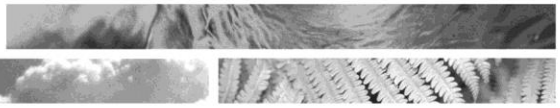
Environmental Protection Authority
Te Mana Rauhī Taiao

EPA staff report

Determining whether eukaryotic cell lines treated with double-stranded RNA are genetically modified organisms

March 2018



Advice to the Decision-making Committee on determination APP203395: – To determine whether eukaryotic cells that have been treated with chemically synthesised double-stranded RNA molecules for the purpose of inducing small interfering RNA (siRNA)-mediated gene silencing are new organisms for the purposes of the Hazardous Substances and New Organisms Act 1996

Executive summary and recommendation

In March 2018, Landcare Research in Lincoln (the applicant) requested that the Environmental Protection Authority (EPA) make a determination on whether or not eukaryotic cells that have been treated with chemically synthesised double-stranded RNA (dsRNA) to produce small interfering RNA (siRNA) molecules that target a specific gene (or genes) of interest, in order to transiently suppress the expression of that gene (or genes) are new organisms for the purposes of the Hazardous Substances and New Organisms (HSNO) Act 1996 (the Act). The applicant contends that eukaryotic cells so treated are not genetically modified, and therefore are not new organisms, because treatment with dsRNA does not integrate into the organism's genome, or cause any other inheritable change to the treated cells.

We have reviewed the information supplied by the applicant and information available in the public domain, and evaluated the eukaryotic cells treated with dsRNAs against the statutory criteria provided in the Act, the relevant regulations and other relevant legislation. We recommend that the dsRNA-treated eukaryotic cells or organisms that are not otherwise considered to be new, prohibited or unwanted are not to be regarded as genetically modified organisms, and therefore are not new organisms for the purpose of the Act.

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1. Introduction

Purpose of this document

1.1. This document has been prepared by EPA staff in the New Organisms team to advise the HSNO Decision-making Committee delegated to determine whether eukaryotic cells, treated with artificially synthesised double-stranded ribonucleic acid (dsRNA) molecules to temporarily suppress the expression of specific genes via small interfering RNA (siRNA)-mediated gene silencing, are considered new organisms for the purposes of the Hazardous Substances and New Organisms Act (1998) (the Act). This document discusses information provided by the applicant as well as information obtained from other readily available sources.

Application summary

1.2. Landcare Research (the applicant) seeks a determination under section 26 of the Act on whether eukaryotic cells treated with artificially synthesised dsRNA to transiently suppress the expression of user-selected genes are new organisms for the purpose of the Act. Section 26 of the Act provides that the Authority may determine whether or not any organism is a new organism.

1.3. The applicant contends that organisms treated with exogenous dsRNA are not genetically modified, since siRNA-mediated gene silencing only transiently suppresses gene expression in a very specific way, and it does not make any change to the genes or genetic material of the eukaryotic organism. The dsRNA is incapable of integrating into the genomes of eukaryotic organisms, and therefore cannot genetically modify the cell or the organism.

2. Summary of background information

RNA interference

2.1. RNA interference (RNAi) is a biological phenomenon first described in 1993, that is common to multicellular eukaryotes, and many, but not all, unicellular eukaryotes (Carthew & Sontheimer 2009). Although the evolution of RNAi mechanisms and proteins has taken widely divergent paths in eukaryotes, particularly between animals and plants, it is thought that all RNAi phenomena evolved from an ancestral

proto-siRNA system in the so-called “Last Eukaryotic Common Ancestor” (LECA) (Shabalina & Koonin 2008).

2.2. Because of the early evolutionary origin of RNAi, it is virtually universal amongst eukaryotes (Shabalina & Koonin 2008; Carthew & Sontheimer 2009). In many species, it is essential in regulating gene expression for proper development and survival of the organism (Shabalina & Koonin 2008), and as a means of protecting the integrity of the germline (Sato & Siomi 2013). However, some single-celled eukaryotic species, such as *Trypanosoma cruzi*, *Leishmania major*, and *Plasmodium falciparum* lack functional RNAi of any kind, and are thought to have lost their RNAi genes independently of each other (Shabalina & Koonin 2008).

2.3. There are three main categories of RNAi mechanisms/phenomena in eukaryotes:

- microRNA (miRNA), derived from eukaryotic non-protein-coding genes, and which regulate the expression of other genes in the same organism
- piwi-interacting RNAs (piRNA), are an animal-specific category of RNAi, which is involved in the suppression of expression of transposable elements in the gonads. Piwi-interacting RNAs are so named because of their specific interaction with Piwi proteins in animal germline cells (Shabalina & Koonin 2008; Sato & Siomi 2013).
- small interfering RNA (siRNA), which mediates either defence against viruses and foreign genes from outside the cell/organism (referred to as “exo-siRNAs” (Carthew & Sontheimer 2009)), or suppression of repetitive expressed sequences in the genome, such as transposons (referred to as “endo-siRNAs” (Carthew & Sontheimer 2009)).

Amongst these three RNAi categories, only siRNA is involved in processing RNAs from outside the cell, and targeting mRNA molecules that can base pair with the siRNA for destruction.

These exo-siRNAs (and *not* endo-siRNAs) are the subjects of this determination, and thus the basis of the remainder of this advice document. For additional clarity, this advice and accompanying draft decision document also do not apply to miRNAs or piRNAs, which only have endogenous sources within the cell.

