered to be risk goods and the conditions under which they could be imported.

Individuals wishing to import a product directly (not through a supplier of biological products holding a permit to import the product) could apply individually for an import permit and supply details of manufacture, purity and source from which the product was derived. A "Permit to import" could then include restrictions applicable to the use of the products.

The importation of products that are intended to be used in animals or plants represents a special case requiring strict control. The importation of these products could be allowed subject to the issuing of an import permit for each batch of product imported. The import permit could specify the specific conditions for importation such as definition of the source of the products (animal species, health status of the herd or flock and country of origin etc.) and tests (specified by MAF) for the presence of particular infectious agents that have been done on each batch of product.

6.7.3.4 Recommendations

i. Companies involved in selling biological products to laboratories should in collaboration with MAF classify all products in their catalogues as risk or non-risk goods Risk goods should be those produced from animal or plant tissue that are manufactured in a manner that does not inactivate or remove contaminating microorganisms. Non-risk goods should be "the rest" and will probably consist of most products in the catalogue. They should include highly purified and inactivated synthesized products produced from animal or plant tissues.

ii. MAF should provide a blanket "Permit to import" for all non-risk goods in the catalogue. Non- risk goods should be traded without restrictions.

iii. a. A separate permit could be issued for risk goods that can be imported under specified conditions. The permit should stipulate that the importer/supplier of biological products should keep these products in a transitional facility and only on-sell them to laboratories that are transitional facilities or;

b. Alternatively separate IHSs should be written for specified catalogues. These IHSs should specify which products in the catalogues are considered to be risk products and have to be imported under a permit.

iv Risk goods sold by suppliers of biological products to facilities that are transitional facilities should contain a warning that the product must be kept in a transitional facility and not be injected or otherwise used in animals or plants unless special clearance has been obtained from MAF for a particular case.

v. Individuals wishing to import products directly (not through a supplier who has a "Permit to import") and laboratories that are not transitional facilities wishing to import risk goods should apply individually for import permits and supply details of manufacture, purity and source from which the product was derived. A "Permit to import" should, where applicable include restrictions applying to the storage and the use of the products.

vi. Each batch of product intended for use in animals or plants should be imported with a separate import permit.

vii. Applications for a "Permit to import" a product for use in animals should be accompanied by any information required by MAF. Information requested could include information on the health status of the donor animals and their herds and countries of origin, methods of manufacture, auditing of the manufacture process in the country of origin, contents of the compounded product and tests for freedom from infectious agents.

viii. Permits for importation of products for use in animals or plants should contain such restrictions and conditions as are appropriate to the particular case and product. They should generally as a minimum include a requirement for provision of documentation of the results of the tests that are required by MAF. These tests should be done by an independent, competent tester not by the manufacturer or supplier.

6.8 Products derived from eggs

6.8.1 Hazard identification

6.8.1.1 Agents of concern

Avian pathogens and *Salmonella* spp. that could contaminate products derived from eggs.

6.8.1.2 General considerations

Fertile hatching eggs are not biological products but living organisms and are not part of this analysis.

Chicken eggs are unlikely to be contaminated with pathogens that can affect animals other than birds. Thirty disease agents that can be carried on or in eggs were identified in a risk analysis for egg powders (Pharo 2003). All organisms in the list are strictly avian pathogens except for *Salmonella* spp. that affect humans and animals and avian influenza. Previously avian influenza virus was regarded as predominantly an avian pathogen but recently direct transmission from birds to humans has occurred although human to human transmission has not been confirmed (CDC. 2005). Salmonellosis is the most common disease transmitted by eggs. It has been estimated that in the USA in 2000, 182,060 $\pm$ 81, 535 (5^{th} percentile) cases of illness caused by *Salmonella enteritidis* were associated with eggs (Schroeder et al 2005).

In the United States, from an annual production of 89.1 billion eggs, 1.6 billion dozen (21%) are broken to produce 212,849,000 pounds of edible liquid and 19,190, 000 pounds of inedible liquid (USDA. 2004). Therefore less than 2.0% of eggs are used to produce inedible products and only some unspecified amount of these eggs is used to produce biological products.

6.8.1.3 Conclusion.

Chicken eggs can carry the infectious agents of 30 diseases of birds and salmonellosis and possibly avian influenza that can infect animals and man. Therefore, biological products derived from eggs are considered potential hazards for the purposes of this analysis.

6.8.2 Risk assessment

6.8.2.1 Release assessment

Some biological products derived from eggs that are found in manufacturers catalogues include: Lecithin, choline, lutein, antibodies produced in eggs, egg yolk emulsions, egg yolks, egg white, cystatin, avidin, conalbumin, lysozyme, eggwhites, egg white powder,

Additional proteins found in egg white have included: ovalbumen, ovotransferrin, ovomucoid, globulins, ovoinhibitor, ovoglycoprotein, ovoflavoprotein, ovomacroglobulin (Froning 1998). Lecithin and choline are widely used as nutritional or health supplements and are freely available outside of laboratories. Lecithin and choline are popular and widely used health supplements.

The considerations that apply to the assessment of the likelihood of release of infectious agents from biological products derived from tissues (Section 6.7) also apply to products derived from eggs. Most products are purified product and are therefore unlikely to be contaminated. The methods of manufacture of crude products, such as egg yolks or egg whites, are not described in manufacturers catalogues. However, it is unlikely that anyone in New Zealand would import egg yolks or egg whites at laboratory reagent prices when they could buy eggs locally. Those products that are sold for use in culture media (e.g. egg yolk emulsions) will not be a threat (See 6.4) as they will be sterile and will be re-sterilised before use in media and again after use before disposal.

It is unlikely that biological products produced from eggs would contain unwanted or notifiable organisms. However, since there are large numbers of products and the production methods are not specified the risk is assessed to be non-negligible.

6.8.2.2 Exposure assessment

Since organisms contained in eggs will not be infectious to plants only the exposure of birds and possibly humans or animals is of concern.

The organisms of concern are 30 infectious agents of birds. Of these 30 organisms only some *Salmonella* spp. and avian influenza virus are zoonotic. Imported products that are derived from eggs are unlikely to contain these organisms but even if they do they would not be transmitted to birds unless birds were injected with them or otherwise exposed to them. Most biological products produced from eggs are likely to be used in laboratories many of which will be registered as transitional facilities. These registered laboratories are prohibited from using biological products in live animals (MAF Standard 154.02.17: *Standard for transitional facilities for biological products*) without the approval of the Chief Technical Officer. In transitional facilities any egg products used for manufacture of culture media would be sterilized in the process of making the medium and again after use. In these laboratories laboratory animals would not be exposed to these products and therefore no infectious agents would be multiplied in the laboratory and the only concern would be the direct infection of laboratory workers with zoonotic agents.

Salmonella spp. of concern in eggs are unlikely to occur in purified products. S*almonella pullorum*, *Salmonella gallinarum* and *Salmonella arizonae* are specific avian pathogens and will not infect laboratory workers. The *Salmonella* spp. most commonly associated

with eggs are *Salmonella enteritidis* and *Salmonella typhimurium.* *Salmonella enteritidis* and *Salmonella typhimurium* are both endemic New Zealand organisms. In 2004 over 140 isolations of *Salmonella enteritidis*, including 11 of phage type 4 and over 900 of *Salmonella typhimurium* were made from human and animal sources (ESR 2004). *Salmonella enteritidis* can be transmitted from infected hens in their eggs (CDC. 2003) but other *Salmonella* spp. are more likely to be transmitted as surface contaminants on the shells of eggs. Since the cleaning of egg shells prior to manufacture of biological products from eggs would be standard practice the likelihood of contamination of products with *Salmonella* spp. that contaminate the surface of the shells would be negligible. In any case at least 77 serotypes of *Salmonella spp.* were isolated in New Zealand in 2004 (ESR 2004) and the likelihood that a new species would be introduced by biological products derived from eggs and transmitted to a laboratory worker during the carrying out of ordinary laboratory procedures is considered to be negligible. Some strains of *Salmonella* such as *Salmonella gallinarum*, *Salmonella pullorum* and *Salmonella arizonae* are specific for poultry and are unlikely to infect humans.

