



**Australian Government**

**Department of Health and Ageing**

**Office of the Gene Technology Regulator**

## **Review of the Gene Technology Regulations 2001**

### **Discussion Paper No. 1 (2010)**

#### **Review of the Classification of Exempt Dealings**

### ***Introduction***

A number of proposed amendments to the Gene Technology Regulations 2001 (the Regulations) relate to scheduling of dealings with GMOs as exempt. The *Gene Technology Act 2000* (the Act) provides for certain dealings with GMOs to be exempt from the requirement for licensing. Exempt dealings represent the lowest risk category for dealings with GMOs. Although the Act provides no specific considerations which must be made before listing a dealing as exempt in the Regulations, a history of safe use has previously been a primary consideration.

The only legislative requirement for conduct of dealings with GMOs scheduled as exempt is that they do not involve intentional release of a GMO into the environment (Regulation 6 (1) (d)). For further information about classification, assessment and containment of exempt dealings see Appendix 1, *Legislative background to exempt dealings*.

Currently there are 4 classes of dealings listed as exempt in Schedule 2 Part 1 of the Regulations (these are numbered items 2 – 5 for historical reasons):

2. dealings with GM *Caenorhabditis elegans*
3. dealings with animals into which GM somatic cells have been introduced
4. dealings with listed host/vector systems with introduced DNA which meets specified criteria
5. dealings with listed host/vector systems involving shot-gun cloning or the preparation of cDNA libraries

Within each class of exempt dealing, limitations on risk-related characteristics of the GMO or donor nucleic acid must be met.

The full listing of exempt dealings and exempt host/vector systems is provided by Schedule 2 of the Regulations (see draft Gene Technology Amendment Regulations 2010). Exempt dealings comprise the vast majority of all dealings with GMOs, the most common being:

- (i) cloning (and to a lesser extent, expression) of genes in *E. coli*; and
- (ii) expression of genes in mammalian cell culture *in vitro*.

## ***Proposed changes***

### **Exempt host/vector systems**

A host/vector system is an experimental system using a vector to facilitate introduction of a foreign gene or nucleic acid sequence into a host organism. Host/vector systems for exempt dealings are listed in Part 2 of Schedule 2 and comprise hosts and corresponding vectors that have been extensively studied and are considered to present negligible biosafety risks to public health and safety, including occupational health and safety, or to the environment. Listed hosts and vectors are essentially non-pathogenic to people, animals, plants or fungi, and offer a level of biological containment of genetic modifications (ie it is unlikely that the GMO could survive outside a laboratory, with the result that it is very difficult for introduced nucleic acid to spread outside the host/vector system or the resulting GMO).

#### **Proposal 1.1 – New hosts**

The following host organisms are proposed for inclusion in the list of host/vector systems for exempt dealings at Schedule 2 Part 2, for use in combination with existing listed vectors:

- *Escherichia coli* Nissle 1917
- *Lactococcus lactis*
- *Streptococcus thermophilus*
- *Synechococcus* strains PCC 7942, PCC 7002 & WH 8102
- *Synechocystis* sp. strain PCC 6803
- *Yarrowia lipolytica*

The Gene Technology Regulator (the Regulator) conducted a targeted consultation with regulated organisations regarding potential hosts. A number of resulting submissions supported inclusion of particular hosts and proposed additional hosts for consideration. All of the proposed additions were considered.

For inclusion in Part 2 of Schedule 2, the host or vector should be sufficiently characterised to reach a conclusion that it is non-pathogenic to people, animals, plants or fungi. This may include debilitated, non-pathogenic strains of a species that includes pathogenic strains, provided there is evidence that dealings permitted as exempt dealings will not restore pathogenicity. Hosts must also provide a suitable level of biological containment of genetic modifications.

Risk assessments were carried out for each host considered for inclusion in the list of host/vector systems for exempt dealings. Schedule 2 Part 1 item 4 places restrictions on the donor nucleic acid which can be used in listed host/vector systems, for the purpose of limiting the potential for exempt dealings to result in the host or vector gaining an increased capacity to cause harm to people or the environment. Risk assessments were undertaken in this context.

