Australian Government

Department of Health and Ageing
Office of the Gene Technology Regulator

Review of the Gene Technology Regulations 2001
Discussion Paper No. 3 (2010)
Review of the Classification of Dealings with Viral Vectors

Issue

Currently, undertaking many dealings involving genetically modified viral vectors, particularly lentiviral vectors, requires case by case assessment and licensing by the Gene Technology Regulator (the Regulator). Some dealings with viral vectors are classified as Notifiable Low Risk Dealings (NLRDs) or exempt dealings.

Over the last two decades, development of replication defective viral vectors has improved their safety profiles, reducing their ability to cause disease in people. This has been reflected by operational experience of the Regulator and the OGTR in assessing licence applications. For some licence applications for dealings involving viral vectors, Risk Assessment and Risk Management Plans have concluded that there is negligible risk to human health and safety and the environment.

The current review of the Regulations therefore included a detailed consideration of the classification of dealings involving GM viral vectors, in order to ensure that the level of regulation of dealings is commensurate with the risks they pose, and is up to date with current scientific understanding. A number of amendments are proposed to the Regulations which would reclassify dealings with some viral vectors. These proposed amendments build on the review undertaken previously which resulted in the Gene Technology Amendment Regulations 2006.

The proposed amendments take into account the range of safety features incorporated into viral vectors, the effect of the introduced nucleic acid and the nature of the procedures being performed. These changes are intended to improve the efficiency of the regulatory system, while ensuring that human health and safety and the environment are appropriately protected.

Introduction

Viral vectors are used extensively as a gene delivery system in gene technology research. Viral vectors make use of the ability of viruses to enter cells and express the genes contained in their genome to create genetically modified (GM) cells of the host organism expressing specific genes of interest.

The current review of the classification of dealings with viral vectors is informed by six overarching considerations, which represent a tiered approach to risk assessment:

1. Whether the viral vector is replication defective or replication competent

An important safety feature incorporated into many viral vectors is genetic modification to render them incapable of replication. Factors required for replication are then supplied from plasmids or a GM cell line. In the event of unintended exposure, replication deficiency limits the number of cells which may be transduced, preventing disease/infection and limiting the potential for harm from vector insertion or transgene expression in transduced cells.

2. The ability of the viral vector to transduce human cells

Unintended exposure to vectors able to enter (transduce) human cells could lead to expression of viral genes in human cells. Potential risks to human health and safety from dealings with GM viral vectors are significantly limited for vectors unable to transduce human cells.

3. Whether the vector is derived from a retrovirus

Retroviruses are RNA viruses which, after entering a cell, can reverse transcribe their genome to DNA which integrates into the host genome, from which progeny viral RNAs are transcribed. Because of this characteristic, viral vectors based on retroviruses are the principal type of vector system used to achieve heritable insertion and stable expression of introduced genes. Non-retroviral vectors enable only non-heritable expression of transgenes, which is generally transient in nature.

4. Safety features of the viral vector

A number of safety features may be incorporated into viral vectors which limit the potential for viral vectors to: regain replication competence, transcribe integrated vector RNA or unintentionally activate expression of host genes. These features reduce the potential for adverse consequences resulting from unintended exposure to viral vectors.

5. Characteristics of the transgene

In addition to the above characteristics, nature of the transgenes carried by the vector will also effect the risk posed to human health and safety by unintended exposure to viral vectors. For example, exposure to a viral vector encoding a protein with immunomodulatory activity is more likely to result in adverse effects than exposure to a viral vector encoding a fluorescent marker.

6. Whether dealings are *in vitro* or *in vivo*

Due to the nature of dealings *in vitro* (eg involving tissue culture) and *in vivo*, the likelihood of unintended exposure of workers to viral vectors is greater for dealings *in vivo* involving the use of sharps to inoculate animals.

An additional consideration in classifying dealings with GMOs as exempt, NLRDs or requiring a licence is the physical containment level of facilities where the dealings will be undertaken. The Regulations take guidance from the Australian/New Zealand Standard 2243.3 *Safety in Laboratories Part 3: Microbiological aspects and containment facilities* (AS/NZS 2243.3), which classifies microorganisms into Risk Groups, each with a corresponding appropriate level of physical containment. Note that NLRDs may be scheduled as suitable for Physical Containment level 1 (PC1) or PC2 facilities. The unmodified viruses from which many viral vectors are derived cause disease in people and animals and meet AS/NZS 2243.3 criteria for a Risk Group 2 organism requiring PC2 containment.