Most avian influenza strains are not zoonotic. However, an H5N1 strain has recently caused at least 55 cases in humans resulting in 42 deaths, in South East Asia. In 1997 an outbreak in Hong Kong resulted in 18 human cases and 6 deaths (CDC. 2005). However, spread of the disease has only occurred by direct bird to human contact. Human to human transmission has not been confirmed (CDC. 2005). Cats, tigers and leopards are susceptible and pigs have also been infected (CDC. 2005). However transmission to all cat species appears to have occurred when the cats ate infected chickens (Anonymous 2004b) or were experimentally exposed to the infection (Marshall 2004) . Therefore, the likelihood of spread from imported biological products, in which the probability of infection with the H5N1 strain is in any case remote, to animals or man in laboratories is negligible.

The likelihood of transmission of avian influenza or *Salmonella* spp. to laboratory workers is negligible and biological products will not be used in birds or other animals in laboratories that are transitional facilities. Therefore the likelihood of introduction of an unwanted or notifiable organism in biological products derived from eggs is negligible in laboratories registered as transitional facilities.

In facilities in which there is no control over the use of biological products the infection of birds is a remote possibility. Therefore in these laboratories the likelihood of exposure is low but non-negligible.

It is unlikely that any applications will be received from importers that wish to use products derived from eggs directly in birds. However, in such cases the likelihood of exposure of birds to infectious agents is non-negligible.

6.8.2.3 Consequence assessment

The consequences of exposure would be dependant on the organism in the particular case. Since organisms identified as being transmissible by eggs could cause disease in

birds or humans the consequences are not negligible. The likelihood of introduction of new bird diseases is very low but non-negligible.

6.8.2.4 Risk estimation

The release, exposure and consequence assessments were considered to be non-negligible when products are used in facilities that are not transitional facilities or are to be used directly in birds. For transitional facilities risk is assessed to be negligible.

6.8.3 Risk Management

6.8.3.1 Risk evaluation

Risk is considered to be non-negligible, for products that will be used in facilities that are not transitional facilities or for products that will be used directly in birds. In these cases risk management measures are justified to reduce the level of risk to an acceptable level.

6.8.3.2 Risk management objective

The objective is to reduce to the lowest possible level the likelihood of introducing unwanted or notifiable pathogens (Ministry of Agriculture and Forestry 2004) and to prevent the release of any pathogens contained in biological products.

6.8.3.3 Risk management options

Control of risk goods could be achieved by co-operation between MAF and companies that supply and manufacture biological products. Products could be classified as risk or non-risk goods. Risk goods could be defined as non-purified products derived from eggs that are manufactured in a manner that does not inactivate or remove contaminating microorganisms. Non-risk goods could be "the rest" and could include highly purified, sterilised and chemically synthesized products produced from animal or plant tissues. MAF could then issue a permit for all non risk goods in a catalogue and these could be imported and sold by the importer without restrictions. A permit for the importation of those products considered to be risk goods could specify that the importer could only import the identified risk goods into a transitional facility. These products could then only be sold to laboratories that are registered transitional facilities. Products sold to these laboratories could contain a warning that under the terms of their registration as transitional facilities they are not permitted to inject or otherwise use risk goods in animals or plants unless special clearance has been obtained from MAF.

Alternatively separate IHSs could be written for specified catalogues. These IHSs could specify those products in the catalogues that are considered to be risk goods and the conditions under which they could be imported.

Individuals wishing to import a product directly (not through an importer /supplier holding a permit to import the product) could apply individually for an import permit and supply details of manufacture, purity and source from which the product was derived. A "Permit to import" could then include restrictions applying to the use of the products.

The importation of products that are intended to be used in birds represents a special case requiring strict control. The importation of these products could be allowed subject to the issuing of an import permit for each batch of product imported. The import permit could specify the specific conditions for importation such as definition of the source of the products (animal species, health status of the herd or flock and country of origin etc.) and tests for particular infectious agents that have been done on the batch of product).

6.7.3.4 Recommendations

i. Companies involved in selling to laboratories should in collaboration with MAF classify all products in their catalogues as risk or non-risk goods. Risk goods should be those produced from eggs that are manufactured in a manner that does not inactivate or remove contaminating microorganisms. Non-risk goods should be "the rest" and will probably consist of most products in the catalogue. They should include highly purified and inactivated products produced from animal or plant tissues.

ii. MAF should provide a blanket "Permit to import" for all non-risk goods in the catalogue. Non- risk goods should be traded without restrictions.

iii. a.. Individual permits should be issued for risk goods that can be imported under specified conditions. The permit should stipulate that the importer should keep these products in a transitional facility and only on-sell them to laboratories that are registered as transitional facilities or:

b. Alternatively separate IHSs should be written for specified catalogues. These IHSs should specify which products in the catalogues are considered to be risk products and have to be imported under a permit.

iv. Risk goods sold by suppliers of biological products to laboratories that are registered as transitional facilities should contain a warning that the product must be kept in a transitional facility and not be injected or otherwise used in animals or plants unless special clearance has been obtained from MAF for a particular case.

v. Individuals wishing to import products directly (not through a registered importer/supplier) and unregistered laboratories wishing to import risk goods should apply individually for import permits and supply details of manufacture, purity and source from which the product was derived. A "Permit to import" would then include restrictions applying to the storage and the use of the products.

vi Each batch of product intended for use in animals or plants should be imported with a separate import permit.

vii Applications for a "Permit to Import" products for use in animals should be accompanied by any such information as MAF may require. Information requested could include information on the health status of the donor animals and their herds and countries of origin, methods of manufacture, auditing of the manufacture process in the country of origin, contents of the compounded product and tests for freedom from infectious agents.

viii Permits for importation of products for use in animals should contain such restrictions and conditions as are appropriate to the particular case and product. They should always include a requirement for provision of documentation of the results of the tests required by MAF for the batch of product. Testing should be done by independent competent testers not by the manufacturer or supplier.

6.9 Products derived from blood

6.9.1 Hazard identification

6.9.1.1 Agents of concern

All animal and human pathogens that could be transmitted by biological products derived from blood.

6.9.1.2 General considerations

Blood products include the following:

- serum
- antisera
- plasma
- hormones
- albumins
- globulins
- antibodies
- proteins derived from blood

Since all the products are derived from a common source the risks involved in importing non-purified products are similar. Serum, antisera and plasma are for all practical purposes the same non-purified products and the likelihood that they will be contaminated with microorganisms depends on the state of health and species of the donor animals. Animals infected with many diseases will only have the infectious agent in their bloodstreams during the acute phase of the infection. Thereafter, they will be immune and their blood will be free from the infection. However, for some diseases infected animals remain chronically infected e.g. when naïve pregnant cows are infected with bovine viral diarrhoea virus (a pestivirus infection) in the first trimester of gestation, their calves remain chronic carriers of the virus for protracted periods or even for their entire lives (Brownlie 2005; Harkness and Van der Lugt 1994). Sera derived from bovine blood from donors of unknown disease status are likely to be contaminated with pestivirus. The prevalence of bovine virus diarrhoea virus in foetuses is 8-10% where the disease is endemic (Lindberg 2005). Bovine virus diarrhoea virus type 2 (a type exotic to New Zealand) has been isolated from imported bovine foetal calf serum (O' Keefe 2004). Bovine polyoma virus is also a common contaminant of bovine serum (Committee-for-Proprietary-Medicinal-Products 2003; Kappeler et al 1996). Human blood may harbour several viruses including hepatitis A, hepatitis B, hepatitis C, HIV, parvovirus B19 (Committee-for-Proprietary-Medicinal-Products 1996) and hepatitis G virus (Alonso-Rubiano et al 2003). In contrast antisera from laboratory mice or rabbits are of minimal biosecurity risk because rabbit and mouse viruses are not considered to be serious biosecurity risks.