The Gene Technology Technical Advisory Committee was consulted on these risk assessments, and their recommendations were taken into account in finalising the risk assessments. For those hosts which are now proposed for inclusion (listed above) the risk assessments concluded that dealings with these organisms pose negligible risk to human health and safety or the environment, with no specific containment being necessary to manage risks, other than the requirement that no intentional release into the environment occur.

In relation to *Synechococcus* strain WH 8102 (which is proposed for inclusion), it was noted that this host cannot be directly transformed and foreign DNA is usually introduced through conjugation with *Escherichia coli*. The use of conjugative plasmids in *E. coli* is not an exempt dealing, therefore the creation of a GM *Synechococcus* strain WH 8102 would remain a notifiable low risk dealing.

A number of other hosts were considered for inclusion, however risk assessments did not support these hosts being included in the list of host/vector systems for exempt dealings. For further detail see Appendix 2.

<b>Proposal 1.2 – tissue culture hosts</b>			
It is proposed that item 4 in the list of exempt host/vector systems in Schedule 2 Part 2 be amended as follows (new text in bold):			
Item	Class	Host	Vector
4	Tissue culture	<p><b>Any of the following that cannot spontaneously generate a whole animal:</b></p> <p>(a) Animal or human cell cultures (including packaging cell lines)</p> <p>(b) <b>isolated cells, isolated tissues or isolated organs, whether animal or human</b></p> <p>(c) <b>early non-human mammalian embryos cultured <i>in vitro</i></b></p> <p>Plant cell cultures, <b>and isolated plant tissues that are incapable of spontaneous vegetative or sexual propagation to generate a whole plant</b></p>	<p>1. Non-conjugative plasmids</p> <p>2. Non-viral vectors, or defective viral vectors unable to transduce human cells</p> <p>3. Baculovirus (<i>Autographa californica</i> nuclear polyhedrosis virus), polyhedrin minus</p> <p>4. None (non-vector systems)</p> <p>1. Non-tumorigenic disarmed Ti plasmid vectors, or Ri plasmid vectors, in <i>Agrobacterium tumefaciens</i>, <i>Agrobacterium radiobacter</i> or <i>Agrobacterium rhizogenes</i></p> <p>2. Non-pathogenic viral vectors</p> <p>3. None (non-vector systems)</p>

Queries to the Office of the Gene Technology Regulator (OGTR) indicate that there is currently a lack of clarity in the regulated community as to what may be classified as a tissue culture host according to the descriptions listed in the host/vector systems for exempt dealings. It is proposed to expand these descriptions to clarify that where tissue culture is mentioned as an exempt host it includes isolated cells, tissues, organs and non-human mammalian embryos, provided that they cannot spontaneously generate a whole animal or plant. Provided that this requirement is met, dealings with all of the described tissue culture hosts pose similarly low risk because of the high level of biological containment of these host/vector systems.

### **Proposal 1.3 – Avipox vectors**

It is proposed that ‘*avipox vectors (attenuated vaccine strains)*’ be removed from item 4 in the list of host/vector systems for exempt dealings (Schedule 2 Part 2).

When the Regulations were made in 2001, avipox vectors (attenuated vaccine strains) were included in the list of host/vector systems for exempt dealings, in combination with animal or human cell culture hosts. If the donor nucleic acid meets the requirements set out in Schedule 2 Part 1 item 4 paragraph (2), dealings with these vectors in tissue culture hosts are currently classified as exempt.

A review of the characteristics of avipox vectors, as part of a wider review of viral vectors, identified that inclusion of these dealings in the exempt category is not in keeping with current viral vector classification, on the basis these vectors are able to transduce human cells. Further discussion of proposed changes to the regulation of dealings with viral vectors is contained in a separate discussion paper, *Review of the classification of viral vectors*. In order to manage potential risks to human health and safety and the environment, it is proposed that avipox vectors be removed from the list of host/vector systems for exempt dealings.

This proposal would result in dealings with avipox vectors being regulated in the same way as for other replication competent viral vectors able to transduce human cells, according to Schedule 3 of the Regulations. Dealings would be classified as either notifiable low risk dealings (NLRDs) or Dealings Not involving Intentional Release (DNIRs) depending on the characteristics of the inserted nucleic acid.