Small interfering RNA and post-transcriptional gene silencing

2.4. As discussed in paragraph 2.1, it is thought that siRNA arose very early in the evolution of eukaryotes as a form of innate immunity/defence against viruses, which often have double-stranded RNA (dsRNA) intermediate forms as part of their replicative life cycle in the host cell (Shabalina & Koonin 2008). The proto-siRNA system is thought to have

consisted of a core triad of genes encoding the following proteins: Argonaute/Piwi (Ago), Dicer, and RNA-dependent RNA polymerase (RdRP). Broadly speaking, these proteins are involved in dsRNA recognition, processing, and amplification respectively. Together, these proteins enabled a flexible defensive response to essentially any dsRNA molecule introduced from outside the cell/organism (Shabalina & Koonin 2008), a function which has endured throughout most of eukaryotic evolution.

2.5. Regardless of whether it is introduced into a cell/organism, either manually, or via a natural viral infection, exogenous dsRNA in eukaryotic cells is recognised in the cell cytoplasm by cellular proteins of the Ago and Dicer families (Fig. 1; Carthew &

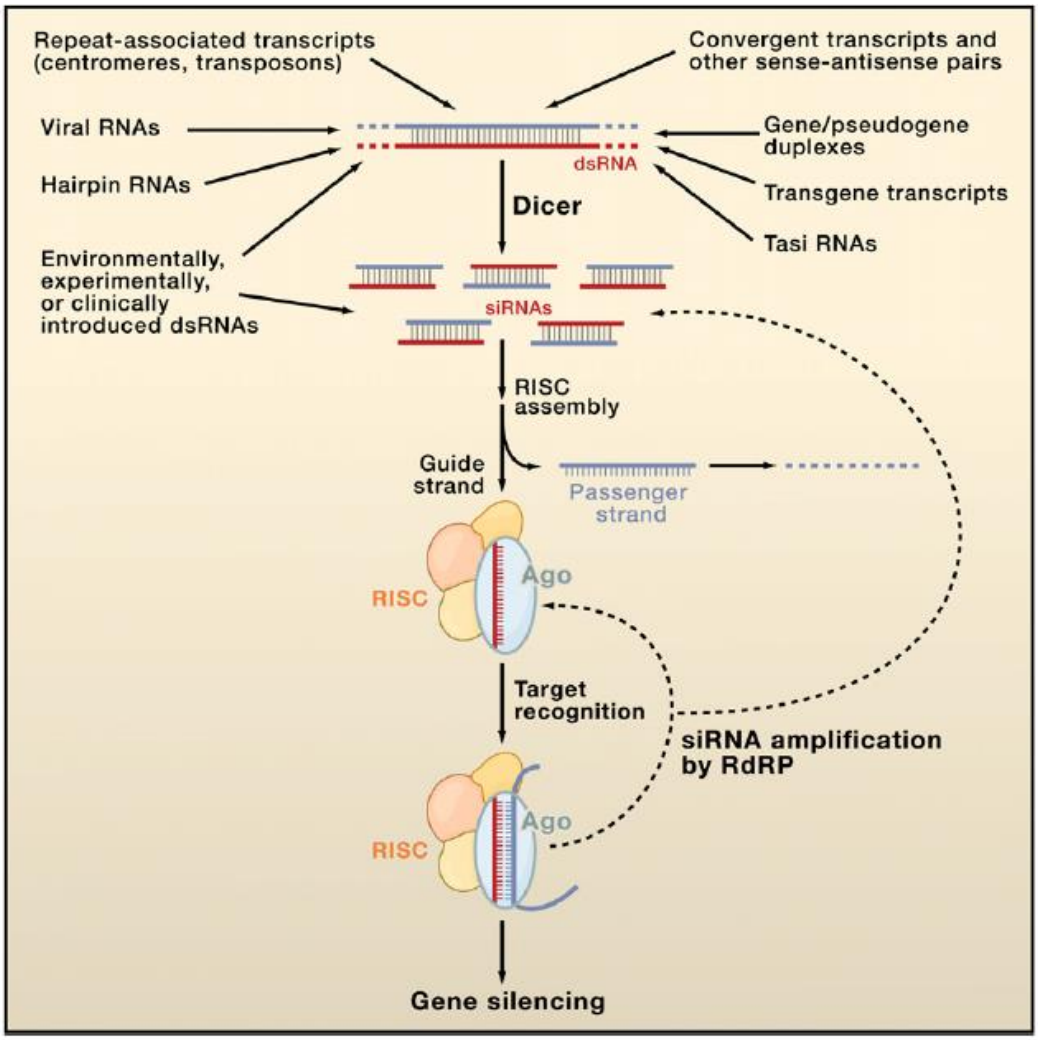

Figure 1: Sources and processing of dsRNA into siRNAs. Double-stranded RNAs can be derived from sources outside (environmental/experimental/clinically introduced dsRNA, including hairpin RNAs (which are the subjects of this determination)), and inside the cell (centromeres, transposons, sense-antisense pairs, gene/pseudogene duplexes, transgene transcripts, tasiRNAs). In all cases, the dsRNA is recognised and processed by a Dicer family member protein into 21-23 nt length dsRNA fragments, which are then assembled into an RNA-induced silencing complex (RISC), in which the “guide strand” is bound to an Ago family protein and other accessory proteins, which binds and recognises the target mRNA, which results in gene silencing. The so-called “passenger strand” is degraded. The RNAi response is amplified by RNA-dependent RNA polymerase (RdRP) to produce more RISCs. Excerpted from Carthew & Sontheimer (2009).