Products that are purified from blood such as plasma proteins (albumin and globulins), hormones and affinity purified antibodies are less likely to be contaminated with microorganisms than non-purified serum and plasma, but in the past several blood products used for treatment of humans are known to have been contaminated with viruses (Franchini et al 2002; Franchini et al 2004; Hayashi et al 2003).There are several methods now commonly used to clear viruses from blood products. Treatments used include pasteurisation (Remington et al 2004), solvent/detergent treatment (Korneyeva et al 2002; Remington et al 2004), treatment with caprylate (octanoic acid) (Dichtelmuller et al 2002; Korneyeva et al 2002; Remington et al 2004), filtration (Aranha-Creado et al 2005; Johnston et al 2000a; Oshima et al 1996; Pall-Corporation 2004), low pH and pepsin treatment (Omar et al 1996) and irradiation (Committee-for-Proprietary-Medicinal-Products 2003; Kurth et al 1999). Products that have not been efficiently treated for removal of viruses may be contaminated with hepatitis G virus (Alonso-Rubiano et al 2003). Despite the considerable progress made no single process is ideal. Heating and irradiation may destroy some of the desirable characteristics of foetal calf serum and proteins, solvent/detergent treatments are only effective for enveloped viruses and filtration methods still require more exhaustive verification and expensive equipment.

The Committee for Proprietary Medicinal Products of the European Agency for the Evaluation of Medicinal Products has issued a "*Note for guidance on the use of bovine serum in the manufacture of human biological medicinal products*" (Committee-for-Proprietary-Medicinal-Products 2003). Testing methods for the quality control of the products are included in the note. However, the use of these methods in the manufacture of biological products is not mentioned in catalogues. Unfortunately manufacturers and suppliers of biological products do not provide any indication that they follow these or similar guidelines in manufacturing their products. Therefore it must be assumed that most biological products are not routinely treated to eliminate viruses, during their manufacture.

Some animal products may be imported with the intention of using them in the manufacture or formulation of products that will be directly used in animals. An example of this is the case of products used in the processing of embryos that are to be transplanted into New Zealand animals. These cases involve high risk.

6.9.1.3 Conclusions

A large number of products are derived from blood. Where the origin and manufacturing procedures are unknown, the likelihood that they may carry infectious pathogens cannot be adequately assessed. For this reason they are classified as potential hazards for this risk analysis.

6.9.2 Risk assessment

6.9.2.1 Release assessment

Foetal calf or other serum is often used in cell culture medium and it is not uncommon that this product is contaminated with pestiviruses such as bovine viral diarrhoea virus. Bovine virus diarrhoea virus type 2, which is a type that is exotic to New Zealand, has been isolated from imported serum (O' Keefe 2004). Serum will be free of microorganisms other than viruses and Mollicutes if it has been filtered (0,2 μm), and will be substantially free of viruses if adequately irradiated or treated by other means or derived from animals of an adequate health status, or sufficiently quality controlled to ensure freedom from virus (Committee-for-Proprietary-Medicinal-Products 2003). However, biological product manufacturer's catalogues seldom give details of manufacture of listed products.

Some antisera are sold as purified gamma globulin fractions. These are commonly produced by precipitation with ammonium sulphate and/or chromatography. Ammonium sulphate precipitation cannot be relied upon to remove viruses. The ability of ion exchange chromatography to remove viruses will vary for different viruses and different chromatography procedures and will be dependant on the capacity of the chromatography column in relation to the amount of product to be purified. Affinity chromatography can be assumed to be highly specific and more likely to separate protein products from viruses.

Some products may have preservatives added to inhibit bacterial growth (phenol, thiomersalate etc.), but these cannot be relied upon to inactivate all microorganims and viruses.

Many other products derived from blood are highly purified by processes that involve several different purification steps; the likelihood that these products will be free from virus is high. In addition the infectious agents of diseases of major economic importance are very unlikely to contaminate blood used as a source material for biological products production. This is because the diseases are rare and often absent from countries that are likely to produce and supply biological products. BVDV 1 and BVDV 2 are the most common contaminants and BVDV 1 is endemic in most parts of the world. Classical swine fever virus which is a pestivirus that can behave in a similar manner to BVDV and would be a disease of concern but it is a very rare disease in developed countries that are likely to be producers of biological products.

In view of the fact that manufacturers do not give adequate guarantees of freedom of blood products from microorganisms and viruses the likelihood that blood products could be contaminated with microorganisms, especially viruses is non-negligible.

6.9.2.2 Exposure assessment

Blood products such as foetal calf serum may be contaminated with viruses. If these products are retained within transitional facilities and inactivated by autoclaving or incineration after use they do not pose a biosecurity threat. In this case they are only a

threat to the quality of the work done within the facility and it is in the facility's interest to ensure that products are adequately quality controlled before use. However, if released from facilities without sterilization they could infect vermin or other animals that were exposed to them and the likelihood of exposure if the products are used in non-approved facilities is non-negligible. Plants and the environment other than wild or feral animals would not be affected by these products.

Blood products that are intended for uses that would involve their direct use in animals represent high risk cases.

6.9.2.3 Consequence assessment.

The consequences of release and exposure depend on the organism concerned and could vary from negligible to catastrophic. The most likely virus to be introduced would be a pestivirus. The introduction of bovine viral diarrhoea type 2 virus into the New Zealand cattle population would represent the introduction of a new economically important disease agent and therefore the consequences are non-negligible.

6.9.2.4 Risk estimation

Because release, exposure and consequence assessments are all non-negligible, according to the methods used in this analysis (Section 4.2) risk is non-negligible.

6.9.3 Risk management

6.9.3.1 Risk evaluation

Since risk is non-negligible, risk management measures should be used to reduce the risk to an acceptable level.

6.9.3.2 Risk management objectives

The objective is to control the introduction and use of blood products in a manner that will ensure that new pathogens are not introduced into the New Zealand environment.

6.9.3.3 Risk management options

The number of products involved is too great to consider drawing up guidelines for each individual product. For the vast majority of the products risk is negligible. Control of risk goods could be achieved by co-operation between MAF and companies that supply and manufacture biological products. Products could be classified as risk or non-risk goods. Risk goods could be non-purified products derived from blood that are manufactured in a manner that does not inactivate or remove contaminating microorganisms. Non-risk goods could be "the rest" and could include highly purified or sterilised products. MAF could issue a permit for all non risk goods in a catalogue and these could be imported and sold by the importer without restrictions. A separate permit for the importation of those

products considered to be risk goods could specify that suppliers of biological products could only import the identified risk goods into transitional facilities. The permit could also specify that these products could only be on-sold to laboratories that are registered transitional facilities. Products sold to these laboratories could contain a warning that under the terms of their registration as transitional facilities they are not permitted to inject or otherwise use risk goods in animals unless special clearance has been obtained from MAF.

Alternatively separate IHSs could be written for specified catalogues. These IHSs could specify those products in the catalogues that are considered to be risk goods and the conditions under which they could be imported

Individuals wishing to import a product directly (not through an importer/supplier holding a permit to import the product) could apply for an import permit and supply details of manufacture, purity and source from which the product was derived. A "Permit to import" could then include restrictions applicable to the use of the products.

The importation of products that are intended to be used in animals or plants represents a special case requiring strict control. The importation of these products could be allowed subject to the issuing of an import permit for each batch of product imported. The import permit could specify the specific conditions for importation such as definition of the source of the products (animal species, health status of the herd or flock and country of origin etc.) and tests for particular infectious agents that have been done on the batch of product).

6.9.3.4 Recommendations

i. Companies involved in selling biological products to laboratories should in collaboration with MAF classify all products in their catalogues as risk or non-risk goods Risk goods should be those produced from blood that are manufactured in a manner that does not inactivate or remove contaminating microorganisms. Non-risk goods should be "the rest" and will probably consist of most products in the catalogue. They should only include highly purified and inactivated products.

ii. MAF should provide a blanket "Permit to import" for all non-risk goods. These products should be imported and traded freely without restrictions.

iii. a. A separate permit should be issued to suppliers of biological products for risk goods. The permit should stipulate that the supplier/importer should keep these products in a transitional facility and only on-sell them to laboratories that are registered as transitional facilities.

b. Alternatively separate IHSs should be written for specified catalogues. These IHSs should specify which products in the catalogues are considered to be risk products and have to be imported under a permit.

iv. Products sold to laboratories should contain a warning that the product must be kept in a transitional facility and should not be injected or otherwise used in animals unless special clearance has been obtained from MAF for a particular case.

v. Individuals wishing to import products directly (not through a registered importer/supplier) should apply individually for import permits and supply details of manufacture, purity and source from which the product was derived. A "Permit to import" would then include restrictions applying to the storage and the use of the products.

vi. Each batch of product intended for use in animals or plants should be imported with a separate import permit.

vii. Applications for a "Permit to Import" products for use in animals should be accompanied by any such information as MAF may require. Information requested could include information on the health status of the donor animals and their herds and countries of origin, methods of manufacture, auditing of the manufacture process in the country of origin, contents of the compounded product and tests for freedom from infectious agents.

viii. Permits for importation of products for use in animals should contain such restrictions and conditions as are appropriate to the particular case and product. They should always include a requirement for provision of documentation of the results of the tests required by MAF for the batch of product. Testing should be done by independent competent testers not by the manufacturer or supplier.