Context of this review

The Regulator's proposals for reclassification of dealings with viral vectors are underpinned by a consideration of the risks they might pose to human health and safety and the environment, and whether any risks can be adequately managed by the requirements for exempt dealings or NLRDs or if case by case assessment and management through licensing is warranted. Consideration has focussed principally on potential risks to personnel undertaking such dealings, as this is the most plausible unintended exposure pathway. Risks to the environment are generally limited by the inability of viral particles or GMOs containing viral particles to survive desiccation in the event of a spill, and the inability of the viral vector to replicate outside a permissive host.

The Regulator has performed an assessment of the risks posed by dealings with replication defective viral vectors, taking into account the factors described above. The assessment also

considered plausible pathways to unintended exposure, including the nature of the procedures being performed and the level of protection provided by undertaking dealings within PC1 and PC2 facilities, according to appropriate laboratory practices.

This review focuses on the classification of dealings with a subset of retroviral vectors, the lentiviral vectors, because experience in administering the regulatory system indicates that some dealings with these vectors are subject to greater regulatory oversight than is warranted, in light of current scientific understanding. Consideration of safety factors and current regulatory requirements indicates lentiviral vector classification is not consistent with classification of other retroviral vectors in the Regulations. It is proposed to make the regulation of dealings with lentiviral vectors more consistent with that of other retroviral vectors, and to clarify safety features critical in determining classification levels. This may result in some increases in classification for other retroviral vectors.

The review was undertaken within the context of requirements for the conduct of NLRDs, which are described in detail in a separate discussion paper, *Review of the classification of Notifiable Low Risk Dealings.*

Proposed Changes

A number of amendments to the Regulations have been proposed that will change the classification or the level of physical containment required to undertake some dealings with GMOs involving viral vectors. The changes are summarised in Tables 1 and 2, which are an indicative aid to understanding the specific changes proposed to the Schedules. Greater detail (including paragraph references for Schedule 3, Parts 1, 2 and 3 of the Regulations) is provided in Tables 3 and 4 in Appendix 1.

The tables indicate current and proposed classifications of dealings involving viral vectors, with references to the specific reclassification proposals which are described in detail below. Proposed amendments which change the levels of containment or classification of dealings have been shaded. The majority of changes in category of classification are from requiring a licence (in practice, usually a DNIR) to NLRD.

It should be noted that the terms “low risk gene”, “lower risk gene” and “higher risk gene” have been employed as comparative descriptors for the purpose of illustration only and do not appear within the Act or the Regulations. ‘Higher risk’ genes are those encoding a protein with immunomodulatory activity in humans or are a growth factor or a component of a signal transduction pathway, that if expressed may lead to cell proliferation in humans. These genes are considered to pose ‘higher risk’ because their expression in human cells following inadvertent exposure has an increased potential to lead to adverse outcomes relative to other genes commonly used in viral vectors. All other genes are considered ‘lower risk’. The current Regulations refer to oncogenic modifications, and depending upon the specific properties of an oncogene it may meet the description of a ‘higher risk’ or ‘lower risk’ gene. A number of proposed amendments replace references to oncogenic modifications with outcome-focused descriptions.

Table 1 describes the classification of dealings with retroviral vectors from which all viral genes have been deleted, and where viral genes required for virion packaging are supplied *in trans* from separate loci (such as plasmids or stably transformed cell lines).

In vitro dealings are those involving tissue culture of an host included in the list of host/vector systems for exempt dealings in Part 2 of Schedule 2. *In vivo* dealings primarily involve introduction of viral vectors into whole animals, and are described in the draft Amendment Regulations as being in hosts other than those listed in Part 2 of Schedule 2.