### **Proposal 1.4 – Non-vector systems**

It is proposed that the definition of a non-vector system be amended to read as follows:

*Non-vector system means a system in which donor nucleic acid is or was introduced into a host cell:*

- (a) in the absence of a nucleic acid-based vector; or*
- (b) using a nucleic acid-based vector in the course of a previous dealing and the vector is:
  - (i) no longer present; or*
  - (ii) present but cannot be remobilised from a host cell.**

It is proposed to amend the definition of a non-vector system to include situations where a vector was used to genetically modify a host but the vector is no longer present in, or able to be remobilised from, the host.

Dealings involving vectors which can be remobilised may pose specific risks associated with the ability of the vector to carry the donor nucleic acid into a new host cell, and it is appropriate that these dealings be regulated more stringently than dealings which do not involve nucleic acid-based vectors. However, once no free vector is present the risks posed by the dealing relate solely to the characteristics of the host and the donor nucleic acid, features which form the basis of classification of dealings with non-vector systems in Schedules 2 and 3. The proposed amendment would facilitate the regulation of dealings in accordance with the level of risk they pose, and allow similar categorisation of dealings which do not involve vectors which

can be remobilised (whether this is because no such vector was used, or because the vector is no longer present).

## **Proposal 2 – Animals with modified somatic cells**

For animals with somatic cells modified *in vivo* by a replication defective viral vector, it is proposed that subsequent dealings when the viral vector is no longer present in the animal and certain conditions are met be classified as exempt. This is proposed to occur by addition of a new item to Schedule 2, Part 1:

3A *A dealing with an animal whose somatic cells have been genetically modified in vivo by a replication defective viral vector, if:*

- (a) the in vivo modification occurred as part of a previous dealing; and*
- (b) the replication defective viral vector is no longer in the animal; and*
- (c) no germ-line cells have been genetically modified; and*
- (d) the somatic cells cannot give rise to infectious agents as a result of the genetic modification; and*
- (e) the animal is not infected with a virus that can recombine with the genetically modified nucleic acid in the somatic cells of the animal.*

Dealings with animals which have had some of their somatic cells modified *in vivo* by GM replication defective viral vectors are currently classified as at least NLRDs requiring physical containment level 2 (PC2). The current classification of such dealings focuses on properties of the viral vector and the donor nucleic acid it is carrying, which largely determine the risk associated with these dealings. However, once the viral vector is no longer present, the dealing no longer poses such risks.

Following inoculation of an animal with a viral vector, viral particles can transduce cells of the animal and the introduced nucleic acid can become stably incorporated into the somatic cells. In time any remaining unincorporated vector will be cleared from the animal through the action of its immune system. Once the viral vector is cleared from the animal, and if the vector is not able to be remobilised from the somatic cells, the only remaining risks from gene technology are those that relate to the modified somatic cells. These are the same risks as for dealings with an animal into which GM somatic cells have been introduced. Dealings with animals into which GM somatic cells have been introduced are classified as exempt provided that the somatic cells are not capable of giving rise to an infectious agent as a result of the genetic modification, and the animal is not infected with a virus which is capable of recombining with the genetically modified nucleic acid in the somatic cells.

It is proposed to include in the exempt category dealings with animals whose somatic cells have been genetically modified *in vivo* which no longer carry the modifying viral vector, and which meet similar requirements for classification as exempt dealings as those with animals into which GM somatic cells have been introduced. This would allow dealings with animals with modified somatic cells to be classified according to risk, regardless of the method by which the somatic cells were modified.

### **Proposal 3 – Volume of GMO culture**

It is proposed that the volume of culture of exempt host/vector systems which can be used in exempt dealings be increased from 10 to 25 litres.

Currently the maximum volume of culture of an exempt host/vector system which can be dealt with as an exempt dealing is 10 litres per single vessel. Above this volume these dealings are classified as PC2 NLRDs and must be conducted in certified PC2 large scale facilities.