6.10 Test kits

6.10.1 Hazard identification

6.10.1.1 Agents of concern

Any live pathogen found in a test kit.

6.10.1.2 General considerations

Test kits are kits used for the diagnosis of diseases of plants and animals, for carrying out analytical procedures, manipulating genetic material etc. For diagnosis of infectious diseases probably the most commonly used kits are ELISA kits that are used for the detection of infectious agents or their antigens or antibodies to them. These kits consist of all the reagents necessary for performing the tests, packaged in a convenient manner. The reagents are represented by the manufacturers as being well defined and standardized. Typically an ELISA kit for detection of an antibody would contain antigen, positive and negative control sera, reagents for detecting bound antibody which typically might be a second antibody bound to a marker enzyme, and a chromogenic substrate for the marker enzyme. Monoclonal antibodies are commonly used in such kits. Antibody preparations and sera contained in kits are in minimal amounts contained in closed vials. In this form it is inconceivable that they would be able to inadvertently infect animals with adventitious agents contained in them. Kitset are also available for several other serological tests used for both antibody and antigen detection. Other available kitsets include kits for polymerase chain reactions to detect specific DNA sequences, kits for cloning DNA sequences, DNA extraction kits, analytical test kits for detection and quantitation of hormones, enzymes and other molecules of interest for diagnosis of diseases or physiological parameters .Most kits are used only in the laboratory and the reagents are used in micro-well plates or tubes. There is no reason to expose any animals to the reagents contained in them.

6.10.1.3 Conclusion

Test kits may contain live organisms and therefore they are potential hazards for the purposes of this analysis

6.10.2 Risk assessment

6.10.2.1 Release assessment

There are a very large number of diagnostic kits for the diagnosis of animal and plant diseases and for other analytical procedures. A quick search on the internet will identify literally hundreds, probably thousands of different test kits and test reagents for the diagnosis or diseases of plants and animals. Little information is given about the reagents or only general information is supplied in laboratory catalogues. However, printed information supplied with the kits often gives more detailed information and manufacturers usually respond to individual enquiries so that the scientists working with the kits are usually well informed about them. The test kits can in principle be regarded as safe provided that they contain no live organisms. Since this information is not available, it must be assumed that the likelihood of a kit containing live organisms is very low but non-negligible. Many vital functions performed by New Zealand laboratories are dependant on the ability to import and use kits for diagnostic and analytical tests.

6.10.2.2 Exposure assessment

Once a kit has been introduced it will generally be used for diagnostic testing in a laboratory. However, some kits are designed for use in the field and it is likely that the use of kits designed for field use will increase in the future. If kits containing live antigens are used in the field this could conceivably result in plants or animals that come into contact with the kit becoming infected with the organism. Effects on the environment would be restricted to the effects on animals or plants. Therefore although the likelihood of exposure is low it is non-negligible.

6.10.2.3 Consequence assessment

The consequences of introducing new pathogens are dependant on the species and strain of organism introduced and may vary from negligible to catastrophic. The consequences are therefore non-negligible.

6.10.2.3 Risk estimation

Since release, exposure and consequence assessment are all non-negligible, according to the methodology used for this analysis (Section 4.2) risk is non-negligible.

6.10.3 Risk management

6.10.3.1 Risk evaluation

Since risk is non-negligible risk management measures should be implemented to reduce the risk to an acceptable level.

6.10.3.2 Risk management objectives

The objectives of risk management are to ensure that live pathogens will not be introduced into the environment by the importation and use of test kits.

6.10.3.3 Risk management options

Importers of diagnostic kits could be required to declare whether a particular kit that they wish to import contains live organisms. If the kit contains live organisms it would be treated as a microorganism and the IHS for the importation of microorganisms would apply. Lists of kits containing live organisms could be negotiated between the companies concerned and MAF as in the case of biological products derived from tissues and blood (Sections 6.7 and 6.9). If a kit contains a new organism that is not known to occur in New Zealand, its importation could be approved by ERMA and MAF could issue a permit with any restrictions applicable to the particular case indicated on the permit.

Kits that contain no live organisms are biological products and would not require ERMA approval to import. Companies supplying such kits could have blanket approval for such products from MAF and import and supply them without restrictions. It should be noted that diagnostic kits are already freely available from pharmacies for the diagnosis of pregnancy in women and that restrictions on the use of kits containing no live organisms are not feasible.

6.10.3.4 Recommendations

i. Applications to import could be made by a laboratory or by a company that produces and sells the product. Importers of diagnostic kits should be required to declare whether a particular kit that they wish to import contains live organisms. If the kit contains live organisms it should be treated as a microorganism and be subject to the conditions in the IHS for the importation of microorganisms.

ii. Kits that do not contain live organisms are biological products. Kits listed in catalogues of suppliers of biological products that contain live infectious agents should be identified. All other kits should be approved for importation without restrictions.

6.11 Amino acids

6.11.1 Hazard Identification

6.11.1.1 Agents or concern

Any infectious agents that could contaminate preparations of amino acids.

6.11.1.2 General considerations

Amino acids are small molecular weight products that can be purified from hydrolysed proteins, or produced by synthesis from simple chemical products, fermentation by microorganisms from simple precursors or enzymatic methods. Purification of the amino acids using these production methods involves standard processes such as filtration, ion exchange, crystalisation etc. These processes can be relied upon to produce purified low molecular weight products that are free from contaminating microorganisms. Amino acids produced synthetically are in the form of equimolar mixtures of D and L isomers. Those produced from animal tissues are in the L isomer form. They are generally sold as highly purified products and the processes of production and purification ensures that they are free from pathogenic agents.

Crude mixtures of amino acids and small peptides produced by hydrolysis of proteins are not classified as amino acids for this analysis. They are described as peptones or hydrolysates (Section 6.4).

6.11.1.3 Conclusion

The likelihood that purified amino acid preparations would contain pathogenic organisms is negligible and they are not considered to be potential hazards in this analysis

6.12 Products derived from microorganisms.

6.12.1 Hazard identification

6.12.1.1 Agents of concern

Any pathogenic agent or new organism that could contaminate products derived from microorganisms.

6.12.1.2 General considerations

A very large number of enzymes and other biological products (mostly proteins) are offered for sale. Products that are extracted directly from tissues of plants, animals (including blood) and eggs have already been discussed in Sections 6.7 - 6.9. Most other products are derived from microorganisms and the majority of these are proteins. However, long chain fatty acids are also sometimes produced from fermentation processes but are more generally derived from plant oils. Starch is generally derived from plant sources such as potatoes and corn. However fermentation processes may be used to obtain small molecular weight products from starch e.g. sugars and alcohol. The selected donor organisms are cultured and the product is extracted and purified from the biomass of cultured organisms or the culture medium containing their excreted products.

Since the medium on which the organisms are grown is sterile the only organisms that could contaminate the end-product are the donor organism. Contamination of products with donor organisms would only be of significance if the organism was an unwanted, notifiable or new organism. Although no donor organism that are pathogens were found in the catalogues investigated this cannot be assumed for all possible products. In addition although all products offered for sale appear to be purified products this could not be verified for all products and all catalogues.

A modern trend is to produce animal or plant proteins that could be contaminated with viruses if produced from animal or plant tissues, by cloning the relevant genes into suitable host-vector systems. If the hosts used are not pathogens or new organisms, expression of the gene by the genetically modified vector guarantees production of a product that is free from contaminating pathogens or new organisms.

6.12.1.3 Conclusions

The likelihood that products produced from microorganisms could be contaminated with viable pathogens is extremely low. However, since all products from all companies could not be investigated it is classified as non-negligible. Therefore, products derived from microorganisms are classified as potential hazards for this analysis.