Table 1. Dealings with replication defective retroviral vectors

<table>
<tr><th colspan="3">Characteristics of the vector</th><th rowspan="3">characteristics of donor nucleic acid (transgene)[3]</th><th colspan="4">Characteristics of the dealings</th></tr>
<tr><th rowspan="2">able to transduce human cells</th><th rowspan="2">self-inactivating[1]</th><th rowspan="2">accessory genes present[2]</th><th colspan="2">In vitro</th><th colspan="2">In vivo</th></tr>
<tr><th>current</th><th>proposed</th><th>current</th><th>proposed</th></tr>
<tr><td rowspan="3">No</td><td rowspan="3">Yes or no</td><td rowspan="3">Yes or no</td><td>low risk gene</td><td colspan="2">exempt</td><td colspan="2">PC2 NLRD</td></tr>
<tr><td>oncogene</td><td>PC1 NLRD</td><td>Exempt</td><td>DNIR</td><td>PC2 NLRD Proposal 1</td></tr>
<tr><td>pathogenic determinant</td><td colspan="2">PC2 NLRD</td><td colspan="2">PC2 NLRD</td></tr>
<tr><td rowspan="9">Yes</td><td rowspan="4">Yes</td><td rowspan="2">No</td><td>lower risk gene</td><td colspan="2">PC2 NLRD</td><td colspan="2">PC2 NLRD</td></tr>
<tr><td>higher risk gene</td><td colspan="2">PC2 NLRD</td><td colspan="2">DNIR</td></tr>
<tr><td rowspan="2">Yes</td><td>lower risk gene</td><td>DNIR</td><td>PC2 NLRD Proposal 2</td><td>DNIR</td><td>PC2 NLRD Proposal 3</td></tr>
<tr><td>higher risk gene</td><td>DNIR</td><td>PC2 NLRD Proposal 2</td><td colspan="2">DNIR</td></tr>
<tr><td rowspan="5">No</td><td rowspan="4">No</td><td rowspan="2">lower risk gene</td><td>Lentiviral: DNIR</td><td rowspan="2">PC2 NLRD Proposal 2</td><td>Lentiviral: DNIR</td><td rowspan="2">PC2 NLRD Proposal 3</td></tr>
<tr><td>other: PC2 NLRD</td><td>other: PC2 NLRD</td></tr>
<tr><td rowspan="2">higher risk gene</td><td>Lentiviral: DNIR</td><td rowspan="2">PC2 NLRD Proposal 2</td><td colspan="2" rowspan="2">DNIR</td></tr>
<tr><td>other: PC2 NLRD</td></tr>
<tr><td>Yes</td><td>any transgene</td><td colspan="2">DNIR</td><td colspan="2">DNIR</td></tr>
</table>

[1] **Self-inactivating deletion:** a deletion in the unique 3′ region of the long terminal repeat (LTR) that eliminates the LTR promoter activity after integration of the provirus into the host genome

[2] **Accessory genes:** only *gagpol*, *env* and *rev* (if a lentiviral vector) are present in the packaging system, no other accessory genes.

[3] **Characteristics of the donor nucleic acid:**

- All dealings involving toxin expression are licensable
- the donor nucleic acid cannot correct a defect in the vector leading to the production of replication competent viral particles
- For vectors unable to transduce human cells:
  - genes other than oncogenes or pathogenic determinants are '**low risk' genes**
- For vectors able to transduce human cells:
  - '**higher risk' genes** are those encoding:
    - a protein with immunomodulatory activity in humans; or
    - a growth factor, or a component of a signal transduction pathway, that if expressed may lead to cell proliferation in humans
  - '**lower risk' genes** are all other genes

Table 2. Dealings with replication defective non-retroviral vectors

Characteristics of the vector	Characteristics of the donor nucleic acid (transgene)[1]	Characteristics of the dealings			
		In vitro		*In vivo*	
		current	proposed	current	proposed
Not able to transduce human cells	low risk gene	exempt		PC2 NLRD	
	oncogene	PC1 NLRD	**exempt**	DNIR	PC2 NLRD **Proposal 1**
	pathogenic determinant	PC2 NLRD		PC2 NLRD	
	higher risk gene	exempt		DNIR	PC2 NLRD **Proposal 1**
Able to transduce human cells, derived from *Human adenovirus* or *Adeno associated virus*	low risk gene	PC1 NLRD		PC2 NLRD	
	oncogene	PC2 NLRD	PC1 NLRD **Proposal 4.1**	DNIR	PC2 NLRD **Proposal 5**
					DNIR
	higher risk gene	PC1 NLRD		DNIR	
Able to transduce human cells, derived from other viruses	low risk gene	PC1 NLRD	PC2 NLRD **Proposal 4.2**	PC2 NLRD	
	oncogene	PC2 NLRD		DNIR	PC2 NLRD **Proposal 5**
					DNIR
	higher risk gene	PC1 NLRD	PC2 NLRD **Proposal 4.2**	DNIR	

[1] **Characteristics of the donor nucleic acid:**

- All dealings involving toxin expression are licensable
- the donor nucleic acid cannot correct a defect in the vector leading to the production of replication competent viral particles
- For vectors unable to transduce human cells:
  - genes other than 'higher risk' genes, oncogenes or pathogenic determinants are **'low risk' genes**
- For vectors able to transduce human cells:
  - **'higher risk' genes** are those encoding:
    - a protein with immunomodulatory activity in humans; or
    - a growth factor, or a component of a signal transduction pathway, that if expressed may lead to cell proliferation in humans
  - **'lower risk' genes** are all other genes

Proposal 1 – *in vivo* dealings with replication defective vectors unable to transduce human cells

It is proposed that all dealings involving inoculation of animals with replication defective vectors (retroviral and non-retroviral) that are unable to transduce human cells be classified as PC2 NLRDs (Schedule 3, Part 2, 2.1 (i)). This would result in such dealings involving 'higher risk genes' being reclassified from requiring a licence to PC2 NLRDs.