Sontheimer 2009). These proteins further process the dsRNAs into siRNAs, which are fragments of 21-23 nucleotides (nt) in length, with 2-base pair (bp) overhangs at their 3' ends (Fig. 1). These proteins eventually produce a single-stranded form of the siRNA that remains bound to an Ago family protein, generally with several accessory proteins, to produce an RNA-induced silencing complex (RISC). The siRNA-Ago RISC binds to cellular messenger RNA (mRNA) molecules via Watson-Crick base pairing. Binding of the RISC complex to its target mRNA blocks the translation of the mRNA into protein, and directs it to degradation by other cellular proteins (Fig.1), thus suppressing the expression of a specific gene (or genes, depending on the dsRNAs that are introduced into the cell).

2.6. When introduced (eg, transfected) into the organism/cell in the form of double-stranded RNA (referred to hereafter as dsRNA-treated eukaryotic cells), the resulting siRNA response can be used to test the effects of the lack of a gene product on the cell, in a transient way. The siRNA and its effect on the cell(s) is short-lived (ranging from 1 to 3 weeks), because it is eventually degraded by the cell's natural metabolic processes (Bartlett & Davis 2006; Mitter et al, 2017a). There is no vertical transmission of the siRNA from external dsRNA to progeny or daughter cells, as neither the dsRNA nor any form of the resulting exo-siRNA can integrate into the DNA of the eukaryotic cell genome, nor can it cause any other DNA sequence changes to the genome(s) of the cell(s) (see paragraph 2.8).

2.7. Small interfering RNA technology has developed to the stage where many companies offer ready-made siRNA kits for the exploration of the effects of gene silencing on aspects of a cell's or organism's functions, eg the Silencer® siRNA library kits (ThermoFisher 2018), or the "Trilencer-27" siRNA kit (OriGene 2018). In the past 10 years, researchers and companies have been exploring the use of siRNA as pesticides in agriculture, and as medicines to improve human and animal health. This includes the use of oral dsRNA uptake by insects (Gong et al, 2013; Borel 2017 and references therein), and as a means of inducing plant viral resistance (Borel 2017 and references therein; Mitter et al, 2017a and references therein). Efforts have also focused on the means of improving the stability of the dsRNA on and in plants (eg, Mitter et al, 2017b). Agrichemical companies such as Monsanto and Syngenta are on record as stating that they will have commercial RNAi-based products on the market by the early- to mid-2020s (Borel 2017).

Endo-siRNA inducers of siRNA responses and heterochromatin formation

2.8. There are many ways in which a siRNA response can be induced within a eukaryotic cell (Figure 1), in addition to viral and exogenous dsRNAs. Some of these can involve the transcription of DNA found within the cell, such as repeat-associated transcripts (ie transposons, centromeres (Carthew & Sontheimer 2009) and so-called paramutable genes (Arteaga-Vazquez & Chandler 2010)), gene/pseudogene[1] duplexes, transcripts from transgenes (ie, so-called "antisense" gene constructs in which the non-protein-coding strand is transcribed to induce gene silencing), so-called "tasiRNAs" (trans-acting siRNAs), and the more recently discovered endogenous hairpin RNAs (Carthew & Sontheimer 2009). Together, these sources of siRNA are referred to as endo-siRNAs, as a shorthand for siRNAs that have an endogenous source within the cell (see also Paragraph 2.3).

2.9. In eukaryotes, the millions to billions of base pairs of DNA in a chromosome are very long. For example, according to the McGraw-Hill Encyclopedia of Science and Technology (1997), the DNA complement of one human cell is estimated to be 2 metres in length if stretched out and laid end to end. In order to enable the chromosomal DNA to be condensed into something as small as a cell nucleus, the DNA is wrapped around proteins called histones (Djupedal & Ekwall 2009). This chromosome-histone complex is generally referred to as chromatin. Heterochromatin is a further condensed, transcriptionally inactive DNA structure that is much more densely packed than chromatin. Endo-siRNAs are known to sometimes induce the formation of heterochromatin (Carthew & Sontheimer 2009; Djupedal & Ekwall 2009; Götz et al, 2016). However, exo-siRNAs are not involved in this process, since heterochromatin formation requires gene transcription from DNA (Djupedal & Ekwall 2009). In contrast, the exo-siRNA-mRNA interaction takes place in the cytoplasm and the exo-siRNA RISC complex is not transported into the nucleus, and therefore transcriptional gene silencing cannot occur (Carthew & Sontheimer 2009; Djupedal & Ekwall 2009).

Double-stranded RNA is not reverse transcribed

2.10. A number of studies have reported a very low-level of random integration of introduced DNA sequences into the somatic cell genomes of mice and non-human primates (eg,

[1]Pseudogenes are generally derived from gene duplication events, after which one gene (the pseudogene) loses its function through mutational events.

Ledwith et al, 2000; Nowrouzi et al, 2012). Integration into the genome is always a possibility when DNA is introduced to a cell. This may occur during natural viral infection, through the action of a viral vector, or when DNA molecules such as plasmids[2] are introduced into the cell. These pieces of DNA can be randomly integrated into the cell's genome as a result of DNA strand breakage and the cell's natural repair mechanisms.