An assessment of risk and its management, based on regulatory experience gained since the commencement of the Act, indicates that dealings involving cultures of up to 25 litres per vessel are suitable for categorisation as exempt. Such a change would result in dealings with cultures of exempt host/vector systems of up to 25 litres being classified as exempt. A corresponding change is proposed in Schedule 3 Part 2 which describes NLRDs suitable for PC2 facilities (see the separate discussion paper *Classification of Notifiable Low Risk Dealings*).

### **Proposal 4 – Oncogenic modifications**

It is proposed that dealings with GMOs involving oncogenic modifications in listed host/vector systems be allowed to be conducted as exempt dealings, by removing relevant prohibiting clauses in Schedule 2 Part 1 and Schedule 3 Part 1.

An oncogenic modification is defined in the Regulations as “*a genetic modification that is capable of inducing unregulated cell proliferation in a vertebrate cell*”. The classification of dealings involving oncogenic modifications has historically been guided by the need to protect laboratory workers. For example, there is a theoretical risk of a laboratory worker developing cancer if a viral vector carrying an oncogene transduces a cell of the laboratory worker, resulting in stable introduction and expression of the oncogene. For this reason, dealings involving both oncogenes and viral vectors able to transduce human cells have consistently been excluded from the exempt category. This has been achieved in a number of ways in subsequent amendments to the Regulations, most recently by excluding dealings involving oncogenic modifications from the exempt category.

It is proposed that the classification of exempt dealings be altered to include the cloning and expression of oncogenes in exempt host/vector systems. With the proposed removal of avipox vectors (see proposal 1.3 above) none of the host/vector systems permitted for use in exempt dealings will be able to transduce human cells. As a result, the potential for *in vivo* expression of an oncogene in a laboratory worker following exposure to a gene construct or a GMO would be extremely low.

The proposed amendment would also clarify the regulatory status of dealings with various cultured human and animal cell lines. Cell lines with an ‘oncogenic transformation’ (ie, that are able to grow and divide indefinitely in culture) are routinely used in laboratory research. However, many long-standing lines have uncertain histories and researchers do not always know how they were originally derived. Immortal cell lines currently in use in research have been produced either by transformation with a non-GM virus or by direct genetic modification. In both cases, the risk to the operator is negligible once the transforming gene is stably integrated, but the regulatory requirements currently differ depending on the provenance of the cell line. If oncogenic modifications were permitted in exempt dealings then dealings involving

these cell lines (provided they meet other requirements for classification as exempt dealings) could be conducted under standard laboratory conditions without the need to obtain specific authorisation, eg as an NLRD.

## **Appendix 1 Legislative background to exempt dealings**

### **What qualifies a dealing for classification as exempt?**

Exempt dealings are defined in the Regulations Part 3 Division 1, regulation 6:

- (1) *For subsection 32 (3) of the Act, a dealing, in relation to a GMO, is an exempt dealing if:*
  - (a) *it is a dealing of a kind mentioned in Part 1 of Schedule 2; and*
  - (b) *it does not involve a genetic modification other than a modification described in Part 1 of Schedule 2; and*
  - (d) *it does not involve an intentional release of the GMO into the environment; and*
  - (e) *it does not involve a retroviral vector that is able to transduce human cells.*
- (2) *For the avoidance of doubt, exemption under subregulation (1) does not apply to a dealing that does not comply with subregulation (1), whether or not that dealing is related to a dealing that does so comply.*

The *Explanatory Statement to Gene Technology Regulations 2001* outlines the approach taken in compiling the original listing of exempt dealings when the legislative scheme commenced in June 2001: “The exempt dealings with GMOs detailed in Schedule 2 are dealings with GMOs that have been assessed over many years by the Genetic Manipulation Advisory Committee as presenting negligible biosafety risks to public health and safety, including occupational health and safety, or to the environment.”

### **Approach to assessing proposals for exempt dealings**

The Regulator may review exemptions at any time under Section 141 of the Act, and the Act does not provide any requirements for such reviews. For the review which resulted in the Gene Technology Amendment Regulations 2006, the same approach as that required for NLRDs was adopted. Before declaring a class of dealings to be NLRDs, the Act requires the Regulator to consider: whether the GMO is biologically contained; whether the dealing with the GMO would involve minimal risk to the health and safety of people and to the environment, taking into account the properties of the GMO as a pathogen or pest and the toxicity of any proteins produced by the GMO; and whether no or minimal conditions would be necessary to be prescribed to manage any such risk (refer to Section 74 of the Act, and for more detail see the separate discussion paper *Review of the Classification of Notifiable Low Risk Dealings*). The same approach is adopted in this review.