Dealings with viral vectors unable to transduce human cells pose negligible risk to laboratory workers because the vector cannot efficiently enter human cells and therefore is unlikely to express genes or integrate into the worker's genome even in the event of unintended exposure.

Dealings **in tissue culture** involving the use of these vectors are currently classified as exempt, PC1 NLRDs or PC2 NLRDs (depending on the risk associated with the donor nucleic acid) because unintended human exposure is considered unlikely. Should laboratory workers be exposed, the viral vectors are unable to efficiently enter human cells and are therefore not expected to express genes or integrate into the worker's genome. However, as a precautionary measure, the previous review of the Regulations resulted in the classification of dealings with vectors expressing oncogenes and 'higher risk genes' such immunomodulatory molecules as licensable, and those involving 'lower risk genes' as PC2 NLRDs.

Ongoing regulatory experience, particularly from assessing DNIR applications involving replication defective viral vectors unable to transduce human cells with 'higher risk genes', has concluded that such dealings pose negligible risk to human health and safety and the environment. This is primarily due to the vectors being unable to transduce human cells, and additionally because of the uptake of newer viral vectors with improved safety features, and also an increase in scientific knowledge regarding these vectors.

In considering reclassification of dealings involving 'higher risk genes' as PC2 NLRDs, NLRD requirements are also of importance:

- All NLRDs must be undertaken in certified physical containment facilities that are subject to certification conditions, including requirements in respect of work practices;
- NLRD assessment by Institutional Biosafety Committees (IBCs) must include consideration of whether the personnel have appropriate training and experience; proposed amendments to Regulation 13 will reinforce this requirement (for more detail about proposed amendments to Regulation 13, refer to the separate discussion paper *Oversight and conduct of NLRDs*).

For these reasons, it is concluded that dealings involving inoculation of animals with replication defective viral vectors that are unable to transduce human cells are suitable for classification as PC2 NLRDs, regardless of the nature of the donor nucleic acid. It is proposed that dealings involving 'higher risk' genes be re-classified from requiring a licence to PC2 NLRDs.

Classification of dealings with retroviral vectors able to transduce human cells

For dealings involving replication competent retroviral vectors, or replication defective retroviral vectors without safety features to sufficiently reduce the likelihood of replication competence being regained, it is proposed that a licence be required to ensure all such dealings are subject to risk assessment and the imposition of specific conditions to manage risk.

Requirements for replication defective retroviral vectors

The specific safety features required of replication defective retroviral vectors ensure that the factors needed for replication are supplied *in trans* in a manner that minimises the potential for a vector to regain replication competence. Replication competence may result from recombination within the packaging cell line creating a vector encoding the genes required for replication in the target host. Alternately, it may occur as a result of recombination with retroviruses present in cell lines or animals.

In decreasing the likelihood of replication competence being regained, the potential for adverse outcomes following unintended exposure to the vector is also reduced, because only a limited number of cells may be transduced. The required features are:

- removal of all viral genes from the vector so that it cannot replicate or assemble into a virion without these factors being supplied *in trans*; and
- the viral genes needed for virion production are expressed from independent, unlinked loci in the packaging cell line, with minimal sequence overlap with the vector.

A packaging system supplies the factors necessary for packaging of viral RNA into viral particles (virions), and may involve either expression of viral genes that have been integrated in a GM cell line or expression of viral genes from plasmids supplied *in trans*. The use of a packaging system allows the production of viral particles from a viral vector which is itself replication defective.

These requirements do not represent a change in classification for dealings with lentiviral vectors. However, for some dealings with other retroviral vectors this will represent an increase in classification, because the safety requirements proposed are more stringent than in the current Regulations. These proposed amendments are incorporated into proposals 2 and 3 below.

Safety features to reduce the likelihood of vectors regaining replication competence

For dealings with replication defective retroviral vectors able to transduce human cells that do not contain any of the safety features described below, the potential for vectors to regain replication competence is increased compared to when safety features are present. These dealings are classified as licensable and will continue to be subject to case by case risk assessment and licensing by the Regulator.

Self-inactivation

Retroviral vectors may include a deletion in one of the DNA signals (the unique 3' long terminal repeat, U3 LTR) which initiates transcription of the introduced DNA following integration into the host genome, known as a self-inactivation. This deletion prevents transcription of the integrated DNA into RNA, thereby blocking production of viral RNA which would be incorporated into new viral particles if factors necessary for packaging are available. This means that the introduced DNA cannot be used to make any further GM viral particles. Self-inactivating deletions also have a secondary benefit, the elimination of long range transcriptional activation from the intact DNA signal, which could otherwise lead to unpredictable changes in the expression of host genes near the site of vector integration.