6.12.2 Risk assessment

6.12.2.1 Release assessment

It is extremely unlikely that manufacturers of biological products chemicals and reagents would choose to use pathogenic organisms as sources for their products when non-pathogenic alternatives are available. The contamination of protein products by new or unwanted organisms is therefore most unlikely. A search of a number of catalogues did not reveal that any organisms, classified by MAF as unwanted or notifiable (Ministry of Agriculture and Forestry 2004), were used as source organisms for production of biological products.

Additionally virtually all products are purified products. Typically the initial step in the purification process would be the separation of microorganisms from spent culture medium by centrifugation, filtration or precipitation. This would usually eliminate virtually all viable microorganisms from the spent culture medium. Either the spent medium or the biomass of organisms would be the source of the protein. In the case where the biomass of organisms is used the cells would be disrupted by mechanical disruption, freezing and thawing, lysis by chemicals or enzymes etc. The disrupted material would then be clarified by filtration or centrifugation and the protein would be purified from the clarified material. Typical purification protocols may include precipitation steps with organic solvents or salts, ultrafiltration, gel, ion exchange or affinity chromatography (including high pressure liquid chromatography), iso-electric focusing, electrophoresis etc (Pierce- Biotechnology 2002). Finally the product is likely to be filtered to render it clear and free of contaminating environmental bacteria and bottled either as a liquid, dried powder or crystalline product.

The likelihood that purified products will contain viable contaminating organisms is negligible. Some non-purified extracts and also freeze dried microorganisms are found in catalogues. For instance the Sigma catalogue advertises the sale of "*Bacillus subtilis* lyophilized cells – produced in pure culture. Not intended for use as a starter culture. Not processed or packaged aseptically". Non-purified extracts are not biosecurity hazards if they are made from non-pathogens and contain no live organisms or toxic substances. Live organisms are considered in Part 2 of this risk analysis. The likelihood that any of the biological products derived from microorganisms that are offered for sale in manufacturers catalogues would be contaminated with pathogenic organisms is negligible.

6.12.2.4 Risk estimation

Since the likelihood of release of pathogens or new organisms in imported proteins derived from microorganism is considered to be negligible, according to the methods used in this risk analysis (Section 4.2), risk is assessed to be negligible.

6.12.3 Risk management

6.12.3.1 Risk management evaluation

Since risk is considered to be negligible the implementation of risk management measures is not justified.

6.13 Small molecular weight fermentation products

6.13.1 Hazard identification

6.13.1.1 Agents of concern

Exotic pathogens or new organisms that could contaminate imported biological products

6.13.1.2 General considerations

Fermentation by microorganisms has a long history and is used to produce a large variety of biological products (Anonymous 2004d). Many large molecular weight organic molecules particularly enzymes and other proteins are produced by processes generally termed fermentation. Large biomasses of microorganisms are commonly produced for vaccine production. Fermentation processes are used for making cheese, yoghurt, bread, alcoholic beverages and for composting organic waste products and producing fuel gasses such as methane. Large molecular weight products derived from microorganisms have been considered in Section 6.12.

This section is restricted to small molecular weight products produced by fermentation. These products could include alcohols (Moreno et al 1995), organic acids (Anonymous 2003a) and esters (Anonymous 2003a; Moreno et al 1995). Aldehydes and ketones are often produced during fermentation or from acids and alcohols that could have been produced by fermentation. These products are purified from spent culture medium by such methods as distillation. Many are commonly contained in beverages (ethanol) cleaning and medicinal products (methanol) and products used in food preparation (tartaric acid, citric acid, acetic acid/vinegar) etc. Products are derived from cultures of harmless organism and are widely traded, often in non-purified form, without restrictions. Many are self sterilising (organic acids, alcohols). Products produced for laboratories are likely to be further purified versions of products already traded without restrictions.

The likelihood that small molecular weight products sold as highly purified products for laboratory use would contain contaminating unwanted organisms is negligible.

6.13.1.3 Conclusions

Small molecular weight purified biochemicals that are produced by fermentation are not potential risk goods for this analysis.

6.14 Antimicrobials/antibiotics

6.14.1 Hazard identification

6.14.1.1 Agents of concern

Unwanted or notifiable microorganisms that could contaminate antimicrobial products.

6.14.1.2 General considerations

Antibiotics are widely used for the treatment of microbial infections in humans and animals. For these purposes they are classed as medicines and are not biological products for the purpose of this analysis. They are also used as growth promotants. Some antibiotics or antimicrobials are sold as biochemicals and are offered for sale in manufacturer's catalogues. These products may be used for fundamental studies on biochemical processes and pathways e.g. actinomycin D may be used as an inhibitor of DNA dependant RNA synthesis. They are also used in diagnostic laboratories for testing the sensitivity of isolated pathogens to antibiotics and in culture media to inhibit the growth of contaminating organisms.

All antibiotics are derived from non-pathogenic microorganisms or are synthesized or semi-synthesised products. Antibiotics produced from microorganisms are always purified. No viruses are involved in their production so the separation of source microorganisms from product is simple.

6.14.1.3 Conclusions

Antibiotics are all purified products produced from harmless organisms and the likelihood that they will be contaminated with unwanted or notifiable organisms is negligible. For this reason they are not classified as potential hazards in this analysis.

7.0 PART 2: MICROORGANISMS

7.1 Hazard identification

There was no necessity to make a preliminary hazard list of microorganisms. Such a list would simply include all microorganisms and would probably run into millions and it would grow continuously as new organisms are described.

7.1.1 Introduction and general considerations

In principle control of the introduction of microorganisms should be designed to ensure that exotic human, animal and plant pathogens are excluded. Additionally exotic organisms with potential to damage the environment should not be introduced.

MAF already has a list of notifiable and unwanted organisms and any attempt to control the importation of microorganisms should as a primary focus be designed to exclude any of these organisms and exotic human, animal and plant pathogens. In addition under the HSNO Act 1996 new organisms can only introduced subject to approval by ERMA. It is the responsibility of a potential importer of an organism to provide evidence to show that an organism that is the subject of an "application to import" is endemic. Applications to introduce new organisms should be referred to ERMA.

There may be good reasons to introduce some unwanted or notifiable organisms into specially constructed secure laboratories where they will be used as positive controls for testing or as diagnostic antigens. These instances represent special cases dependant on the type of laboratory concerned and will usually involve approval from ERMA since they are likely to be new organisms.

Most applications to import microorganisms are likely to be from laboratories and many of these will be transitional facilities. However, some applications could be for harmless organisms that will be used in harmless applications such as yoghurt making.

7.1.2 Conclusions

The numbers and diversity of micro-organisms is so great that individual consideration of organisms is not possible. Therefore a formal risk analysis would not be useful. This risk analysis therefore proceeds directly to outlining the options that are available for controlling importation of organisms.

7.2 Risk management considerations and options

MAF could operate a permit system.

Granting a permit to import could be subject to the following conditions:

- Provision of evidence that the organism is endemic. All applications for new organisms could be referred to ERMA.

- Provision of written certification that the organism is in the form of a pure culture i.e. one that consists of a single species of organism that has been shown to contain no contaminating organisms.

- Unambiguous identification down to species level.

- The organism should have a negligible potential to damage the environment, economy, or health status of New Zealand's human, animal or plant populations. Specifically it should not be classified by MAF as a notifiable or unwanted organism or human, animal or plant pathogen or new organism.

Harmless organisms such as cultures for the manufacture of yoghurt could be granted clearance, provided they are not new organisms requiring ERMA approval.

The suitability of the facilities to contain an organism could be considered when an application to introduce an organism is made.

Applications to introduce unwanted or notifiable organisms should be accompanied by a detailed and well constructed case for consideration by ERMA and MAF.

A decision tree could be used to assist in and formalize the decision making process (See Figure 2).

7.3 Recommendations

It is recommended that:

i. MAF should operate a permit system for the importation of microorganisms.

ii. Evidence should be provided that the organism is endemic. All applications for new organisms should be referred to ERMA.