2.11. In order for any external dsRNA to be integrated into a cell's genome, it would first have to be reverse transcribed into DNA. Although siRNA phenomena involve the amplification of RNAi sequences via RNA-dependent RNA polymerase, there is always only a single strand of the RNA that is bound by an Ago family protein that mediates the translational silencing in the cytoplasm (Shabalina & Koonin 2008; Carthew & Sontheimer 2009). The siRNA construct and amplified siRNA molecules within the cell are eventually degraded, and translation of the targeted genes eventually returns to normal in the transfected cells, generally after one to three weeks, although this can vary, dependent on organism and cell type (Bartlett & Davis 2006; Mitter et al, 2017a).

2.12. Since there is no known mechanism for the introduced double-stranded RNA, or resulting siRNAs to be reverse transcribed into DNA, it is highly unlikely for the dsRNA or exo-siRNA to undergo reverse transcription and integration into a cell's genome that would allow replication by the eukaryotic cell. Therefore, in the event that a transfected eukaryotic cell divides while carrying dsRNA, the dsRNA would simply be diluted between the daughter cells.

The transfected dsRNA does not persist in the cell

2.13. As stated earlier at several points in this staff advice, introduced dsRNA has a short duration of approximately one to three weeks in eukaryotic cells and organisms, depending on the specific circumstances of the organism, the dsRNA, and how it is applied (Bartlett & Davis 2006; Mitter et al, 2017b; Mitter et al, 2017a). Following Watson-Crick binding of the RISC to its cognate mRNA in the cytoplasm, the entire complex is directed to degradation by internal cellular mechanisms, which are part of normal cell processes for the regulation of gene expression.

[2]A genetic structure in a cell that can replicate independently of the chromosomes, typically a small circular DNA strand in the cytoplasm of a bacterium.

2.14. Although there is an RdRP-mediated amplification process of siRNAs, (Fig. 1), so called primary siRNAs made from the initial processing of the dsRNA, are distinguishable in the cell from secondary siRNAs. Specifically primary siRNAs have 5'-monophosphate groups at their 5' ends, while secondary siRNAs have triphosphate groups at their 5' ends (Djupedal & Ekwall 2009). The secondary siRNAs are not used as templates for further activation by RdRP, thus avoiding a "runaway" amplification response. Thus, as the primary siRNA is degraded, the secondary siRNA amplification gradually becomes attenuated to the point of extinction. Therefore, suppression of expression of the user-targeted gene is transient and self-limiting, and the suppressed gene expression is not inheritable.

3. Evaluation against statutory criteria

3.1. We considered the dsRNA-treated eukaryotic cells against the statutory criteria set out in the Act to determine whether these cells are new organisms. To make a determination on the status of a new organism under section 26 of the Act, we must first determine whether the treated eukaryotic cells fit the definition of an "organism". The Act defines an organism as:

> ***Organism-***
>
> *(a) does not include a human being:*
>
> *(ab) includes a human cell:*
>
> *(b) includes a micro-organism:*
>
> *(c) includes a genetic structure, other than a human cell, that is capable of replicating itself, whether that structure comprises all or only part of an entity, and whether it comprises all or only part of the total genetic structure of an entity:*
>
> *(d) includes an entity (other than a human being) declared to be an organism for the purposes of the Biosecurity Act 1993:*
>
> *(e) includes a reproductive cell or developmental stage of an organism*

3.2. The relevant description which pertains to the dsRNA-treated eukaryotic cells described here is (ab) – an organism includes a human cell (which is also a eukaryotic cell), and (c) – an organism includes a genetic structure, other than a human cell, that is capable of replicating itself, whether that structure comprises all or only part of an entity, and whether it comprises all or only part of the total genetic structure of an entity. This includes any eukaryotic cell other than a human cell, regardless of whether that

cell is grown *in vitro*, or is an intact cell in a multicellular eukaryotic organism. The human and other eukaryotic cells to be treated thus fulfil the description of an organism for the purpose of the Act.

3.3. We next considered whether a dsRNA-treated eukaryotic cell fulfilled the definition of a new organism under the Act.

3.4. For an organism to be determined as "not new" under section 26 of the HSNO Act, the organism must be shown to lie outside the bounds of the definition of a new organism as defined in section 2A(1) of the HSNO Act; specifically, 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 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.

3.5. We further considered whether any other relevant legislation applied to the organisms under consideration. We found that Prohibited Organisms (Schedule 2 of the HSNO Act and Unwanted Organisms (section 2 of the Biosecurity Act 1993) are also beyond the scope of this determination.

3.6. Therefore, for the purpose of this determination, the organisms under assessment are eukaryotic organisms that are not already considered to be new, prohibited, or unwanted organisms, specifically: eukaryotic cells that have been treated with dsRNA, and:

- *were present in New Zealand immediately before 29 July 1998 (section 2A(1)(a));*
- *have not been prescribed as risk species (section 2A(1)(b));*
- *have not been approved to be held in containment or released with controls for treatment with dsRNAs (sections 2A(1)(c), (ca) and (cb));*

- *have not previously been genetically modified (section 2A(1)(d));*
- *are not members of species that have been eradicated from New Zealand (section 2A(1)(e));*
- *are not named in Schedule 2 of the HSNO Act, as set out in section 50(1) of the HSNO Act;*
- *are not unwanted organisms as defined in section 2 of the Biosecurity Act 1993;*

3.7. We next considered whether eukaryotic cells/organisms, that are not otherwise already considered to be new organisms, and that have been treated with externally applied dsRNA, would meet the definition of genetically modified organisms. We analysed the treated eukaryotic cells against the statutory criteria set out in the Act to determine whether such cells constitute genetically modified organisms.
The Act defines a genetically modified organism as:

> *"unless expressly provided otherwise by regulations, any organism in which any of the genes or other genetic material-*
>
> *(a) have been modified by* in vitro *techniques; or*
>
> *(b) are inherited or otherwise derived, through any number of replications, from any genes or other genetic material which has been modified by* in vitro *techniques."*

3.8. The Act does not provide a definition for genes or genetic material, however the Oxford English Dictionary defines 'genetic' as *"of genetics or genes; inherited. Of, in, or concerning origin; causal"*. Gene is defined as *"a unit of heredity composed of DNA or RNA and forming part of a chromosome etc., that determines a particular characteristic of an individual"*.