Where a replication defective vector has a self-inactivating deletion, the likelihood of replication competence being regained is reduced because even if packaging genes were introduced into the vector by recombination, they would not be expressed.

Accessory genes

The potential for adverse consequences from unintended exposure to a replication defective retroviral vector able to transduce human cells is affected by whether recombination between the vector and the genes supplied for packaging could produce a replication competent virus. For systems where only the minimal set of genes *gagpol*, *rev* (in the case of lentiviral vectors) and an

envelope gene are supplied from independent loci with minimal sequence overlap, recombination is unlikely. If this requirement is met, the potential for a vector to regain replication competence is reduced.

Additionally, accessory genes may strongly increase transcription of viral genes or host genes adjacent to an integrated viral vector, or their expression may have direct adverse effects on infected cells (eg through oncogenic or immunomodulatory effects). These additional effects do not occur for viral vectors with only a minimal set of accessory genes.

Proposal 2 – *in vitro* dealings with replication defective lentiviral vectors able to transduce human cells

It is proposed that dealings in tissue culture involving replication defective lentiviral vectors able to transduce human cells either:

- with a self-inactivating deletion; or
- where only the genes *gagpol*, *rev* and an envelope protein (or a subset of these) are expressed from independent loci in the packaging system

be classified as PC2 NLRDs (Schedule 3, Part 2, 2.1 (l)).

The Regulations currently schedule dealings with replication defective lentiviral vectors able to transduce human cells as licensable unless:

- the vector has a self-inactivating deletion; and
- all structural and accessory genes have been removed from the vector and must be supplied *in trans* for replication to occur; and
- the genes supplied *in trans* include only *gag*, *pol*, *rev* and an envelope protein gene.

Dealings involving the introduction into tissue culture hosts of other replication competent retroviral (non-lentiviral) vectors able to transduce human cells are classified as PC2 NLRDs if the donor nucleic acid cannot restore replication competence.

It is proposed that *in vitro* dealings with all retroviral vectors, including lentiviral vectors, where the vector is able to transduce human cells be classified as PC2 NLRDs if the vector contains one of two safety features: a self inactivating deletion or restrictions on accessory genes, as described above. This proposal takes into account the limited potential for adverse outcomes from unintended exposure to replication defective vectors, the reduced potential for vectors to regain replication competence due to inclusion of safety features, and the limited potential for laboratory workers to be exposed to viral vectors used in *in vitro* dealings.

For these reasons, it is considered unnecessary to place restrictions on the types of transgenes which may be included in these dealings, other than toxin genes.

This proposed amendment would enable consistent regulation between lentiviral and other retroviral vectors. The classification of dealings with non-lentiviral retroviral vectors would be unchanged except where vectors previously considered replication defective do not meet the requirements of replication defective vectors as described in the proposed amendments.

Proposal 3 – *in vivo* dealings with replication defective lentiviral vectors able to transduce human cells

It is proposed that dealings *in vivo* involving replication defective lentiviral vectors able to transduce human cells either:

- with a self-inactivating deletion; or
- where only the genes *gagpol*, *rev* and an envelope protein (or a subset of these) are expressed from independent loci in the packaging system

be classified as PC2 NLRDs where the donor nucleic acid is a 'lower risk' gene (Schedule 3, Part 2, 2.1 (m)).

In vivo dealings with replication defective retroviral vectors able to transduce human cells are currently classified as PC2 NLRDs if the donor nucleic acid does not confer an oncogenic modification, except if they involve a lentiviral vector, in which case a licence is required (as described above, see Proposal 2).

In classifying *in vivo* dealings involving lentiviral vectors in this way, safety features which may be present in the vector to limit the potential for replication competence to be regained were considered. However, recent scientific progress and ongoing regulatory experience in assessing relevant licence applications has lead to the view that safety features are more effective at reducing risks than was thought at the time of the previous review of the Regulations.

Compared to *in vitro* dealings, dealings involving inoculation of animals with viral vectors require an additional consideration: with the use of sharps to inoculate animals, inadvertent needle-stick injuries become a plausible pathway to unintended exposure of laboratory workers to the viral vector. Should such an unintended exposure occur the magnitude of potential consequences would be determined by the transgene involved and the safety features of the vector.