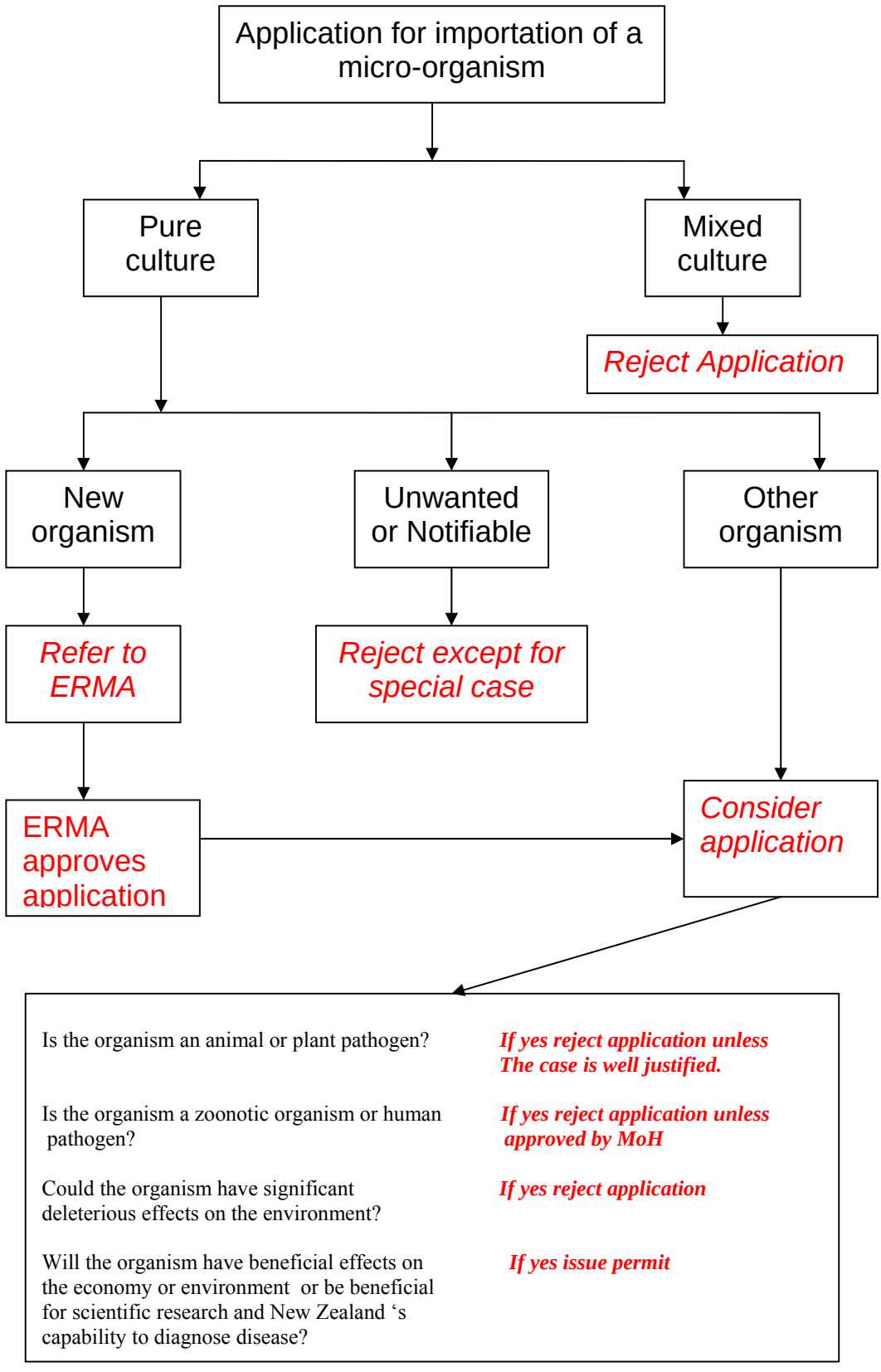
iii. Microorganisms should be accompanied by a certificate that certifies they are pure cultures that have been identified to the species level.

iv. The decisions relating to the issuing of permits should be formalized by making use of decision making tree such as the one shown in Figure 2.

v. Each step in the decision making process should be signed off on a check sheet and the check list and relevant additional notes should be kept as auditable records.

vi. Subject to ERMA approval new organisms should be permitted entry with a permit to import which will direct them on arrival to an appropriate transitional facility or should be granted clearance in the case of harmless organisms.

Figure 2. Decision tree for the issuing of permits for the importation of microorganisms



8.0 LIVING CELLS DERIVED FROM ANIMALS OR PLANTS.

8.1 General introduction and preliminary hazard list

Living cells derived from animals are commonly grown as cell (tissue) cultures *in vitro* in the laboratory. Cell cultures are either primary cells derived directly from animal tissues that grow for a limited number of generations in culture or cell lines, which grow indefinitely. These tissue cultures are most commonly used for isolating animal viruses for the diagnosis of disease or propagating viruses or other microorganisms for production of antigens, vaccines etc. To isolate viruses tissue cultures that are free from viruses are inoculated with material that contains or is suspected to contain viruses. Viruses then multiply in the tissue culture and can be identified in the cultured cells.

In the case of plants virus isolation is done by directly culturing cells from the infected or suspected infected plants and identifying the virus that is growing in them.

Cell lines of both plant and animal cells are also used for other purposes such as studies on physiology, metabolism, toxicology, immunology etc. Cell lines are rarely used by plant biologists and requests to import plant cell lines could not be recalled by MAF workers in this field (Clover 2005). For these reasons this risk analysis focuses mainly on the importation of animal cells.

The term “tissue culture” as used by plant biologists implies the propagation of plant tissues from living plant material for the purposes of cloning plants. This is common practice and the importation of plants or plant cuttings is not covered by this risk analysis since separate control procedures are in place that cover these cases.

Importation of viruses may be accomplished by importation of cells that have been infected with the virus. In these cases the importation will be an importation of both a virus and the importation of living cells and will have to meet the requirements of the IHSs for both microorganisms and cell cultures.

In the future there may be applications to import living cells from animals for the purposes of cloning animals from them. One such case is already under consideration by MAF and ERMA. In this case the tissue has been imported and is being held in a transitional facility pending some uncertainty about the legal and regulatory ramifications of the case. The rapid development of biology may in the future result in the development of new areas of technology for which living cells are required to be used in novel ways. Applications to import cells for cloning or the purposes are likely to be rare. However, it is appropriate for the regulatory controls for the importation of all living cells to be considered at this stage.

Importation of living human cells is not considered in this risk analysis as it is a matter for control by the MoH. Applications for human cells may in the future include such things as use of cells or organs for transplantation into humans.

Importation of plants derived from tissue cultures is already controlled by MAF and is not included in this risk analysis.

A preliminary hazard list therefore only contains two items:

- Cell cultures
- Animal cells for other applications.

8.2 Cell cultures

8.2.1 Hazard identification

8.2.1.1 Agents of concern

Cell cultures may be contaminated with adventitious organisms such as viruses, Mollicutes (*Mycoplasma* spp), bacteria and fungi and even in some cases protozoal parasites. Some contaminating organisms may be zoonotic agents or pathogenic animal pathogens that are unwanted or notifiable organisms. Oncogenic cells or cells contaminated with oncogenic viruses also constitute a risk to the laboratory personnel that will work with them.

8.2.1.2 General considerations

Contaminating bacteria (other than Mollicutes), fungi and protozoa are generally easily recognised and should be identified by the suppliers of cell cultures or after importation by the user of the cells. These organisms are therefore of little concern. The main organisms of concern are viruses and Mollicutes, particularly *Mycoplasma* spp.

Adventitious viruses contaminating cell cultures are readily identifiable when they are cytopathic and the cell damage they cause is easily recognised. However, non-cytopathic viruses and *Mycoplasma* spp may be easily overlooked and extensive testing procedures are required to prove that cell lines are free from contaminating viruses (Onions 1993). No single testing regimen can identify all viruses and combinations of testing such as co-culture on susceptible cell lines, injection of animals and measurement of antibody response, PCRs for particular organisms or groups of organisms and tests for reverse transcriptase for the identification of retroviruses are used (FDA 1998). When deciding on whether a cell culture is likely to be free from adventitious agents good records of the test procedures that have been carried out on the cells will be helpful.