3.9. Given these definitions, we consider that "genes or genetic material" must be capable of being inherited by the progeny of the organism, or be capable of causing a characteristic or trait that can be inherited. We consider that the introduction of dsRNA into tissues or cells of a eukaryotic organism does not modify its genome or genetic material, as discussed in Paragraphs 2.10-2.12.

3.10. The Act does not define what is meant by *"in vitro* techniques*". In vitro* literally means 'in glass'. We consider that *in vitro* techniques are scientific techniques that occur outside a cell or organism in an artificial environment.

3.11. Information provided by the applicant indicates that the dsRNA may be created using *in vitro* techniques (see Section 2.1 of the application). Regardless of whether the dsRNA is created *in vitro* or *in vivo*, such molecules are not organisms, as they are unable to be replicated by the cell or any other mechanism, and the dsRNA molecules do not cause any permanent or heritable change to the treated cell or organism.

3.12. Therefore, we conclude that the treatment of eukaryotic organisms with dsRNA does not modify the 'genes or genetic material' of the organism by *'in vitro* techniques', and so dsRNA-treated eukaryotic organisms do not meet the definition of a GMO in the HSNO Act. Therefore eukaryotic organisms treated with dsRNA are not new organisms if the organisms satisfy the criteria described in paragraph 3.6 above.

3.13. The decision pathway for a section 26 determination is provided in Appendix 1.

4. Comments from MPI and DOC

4.1. EPA staff advised the Ministry for Primary Industries (MPI), and the Department of Conservation (DOC), of this request for a determination, and invited them to provide comments. DOC replied that the application did not appear to have any biodiversity implications, and therefore had no further comments. MPI did not respond to the invitation.

5. Prior EPA determination of relevance to APP203395

5.1. One determination (APP202786) was made by the EPA regarding the new organism status of primary human immune cells taken from a cancer patient, treated *in vitro* with specific mRNA molecules predicted to have an effect on the cancer, then returned to the patient for treatment. While the Decision-Making Committee (the Committee) evaluates each section 26 determination on the basis of its own merits, this earlier determination may be informative to the Committee, and may have bearing on this determination. The reasoning of the earlier Committee in this determination is discussed in greater detail in the following paragraph.

5.2. The Auckland District Health Board requested a determination as to whether human T lymphocyte cells (T cells) transfected with messenger RNA were considered to be genetically modified organisms for the purpose of the Act, and therefore new organisms. The Committee considered a human T cell to be an organism as defined in

the Act, but considered that the transfected RNA could not be integrated into the host T cell's genome, as the mRNA would first need to be reverse transcribed into DNA. The Committee noted that the T cell did not possess the necessary proteins for reverse transcription. The Committee noted that such exogenous mRNAs would be completely degraded by the cell within three to four days after transfection. The Committee concluded that the transfected RNA did not meet the definition of 'genes or genetic material' in the Act, and therefore the transfection of the mRNA into the T cell did not constitute 'genetic modification' as defined in the Act. Such transfected T cells were therefore determined not to be new organisms for the purpose of the Act.

6. Recommendation

6.1. Externally applied dsRNA, as described in the application and this advice document, cannot integrate into a treated eukaryotic organism's genome, and it only transiently and reversibly suppresses protein translation in the organism. Therefore, we recommend that eukaryotic organisms that are not otherwise already considered to be new, prohibited or unwanted organisms that have been treated with dsRNA do not meet the definition of genetically modified organisms, and therefore cannot be considered as new organisms for the purpose of the Act.

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8. Appendix 1: Decision path for section 26 determination

Figure 17: Decision pathway for applications under Section 26 for determination as to whether an organism is a new organism

Context

This decision pathway describes the decision-making process for applications under Section 26 for determination as to whether an organism is a new organism.

Introduction

The purpose of this decision pathway is to provide the HSNO decision maker[3] with guidance so that all relevant matters in the Hazardous Substances and New Organisms Act (1996) (the *Act*) and the Hazardous Substances and New Organisms (Organisms Not Genetically Modified) Regulations (1998) (the *Regulations*) have been addressed. It does not attempt to direct the weighting that the HSNO decision maker may decide to make on individual aspects of an application.