For a replication defective vector, safety features (ie a self-inactivating deletion or limitations on accessory genes) limit the possibility of a vector regaining replication competence. Safety features reduce the potential for harm due to insertion of the vector or expression of the introduced genes because only a limited number of cells can be transduced. However, the transgene may pose a risk if its expression could lead to cell proliferation or the encoded protein has immunomodulatory activity.

The risk to the operator following unintended exposure to a replication defective retroviral vector are minimal if the transgene is 'lower risk' and the vector incorporates safety features to prevent replication competence being regained, because only a limited number of cells could be transduced and the effects on those cells would also be limited. Conversely, transduction of cells with a retroviral vector expressing a 'higher risk' gene, such as a growth factor, may lead to uncontrolled cell proliferation, with the trait being passed to daughter cells.

An additional consideration, in the context of PC2 NLRDs, is that the likelihood of such an unintended exposure occurring is somewhat mitigated by behavioural requirements contained in facility certification guidelines, and the requirement that IBCs assess personnel as having adequate training to undertake a dealing.

For these reasons it is proposed that dealings involving introduction into animals of replication defective retroviral vectors where the gene is considered 'lower risk' and the vector incorporates specific safety features be scheduled as PC2 NLRDs. This proposed amendment would enable consistent regulation between lentiviral and other retroviral vectors. Dealings involving 'higher

risk genes' would continue to require a licence, and would be subject to case by case assessment and management of risks.

Classification of dealings with non-retroviral vectors able to transduce human cells

Proposal 4.1 – *in vitro* dealings with replication defective non-retroviral vectors able to transduce human cells derived from *Human Adenovirus* or *Adeno associated virus*

It is proposed that all dealings with vectors derived from *Human Adenovirus* or *Adeno associated virus* in tissue culture be scheduled as PC1 NLRDs, including those involving transgenes conferring oncogenic modifications.

Proposal 4.2 – *in vitro* dealings with replication defective non-retroviral vectors able to transduce human cells not derived from *Human Adenovirus* or *Adeno associated virus*

It is proposed that *in vitro* dealings with replication defective non-retroviral vectors able to transduce human cells other than those derived from *Human Adenovirus* or *Adeno associated virus* be removed from Part 1 of Schedule 3, and classified as PC2 NLRDs.

Dealings with replication defective non-retroviral vectors able to transduce human cells in tissue culture, where the donor nucleic acid does not confer an oncogenic modification, are currently scheduled as PC1 NLRDs. This scheduling was based on assessment of replication defective viral vector systems based on *Human Adenovirus* and *Adeno associated virus*, which were prevalent at the time of the previous review, which had been subject to extensive characterisation.

More recently, new non-retroviral vector systems have been developed which are derived from viruses that cause disease in people and animals and which meet AS/NZS 2243.3 criteria for Risk Group 2 organisms (or higher) requiring at least PC2 containment. These systems do not have the same long history of safe use as those developed from *Human Adenovirus* or *Adeno associated virus* and the potential risks they pose are less well characterised.

The potential risks posed by replication defective non-retroviral vectors differ from retroviral vectors, because non-retroviral vectors do not integrate into the genome of the host cell. Such non-retroviral vector systems generally provide for transient transfection and expression of transgenes in host cells. It is considered unlikely that laboratory workers would be inadvertently exposed to viral vectors in the course of *in vitro* dealings. However, should this occur the potential consequences would be minor, regardless of the nature of the transgene, based upon the extensive information available about vectors derived from *Human Adenovirus* and *Adeno associated virus*. This is because replication deficiency limits the number of cells that could be transfected, and any effects from the transgene would be transient because the vector would be unable to integrate into the host cell genome. In the case of the transgene conferring an oncogenic modification or a 'higher risk' gene, the potential for uncontrolled cell proliferation is limited because the transformed cells would not be able to proliferate indefinitely. An important consideration is that the first step of this pathway to an adverse outcome, inadvertent exposure of an operator to a vector in the course of conducting dealings in tissue culture, is unlikely.

It is therefore proposed that all dealings with replication defective non-retroviral vectors able to transduce human cells derived from *Human Adenovirus* or *Adeno associated virus* in tissue

culture, including oncogenic modifications but excluding toxin transgenes, be classified as a PC1 NLRDs.

For dealings with replication defective non-retroviral vectors able to transduce human cells not derived from *Human Adenovirus* or *Adeno associated virus*, there is not sufficient information available to support classification as PC1 NLRDs. It is proposed that dealings involving these vectors in tissue culture be removed from Part 1 of Schedule 2 (PC1 NLRDs), and classified as a PC2 NLRDs, except where the transgene is a toxin gene in which case a licence would be required.