### **Containment of exempt dealings**

The prescribed containment requirements for exempt dealings have changed with successive amendments to the Regulations, however a constant requirement has been that no intentional release of GMOs into the environment be involved. Under the original Regulations that operated from 21 June 2001 until 30 March 2007, exempt dealings were required to be conducted in accordance with Australian Standards AS/NZS 2243.3:1995 (Safety in laboratories: microbiology) for physical containment level 1 (PC1).

From 31 March 2007 to 30 June 2007, as a result of the Gene Technology Amendment Regulations 2006, exempt dealings had to be conducted in accordance with applicable

guidelines issued by the Regulator. These were based around PC1 in the Australian Standard and specifically modified to suit this class of dealings.

With the commencement of the Gene Technology Amendment Regulations 2007 on 1 July 2007 (which are currently in force), specific containment requirements for exempt dealings were removed, and as a result exempt dealings now must simply not involve intentional release of a GMO into the environment. This change implemented recommendation 6.1 from the Statutory Review of the Act, that there should be no legislative requirements on exempt dealings beyond listing in the Regulations. Exempt dealings can therefore be conducted under the same conditions as dealings with similar non-GM organisms, as determined to be appropriate by the operator and their organisation, provided that this does not involve release of GMOs into the environment.

Exempt dealings are not required to be conducted in facilities certified by the Regulator, and they may be conducted in a range of situations including, for example, university teaching laboratories or high school laboratories. However, exempt dealings should not be carried out in such a way that environmental release of a GMO is an inevitable or foreseeable consequence of the dealing. The OGTR has developed guidance on the containment of exempt dealings for the assistance of researchers and organisations, available on the OGTR website ([www.ogtr.gov.au](http://www.ogtr.gov.au)). This guidance is effectively equivalent to PC1 conditions. PC1 is the lowest containment level described in Australian Standards AS/NZS 2243.3:1995 (Safety in laboratories: microbiology) and is suitable for work with non-pathogenic organisms. Exempt dealings are limited to dealings with GMOs that are low risk, for which standard laboratory practice, as applied to non-GM non-pathogenic organisms, is considered appropriate.

## Appendix 2

### Hosts considered and not proposed for inclusion in the list of host/vector systems for exempt dealings

For a number of the hosts considered, risk assessments did not support these hosts being included in the list of host/vector systems for exempt dealings. These conclusions were reached for a range of reasons, including:

- the host may pose risks to the health and safety of some individuals (eg *Streptococcus gordonii* and *S. salivarius*)
- the host being a human pathogen (eg several *Leuconostoc* spp. are minor opportunistic pathogens, *Escherichia coli* 83972 has been linked with a case of urinary tract infection)
- the host may pose risks to the environment which require management (eg *Lactococcus garvieae* and *L. piscium*)
- there is a lack of information about potential negative effects for human health or the environment (eg *L. plantarum* and *L. raffinolactis*)
- there is a lack of information about the basis for attenuation of the host (eg *E. coli* W)
- the host can persist in the environment for extended periods of time (eg several *Leuconostoc* spp., T7 phage)
- the host provides poor biological containment (eg T7 phage can readily exchange genetic material with other T phages)
- only a short history of safe use is available (eg *E. coli* 83972)

One submission requested inclusion of several hosts which were considered and excluded during the 2006 review of the Regulations (*Salmonella typhi* Ly21a, *S. typhimurium* LT2, *Shigella flexneri* SFL124, *Xenorhabdus* spp., *Emericella nidulans*). Risk assessments prepared at that time were reviewed and the conclusions that these hosts not be included remain valid.

For host organisms for which risk assessments do not support inclusion in the list of host/vector systems for exempt dealings, further information would be required to support future proposals to schedule these hosts as exempt.