Adventitious viruses: The best known example of an adventitious virus infecting cell cultures is the contamination of monkey cells that were used for production of polio vaccine. Up to 1963, many cell cultures were contaminated with Simian virus 40 and as a consequence an estimated 10-30 million people may have received contaminated polio vaccine in the USA alone (CDC. 2002). Fortunately SV40 is not a human pathogen

although the ramifications of the exposure of people to the virus are still being debated with some claims that the virus may have oncogenic effects (Fisher et al 1999). A large number of adventitious viruses have been found in human cells. Some of those that are of concern for human health include: hepatitis B virus, hepatitis C virus, human immunodeficiency virus (HIV) and human T-cell lymphotrophic viruses (Belgian-Biosafety-Server 2005). Such deadly viruses as Marburg and Ebola (Peters et al 1992) are also a threat to cell culture laboratory workers but are unlikely to contaminate established cell lines and the possibility of them being introduced when importing cell lines is negligible. Many mouse viruses have been found in murine cells.

BVDV virus commonly contaminates cell cultures often due to the use of contaminated foetal calf serum in the culture medium (Falcone et al 2003; Makoschey et al 2003; Zabal et al 2000). Because many strains of BVDV are not cytopathic their presence in cell cultures may not be obvious. Other contaminating viruses found in cell cultures or vaccines (where the contamination presumably originated from the cell cultures) include IBR in bovine cells, guinea pig herpes viruses in guinea pig embryo or kidney cells and equine herpes virus in horse kidney cells (Fong and Landry 1992) and parvovirus in pig cells (Mengeling 1975). Both endogenous retroviral sequences and avian leucosis occur in avian cells (Weiss 2001) and other avian viruses have been described.

Other adventitious viruses have been found in cell cultures but it is not necessary to review them all here

Mollicutes.

Contamination of cell cultures by *Mycoplasma* spp and other Mollicutes are common and workers using cell cultures have to constantly guard against getting their cultures contaminated. One publication lists nine species of *Mycoplasmas* and one of *Acholeplasma* that have been isolated from cell cultures (Anonymous 2005a). However seven species including the human pathogen *Myccoplasma pneumoniae* and animal pathogens *Mycoplasma gallisepticum* and *Mycoplasma hyorhinis* are believed to account for 96% of contaminations (Belgian-Biosafety-Server 2005). Cell cultures readily become contaminated with Mollicutes that originate from human lab workers or the environment. Cell cultures infected with mollicutes are not considered to be a health hazard for laboratory workers but they affect the quality of the tissue cultures and their suitability for propagation of viruses. Mollicute contamination of cell cultures is not uncommon in New Zealand laboratories. Therefore, contamination of cell cultures by these organisms is a quality issue for the laboratories concerned and rarely a biosecurity issue.

8.2.1.3 Conclusions

Adventitious viruses may be present in cell cultures and are potential biosecurity hazards. They are classed as potential hazards in this risk analysis. However, contaminating Mollicutes, bacteria fungi and protozoa are primarily quality issues to be addressed by the

laboratory using the cell cultures, rather than biosecurity hazards and are not included in the risk analysis.

8.2.2 Risk assessment

8.2.2.1 Release assessment

Many laboratories use both primary cell cultures and well characterised cell lines. Primary cells are more likely to carry adventitious viruses than well characterised cell lines that have been extensively used and tested in many laboratories.

Importation of primary cells involves greater risks than importation of established cell lines. However animal and plant primary cell cultures used in New Zealand are usually derived from New Zealand animals and in this case because they could only contain New Zealand endemic pathogens are not a biosecurity risk. Risks to laboratory workers are minor since zoonotic diseases are rare in New Zealand livestock. Hazards to laboratory workers from endemic disease agents in cells and diagnostic specimens are a safety issue for the laboratory not a biosecurity issue and will not be considered in this risk analysis. Similarly, if laboratories are using primary cell cultures derived from human tissues the laboratories are responsible for all safety issues related to the use of such tissues. However, importation of cell lines that may contain human pathogens is a biosecurity issue. The risks to laboratory workers involved in the use of human and primate cells cultures are greater than those working with non-primate animal cell cultures. Humans and primate cell cultures may potentially contain human pathogens such as Hepatitis B and C viruses and HIV. All human lymphoid cells should be viewed with some suspicion as possibly carrying oncogenic viruses and cultures of tumour cells or cultures that contain oncogenic viruses are a potential hazard to laboratory staff handling them.

Because of the potential dangers, standards for working safely with cell cultures are usually set (Belgian-Biosafety-Server 2005). One university safety manual goes so far as to suggest that all cell cultures should be regarded as possibly infected with viruses (Anonymous-Iowa-State-University 2002). There is extensive regulatory control of the use of cells and cell cultures in the USA (Anonymous 2004c). However, it is necessary to keep the dangers in perspective and since infection of laboratory staff from cell cultures is very rare.

Standards set for working safely with cell cultures vary depending on the types of cells being used. The most dangerous cells requiring the most stringent working conditions are human cells, followed by non-human primate cells and non-primate mammalian cells with the least dangerous cells being non-mammalian cells (Anonymous 2003b). The assessment of risk and resulting requirements for safe working procedures is also affected by:

- *Cell types* - with risk being in the following order: epithelial and fibroblastic cells (least risk), gut mucosa, endothelium, neural cells, haemopoietic (highest risk).

- *Culture type* – risk is in the order: well characterised cell lines (least risk), continuous cell lines, primary cell lines (highest risk) (Belgian-Biosafety-Server 2005)

Non-human mammalian primary cells could contain animal pathogens. BVDV is a common contaminant of animal cells cultures and BVDV 2 does not occur in New Zealand so the likelihood of introducing this virus is non-negligible. The likelihood of release of adventitious agents (particularly human pathogens) in cell cultures varies according to the types of cell cultures and the types of cells introduced.

Since several human and animal pathogens could be introduced in cell cultures the risk of release of significant viruses in cell cultures is non-negligible.

8.2.2.2 Exposure assessment

If it is assumed that cell cultures will only be introduced into facilities that are registered transitional facilities and will be kept in those facilities, it follows that they will be, handled and disposed of as specified in the MAF Standard 154.02.17: *Standard for transitional facilities for biological products*. This will in effect ensure that all cell cultures are kept in secure transitional facilities areas and will be sterilised before disposal. In these conditions the likelihood of exposure of animals, plants, humans or any component of the environment outside of the laboratory is negligible. However, laboratory workers could be exposed to agents in cell cultures and therefore the likelihood of exposure in laboratories working with human or primate cell cultures that are not well established cell lines, depends on the safety procedures used in the laboratory and the agent and cell culture concerned. The likelihood of laboratory workers becoming infected is low but non-negligible.

8.2.2.3 Consequence assessment

As discussed in the exposure assessment the likelihood of infecting animals or plants outside the laboratory is remote. The consequences of exposure of a laboratory worker to a particular agent in a cell culture depends on the agent concerned and could vary from a trivial to a life threatening infection. In a worst case scenario such as infection with SARS virus the infected laboratory worker could transmit a dangerous virus to people outside of the laboratory. The likelihood of such an infection occurring is very low but non-negligible.

8.2.2.4 Risk estimation

Release, exposure and consequence assessment are all non-negligible. Therefore risk is non-negligible.

8.2.3 Risk Management

8.2.3.1 Risk evaluation

Since risk is considered to be non-negligible, risk management measures should be adopted to reduce risk to an acceptable level.

8.2.3.2 Risk management objective

The objectives are to ensure that imported cell cultures are free from harmful adventitious agents and are not released from a transitional facility.

8.2.3.3 Risk management options.

A permit system could be operated by MAF. Importers could be asked to supply full details about the culture they wish to import including information on testing for adventitious agents. MAF could use a decision tree to judge the appropriateness of the importation of cell cultures before issuing a "Permit to Import". Conditions relating to the importation of the cells could be individually tailored to the particular importations and could be attached to the permit. Importation of cell cultures could be restricted to importation by laboratories that are transitional facilities. In cases involving human or primate cell lines MAF could take advice from MoH before issuing a "permit to import". A decision tree such as that shown in Figure 3 could be used to formalise decision making.