Proposal 5 – *in vivo* dealings with replication defective non-retroviral vectors able to transduce human cells

It is proposed that *in vivo* dealings involving replication defective non-retroviral vectors that are able to transduce human cells, where the donor nucleic acid does not meet the description of 'higher risk' genes, be classified as PC2 NLRDs (Schedule 3, Part 2, 2.1 (k)).

Dealings involving the inoculation of animals with replication defective non-retroviral vectors able to transduce human cells currently require a licence if the donor nucleic acid confers an oncogenic modification or encodes a 'higher risk' gene. For 'lower risk' transgenes, these dealings are currently scheduled as PC2 NLRDs.

In reviewing the classification of dealings with viral vectors the potential adverse effects particular transgenes may have has been examined. The current Regulations classify some dealings involving oncogenic modifications in higher categories than similar dealings with what are described in this paper as 'higher risk' genes (which includes oncogenes where they are known to induce uncontrolled cell proliferation). However, many known oncogenes encode proteins which may not result in an adverse outcome if expressed in a human cell.

The current review has concluded that descriptions focusing on potential adverse outcomes would better inform the classification of dealings commensurate with the potential risks they pose. As a result, an outcome-focused description of 'higher risk' genes is used, which is intended to encompass those oncogenes known to pose greater risk, ie genes encoding:

- a protein with immunomodulatory activity in humans; or
- a growth factor, or a component of a signal transduction pathway, that if expressed may lead to cell proliferation in humans.

As discussed for Proposal 4, if a laboratory worker is exposed to a non-retroviral replication defective vector able to transduce human cells, the potential for adverse outcomes is limited by the vector being unable to replicate and unable to integrate into the host cell genome, particularly for 'lower risk' transgenes.

Therefore it is proposed that dealings involving the introduction into animals of non-retroviral vectors able to transduce human cells, where the donor nucleic acid is a 'lower risk' gene, be scheduled as PC2 NLRDs. This proposal would result in the reclassification of dealings involving oncogenic modifications that do not meet the description of 'higher risk' genes. Dealings where the donor nucleic acid is any other 'lower risk' gene would continue to be scheduled as PC2 NLRDs. Dealings where the donor nucleic acid is a 'higher risk' gene (including oncogenes that meet the requirements) would continue to be subject to case by case risk assessment and licensing by the Regulator.

Appendix 1

Table 3. Dealings with replication defective[1] retroviral vectors

Characteristics of the vector			Characteristics of donor nucleic acid (transgene)[4]	Characteristics of the dealings			
				In vitro		*In vivo*	
Able to transduce human cells	Self-Inactivating[2]	Accessory genes present[3]		Current	Proposed	Current	Proposed
No	Yes or no	Yes or no	low risk gene[5]	exempt	exempt	PC2 NLRD 2.1 (c) & (d)	PC2 NLRD 2.1 (i)
			oncogene	PC1 NLRD 1.1 (b)	**Exempt** [6]	DNIR 3.1 (d)	PC2 NLRD **Proposal 1** 2.1 (i)
			pathogenic determinant	PC2 NLRD 2.1 (e)	PC2 NLRD 2.1 (e)	PC2 NLRD 2.1 (c)	PC2 NLRD 2.1 (i)
Yes	Yes	No	lower risk gene[7]	PC2 NLRD 2.1 (i)	PC2 NLRD 2.1 (l)	PC2 NLRD 2.1 (d)	PC2 NLRD 2.1 (d) & (m)
			higher risk gene[8]	PC2 NLRD 2.1 (i)	PC2 NLRD 2.1 (l)	DNIR 3.1 (d)	DNIR 3.1 (j)
		Yes	lower risk gene[7]	DNIR 3.1 (i)	PC2 NLRD **Proposal 2** 2.1 (l)	DNIR 3.1 (i)	PC2 NLRD **Proposal 3** 2.1 (m)
			higher risk gene[8]	DNIR 3.1 (d) & (i)	PC2 NLRD **Proposal 2** 2.1 (l)	DNIR 3.1 (d) & (i)	DNIR 3.1 (j)
	No	No	lower risk gene[7]	Lentiviral: DNIR 3.1 (i) other: PC2 NLRD 2.1 (i)	PC2 NLRD **Proposal 2** 2.1 (l)	Lentiviral: DNIR 3.1 (i) other: PC2 NLRD 2.1 (i)	PC2 NLRD **Proposal 3** 2.1 (m)
			higher risk gene[8]	Lentiviral: DNIR 3.1 (i) other: PC2 NLRD 2.1 (i)	PC2 NLRD **Proposal 2** 2.1 (l)	DNIR 3.1 (d) & (i)	DNIR 3.1 (j)
		Yes	lower risk gene[7]	DNIR 3.1 (i)	DNIR 3.1 (j)	DNIR 3.1 (i)	DNIR 3.1 (j)
			higher risk gene[8]	DNIR 3.1 (d) & (i)	DNIR 3.1 (j)	DNIR 3.1 (d) & (i)	DNIR 3.1 (j)