The decision pathway has two parts –

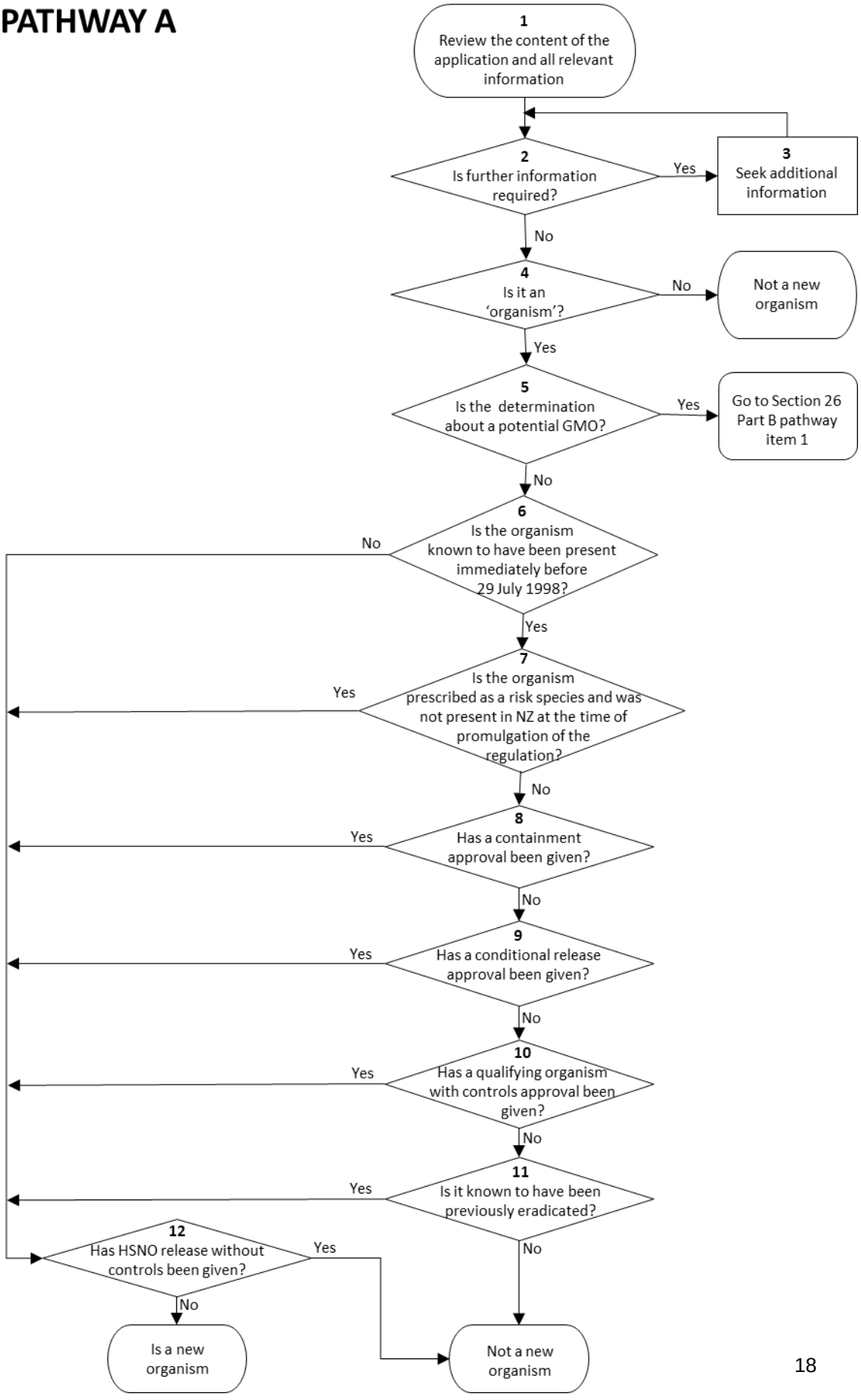
- Flowchart (a logic diagram showing the process prescribed in the HSNO Act and the Methodology to be followed in making a decision), and
- Explanatory notes (a discussion of each step of the process).

Of necessity the words in the boxes in the flowchart are brief, and key words are used to summarise the activity required. The explanatory notes provide a description of each of the numbered items in the flowchart, and describe the processes that should be followed.

For proper interpretation of the decision pathway it is important to work through the flowchart in conjunction with the explanatory notes.

[3] The HSNO decision maker refers to either the EPA Board or any committee or persons with delegated authority from the Board.

PATHWAY A


1
Review the content of the application and all relevant information
2
Is further information required?
Yes
3
Seek additional information
No
4
Is it an 'organism'?
No
Not a new organism
Yes
5
Is the determination about a potential GMO?
Yes
Go to Section 26 Part B pathway item 1
No
6
Is the organism known to have been present immediately before 29 July 1998?
No
Yes
7
Is the organism prescribed as a risk species and was not present in NZ at the time of promulgation of the regulation?
Yes
No
8
Has a containment approval been given?
Yes
No
9
Has a conditional release approval been given?
Yes
No
10
Has a qualifying organism with controls approval been given?
Yes
No
11
Is it known to have been previously eradicated?
Yes
No
12
Has HSNO release without controls been given?
Yes
No
Is a new organism
Not a new organism