8.2.4 Recommendations

It is recommended that:

i. MAF should operate a permit system for the importation of cell cultures

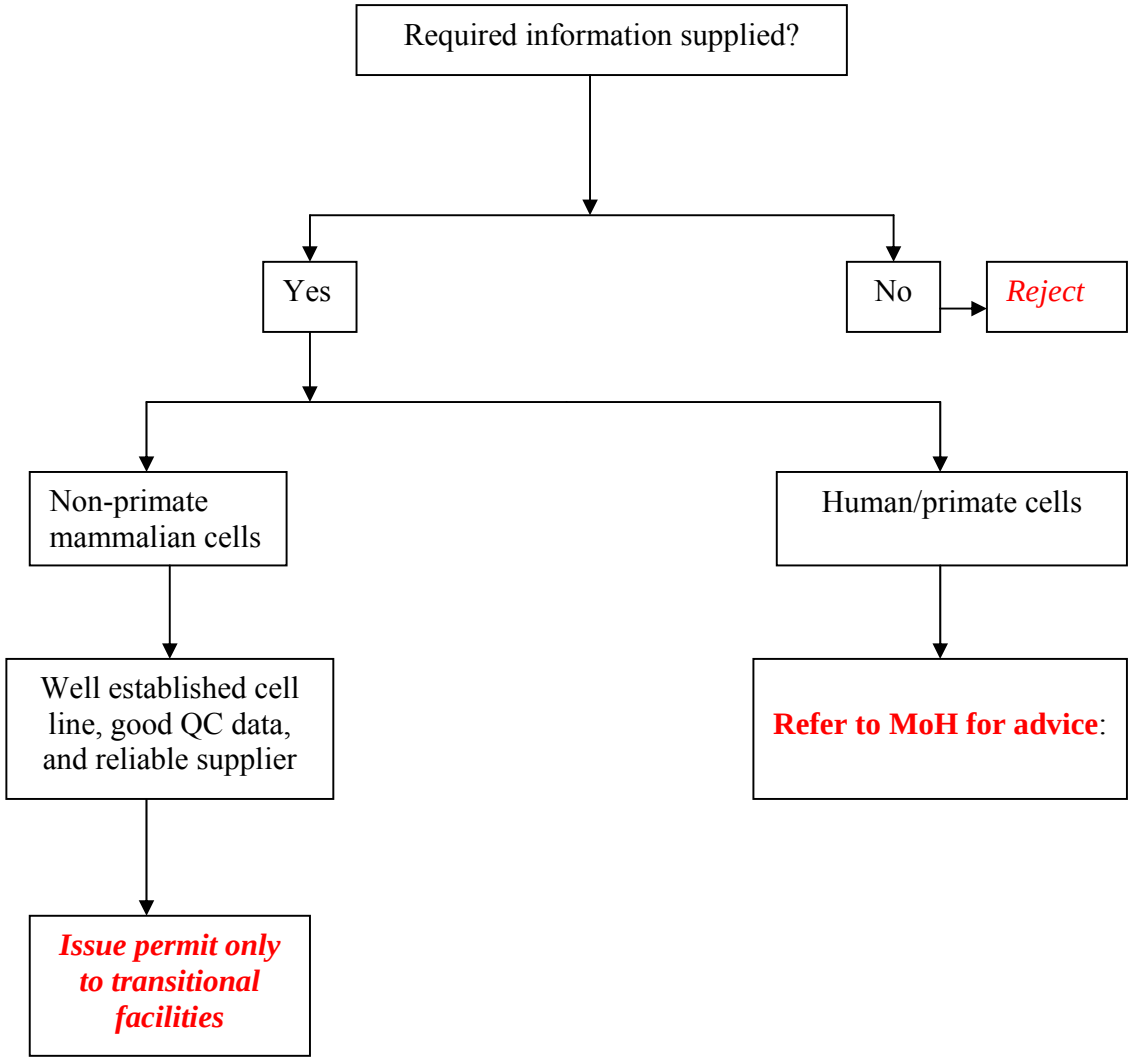
ii. Applications for importation of the cell cultures should provide details about the cells to be imported the supplier of the culture and quality control testing that has been done on the cells culture.

iii. Importation of cell cultures should be restricted to transitional facilities and it should be a requirement that cell cultures should be retained in the transitional facility.

iv. Decisions to issue a permit should be based on a decision tree such as that given below.

v. MAF should take advice from MoH before issuing a "Permit to Import" human of primate cell lines. Alternatively MoH could take over the administrative responsibility for importations of these cell lines.

Table 3. Decision tree for the issuing of permits for the importation of cell cultures.



8.3 Animal cells for other applications

8.3.1 Hazard identification

8.3.1.1 Agents of concern

Infectious agents that can infect animals and cause diseases of concern.

8.3.1.2 General considerations and recommendations.

Requests for importation of live cells could be for cloning of organisms or other as yet undefined uses. Importing biopsy material is not a greater biosecurity risk than importing the donor animal itself. For this reason donors of animal cells should meet all the health standards specified in the IHS for importation into New Zealand of live animals from the country concerned. In cases where an IHS does not exist for a particular animal species and a particular country, a request for the development of an IHS should be made in the same way as a request would be made to import live animals. A formal risk analysis would be required before an IHS could be written.

9.0 NOVEL SUBSTANCES

There could be applications to import novel substances that do not fit into any of the categories discussed above. Since it is not possible to design a system to cope with unknown substances it is recommended that applications to import such substances should be controlled by an import permit system. MAF should require that the proposed importer should supply whatever information is required for consideration of the case. A permit would only be issued after consultation with DoC and ERMA and if appropriate the MoH. If a permit to import is granted it should contain appropriate restrictions as agreed between the appropriate agencies.

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11.0 APPENDICES

11.1 Appendix 1

Product information :Restriction endonucleases

The following information was given about the restriction endonucleases produced by individual suppliers/manufacturers.

Fermentas UAB

Please, excuse us for delay in responding.
Answering to your inquiry we would like to inform you that all Fermentas enzymes are highly purified with quality parameters matching or even surpassing those of the main wholesalers of molecular biology enzymes, such as Invitrogen, Roche, etc. As you know, our products are designated for molecular biology research, where even trace contaminants, including microbial contamination, may have detrimental effect to the experimental outcome. Therefore we purify the enzymes to near homogeneity and subject them to very strict quality assurance procedures. Purification schemes developed for our products routinely comprise from three to six chromatography steps followed by precipitation in few cases.
As regards the sterility, similarly to the products of our competitors, we do not apply sterility testing for our products, since no such regulations were relevant so far to this product group in the industry, as is the case with pharma products.
For each particular product information is given in our catalogue, indicating whether the enzyme is produced from the native strain or from the E. coli recombinant strain.
We have also contacted our representatives in Australia and New Zealand Progen Biosciences and they have confirmed that our products conform to AQIS import requirements. I am not sure how much these regulations can be extended to the import procedures in New Zealand, but thought you could find this information useful.
If you need more information, or would like to have our catalogue, please, do not hesitate to contact me.
Sincerely,
Egle Cesnaviciene

_____

Dr. Egle Cesnaviciene (Ms.)
Head of IP Group
FERMENTAS UAB
V.Graiciuno 8
LT-02241 Vilnius
Tel.: +370-52-602139
Fax.: +370-52-602142
Internet: www.fermentas.com

Invitrogen

Thank you for contacting Invitrogen. Our restriction enzymes are not specifically filter sterilised but given their highly purified state we do not expect any bacteria or other organisms to be present. This is somewhat confirmed by the lack of other endo- and endo-nucleases which forms part of the QC. I hope this helps.
Regards,

Michael Bateson
Technical Service Australasia
0800 335 997

Jena bioscience

thank you very much for your interest in our products.

Jena Bioscience Restriction Enzymes are purified from
crude extracts, that have been freed of cellular nucleic acids, by
Chromatographic procedures.
A variety of chromatographic methods are used. These include size exclusion
chromatography, ion exchange, affinity or dye-ligand chromatography. Some of
the proteins are purified to 98% homogeneity, but in general the criterion is the absence of non-specific
endo- exo- nucleases and phosphatases from the final product up to
1000-6000 fold overdigestion.

The enzymes are produced from the organism specified in the Technical Data
Sheets.
Some of the enzymes produced from E. coli strain that carries in plasmid the
cloned enzyme (HinfI, HpaI, PstI, PvuII, StyI). These are described in the datasheets.

All of our restriction endonucleases are not hazardous and non-toxic.

Hope, this helps.

Please contact me, if you need additional information.

Best regards,
Christiane

Christiane Kohls christiane.kohls@jenabioscience.com