[1] Any dealing where the introduced gene can correct a defect in the vector leading to the production of replication competent viral particles must be assessed as for a replication competent virus; replication defective retroviral vectors must include safety features to reduce the likelihood of recombination leading to replication competence being regained, including that all viral genes must be removed from the retroviral vector so that it cannot replicate or assemble into a virion without these functions being supplied *in trans*, and that viral genes needed for virion production must be expressed from independent, unlinked loci with minimal sequence overlap

[2] Indicates the presence of a 'self inactivating' deletion in the unique 3' region of the long terminal repeat (LTR) that eliminates the LTR promoter activity after integration of the provirus into the host genome

[3] Only *gagpol*, *env* and *rev* (if a lentiviral vector) present in the packaging system

[4] All dealings involving toxin expression are DNIRs irrespective of the nature of the vector in which they are expressed

[5] Low risk genes are those encoding proteins which are not oncogenes or pathogenic determinants

[6] For further detail see the separate discussion paper *Review of the classification of exempt dealings*

[7] Lower risk genes are those encoding proteins other than those with immunomodulatory activity in humans, and proteins (growth factors or components of a signal transduction pathway) that if expressed may lead to cell proliferation in humans

[8] Higher risk genes are those encoding proteins with immunomodulatory activity in humans, or proteins (growth factors or components of a signal transduction pathway) that if expressed may lead to cell proliferation in humans

Table 4. Dealings with replication defective non-retroviral vectors

Characteristics of the vector	Characteristics of the donor nucleic acid (transgene)[1]	Characteristics of the dealings			
		In vitro		*In vivo*	
		current	proposed	current	proposed
Not able to transduce human cells	low risk gene[2]	exempt	exempt	PC2 NLRD 2.1 (c) & (d)	PC2 NLRD 2.1 (i)
	oncogene	PC1 NLRD 1.1 (b)	**exempt** (see exempt dealings paper)	DNIR 3.1 (d)	PC2 NLRD **Proposal 1** 2.1 (i)
	pathogenic determinant	PC2 NLRD 2.1 (e)	PC2 NLRD 2.1 (e)	PC2 NLRD 2.1 (c)	PC2 NLRD 2.1 (i)
	higher risk gene[3]	exempt	exempt	DNIR 3.1 (d)	PC2 NLRD **Proposal 1** 2.1 (i)
Able to transduce human cells, *Human adenovirus* or *Adeno associated virus*	low risk gene[2]	PC1 NLRD 1.1 (c)	PC1 NLRD 1.1 (c)	PC2 NLRD 2.1 (d)	PC2 NLRD 2.1 (k)
	oncogene	PC2 NLRD 2.1 (i)	PC1 NLRD **Proposal 4.1** 1.1 (c)	DNIR 3.1 (d)	PC2 NLRD 2.1 (k) **Proposal 5** DNIR 3.1 (d)
	higher risk gene[3]	PC1 NLRD 1.1 (c)	PC1 NLRD 1.1 (c)	DNIR 3.1 (d)	DNIR 3.1 (d)
Able to transduce human cells other viruses	low risk gene[2]	PC1 NLRD 1.1 (c)	PC2 NLRD **Proposal 4.2** 2.1 (i)	PC2 NLRD 2.1 (d)	PC2 NLRD 2.1 (k)
	oncogene	PC2 NLRD 2.1 (i)	PC2 NLRD 2.1 (j)	DNIR 3.1 (d)	PC2 NLRD 2.1 (k) **Proposal 5** DNIR 3.1 (d)
	higher risk gene[3]	PC1 NLRD 1.1 (c)	PC2 NLRD **Proposal 4.2** 2.1 (j)	DNIR 3.1 (d)	DNIR 3.1 (d)

[1] All dealings involving toxin expression are DNIRs irrespective of the nature of the vector in which they are expressed, additionally any dealing where the introduced gene can correct a defect in the vector leading to the production of replication competent viral particles should be assessed as for a replication competent virus

[2] Low risk genes are those encoding proteins other than immunomodulatory activity in humans or growth factor, or proteins (growth factors or components of a signal transduction pathway) that if expressed may lead to cell proliferation in humans and which are not pathogenic determinants

[3] Higher risk genes are those encoding proteins with immunomodulatory activity in humans or proteins (growth factors or components of a signal transduction pathway) that if expressed may lead to cell proliferation in humans