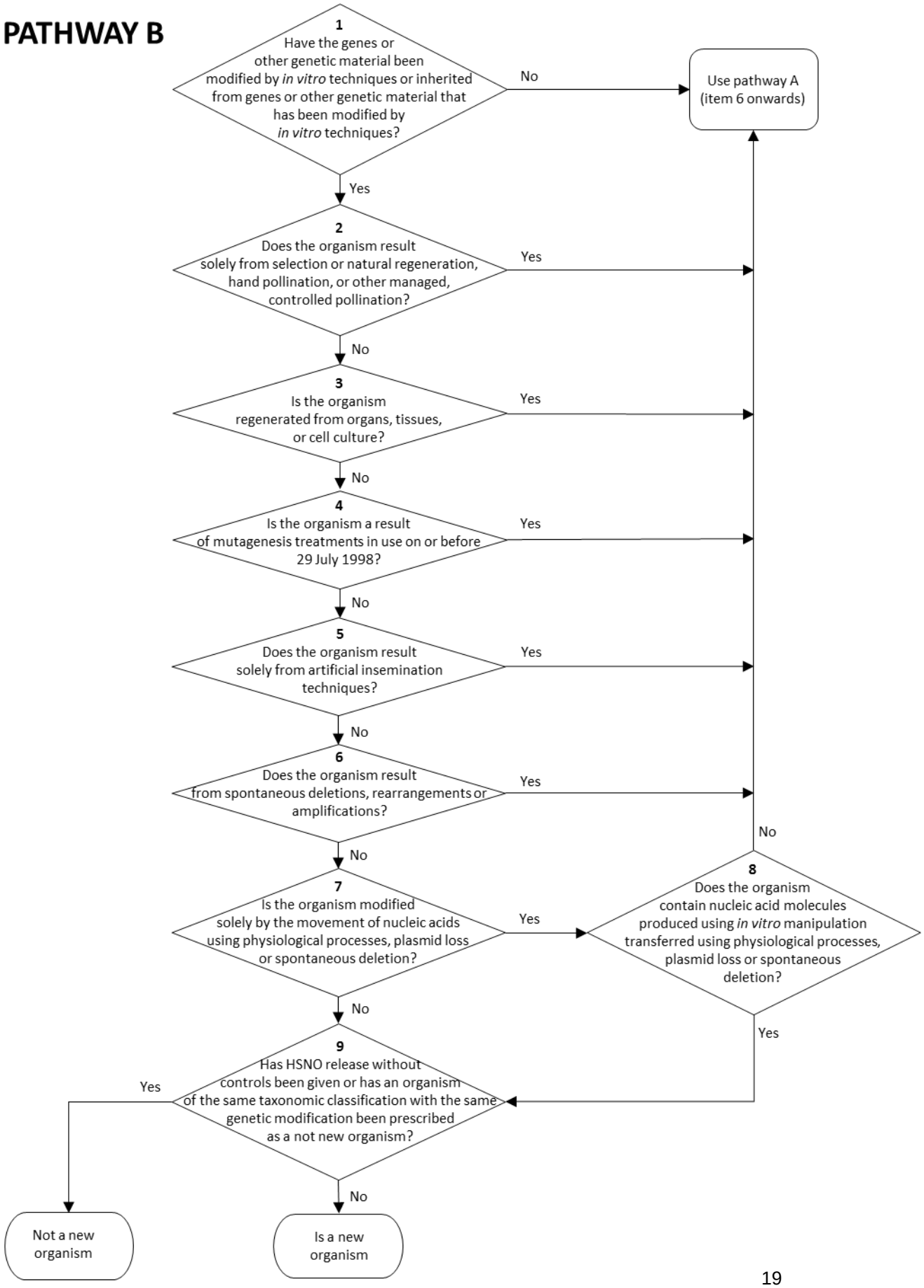

PATHWAY B
1
Have the genes or other genetic material been modified by *in vitro* techniques or inherited from genes or other genetic material that has been modified by *in vitro* techniques?
No
Use pathway A (item 6 onwards)
Yes
2
Does the organism result solely from selection or natural regeneration, hand pollination, or other managed, controlled pollination?
Yes
No
3
Is the organism regenerated from organs, tissues, or cell culture?
Yes
No
4
Is the organism a result of mutagenesis treatments in use on or before 29 July 1998?
Yes
No
5
Does the organism result solely from artificial insemination techniques?
Yes
No
6
Does the organism result from spontaneous deletions, rearrangements or amplifications?
Yes
No
No
7
Is the organism modified solely by the movement of nucleic acids using physiological processes, plasmid loss or spontaneous deletion?
Yes
8
Does the organism contain nucleic acid molecules produced using *in vitro* manipulation transferred using physiological processes, plasmid loss or spontaneous deletion?
No
Yes
9
Has HSNO release without controls been given or has an organism of the same taxonomic classification with the same genetic modification been prescribed as a not new organism?
Yes
No
Not a new organism
Is a new organism

Figure 17 Explanatory Notes

Section 26 pathway A

Item 1	**Review the content of the application and all relevant information** Review the application, staff advice and any relevant information held by other Agencies, and advice from experts.
Item 2	**Is further information required?** Review the information and determine whether or not there is sufficient information available to make a decision.
Item 3	**Seek additional information (Section 52 and Section 58)** If the HSNO decision maker considers that further information is required, then this may be sought either from the applicant (if there is an external applicant) or from other sources. If the HSNO decision maker considers that the information may not be complete but that no additional information is currently available, then the HSNO decision maker may proceed to make a determination. If the application is not approved on the basis of lack of information (or if the organism is considered new) and further information becomes available at a later time, then the HSNO decision maker may choose to revisit this determination.
Item 4	**Is it an organism (i.e. fits the "organism" definition in Section 2)?** An organism (a) does not include a human being: (ab) includes a human cell: (b) includes a micro-organism: (c) includes a genetic structure, other than a human cell, that is capable of replicating itself, whether that structure comprises all or only part of an entity, and whether it comprises all or only part of the total genetic structure of an entity: (d) includes an entity (other than a human being) declared to be an organism for the purposes of the Biosecurity Act 1993: (e) includes a reproductive cell or developmental stage of an organism If yes, go to item 5. If no, as this is not an organism, it is not regulated under the new organism provisions of the HSNO Act.
Item 5	**Is the determination about a potential GMO (Section 2A(1)(d))?** If the determination relates to whether an organism is a potential GMO, go to pathway B. If the organism is not a GMO, go to item 6.
Item 6	**Does the organism belong to a species that was known to be present in NZ immediately before 29 July 1998 (Section 2A(1)(a))?**

	Determine on the basis of the available information whether on balance of probabilities the organism is known to belong to a species that was present in New Zealand immediately prior to 29 July 1998. For the purposes of making a Section 26 determination an organism is considered to be present in New Zealand if it can be established that the organism was in New Zealand: (a) immediately before 29 July 1998; and (b) not in contravention of the Animals Act 1967 or the Plants Act 1970 (excluding rabbit haemorrhagic disease virus, or rabbit calicivirus). If yes, go to item 7 to test the organism against the next criterion. If no, go to item 12.
Item 7	**Is the organism prescribed as a risk species and was not present in New Zealand at the time of promulgation of the relevant regulation (Section 2A(1)(b))?** Determine whether the organism belongs to a species, subspecies, infrasubspecies, variety, strain, or cultivar that has been prescribed as a risk species by regulation established under Section 140(1)(h) of the Act. If the organism is prescribed as a risk species, determine whether it was present in New Zealand when it was prescribed. The organism is a new organism if it was not present in New Zealand at the time of the promulgation of the relevant regulation. Note: at this point it may become apparent that the organism is an unwanted organism under the Biosecurity Act. If this is the case, then MPI and DOC may be advised (they may already have been consulted under items 1, 2 and 3). If yes, go 12. If no, go to item 8 to test the organism against the next criterion.
Item 8	**Has a containment approval been given for the organism under the Act (Section 2A(1)(c))?** For the purposes of making a Section 26 determination, this will also include the following organisms which are “deemed” to be new organisms with containment approvals under the HSNO Act: (a) animals lawfully imported under the Animals Act 1967 before 29 July 1998 pursuant to Section 254 of the HSNO Act; (b) animals lawfully present in New Zealand in a place that was registered as a zoo or circus under the Zoological Garden Regulations 1977 pursuant to Section 255 of the HSNO Act (except where other organisms of the same taxonomic classification were lawfully present outside of a zoo or circus –see section 2A(2)(c)); (c) hamsters lawfully imported under the Hamster Importation and Control Regulations 1972 pursuant to Section 256 of the HSNO Act; or (d) plants lawfully imported under the Plants Act 1970 before 29 July 1998 pursuant to Section 258 of the HSNO Act. If yes, go to item 12. If no, go to item 9 to test the organism against the next criterion.
Item 9	**Has a conditional release approval been given for the organism (Section 2A(1)(ca))?** If yes, go to item 12. If no, go to item 10 to test the organism against the next criterion.
Item 10	**Has a qualifying organism with controls approval been given for the organism (Section 2A(1)(cb))?**

	A "qualifying organism" is an organism that is or is contained in a "qualifying medicine" or "qualifying veterinary medicine". These terms are defined in Section 2 of the HSNO Act. If yes, go to item 12. If no, go to item 11 to test the organism against the next criterion.
Item 11	**Is the organism known to have been previously eradicated (Section 2A(1)(e))?** Determine whether the organism belongs to a species, subspecies, infrasubspecies, variety, strain, or cultivar that is known to have been previously eradicated. Eradication does not include extinction by natural means but is considered to be the result of a deliberate act. If yes, go to item 12. **If no, then the organism is not a new organism.**
Item 12	**Has HSNO release approval without controls been given for an organism of the same taxonomic classification under Sections 35, 38 or 38I of the Act or has an organism of the same taxonomic classification been prescribed as a not new organism (Section 2A(2)(a))?** If a release approval has been given for an organism of the same taxonomic classification under Section 35 or 38 of the Act then the organism is not a new organism. If a release approval has been given for an organism of the same taxonomic classification under Section 38I of the Act **without controls** then the organism is not a new organism, however, if this approval has been given **with controls** then it is a new organism. If an organism of the same taxonomic classification has been prescribed by regulations as not a new organism[4] then it is not a new organism. **If yes, the organism is not a new organism.** **If no, the organism is a new organism.**

[4] http://www.legislation.govt.nz/regulation/public/2009/0143/latest/whole.html#DLM2011201

Section 26 pathway B

Item 1	**Have the genes or other genetic material been modified by *in vitro* techniques or inherited from genes or other genetic material that has been modified by *in vitro* techniques?** If yes, go to item 2. If no, the organism is not a genetically modified organism. However, you must check whether it meets the other new organism criteria so go to Pathway A item 6 onwards.
Item 2	**Does the organism result solely from selection or natural regeneration, hand pollination, or other managed, controlled pollination (Regulation 3(1)(a) of the Regulations)?** Is the organisms solely the result of selection or natural regeneration, hand pollination, or other managed, controlled pollination? If yes, the organism is not a GMO. However, you must check whether it meets the other new organism criteria so go to Pathway A item 6 onwards. If no, go to item 3.
Item 3	**Is the organism regenerated from organs, tissues, or cell culture (Regulation 3(1)(b) of the Regulations)?** Is the organism regenerated from organs, tissues, or cell culture, using any of the following techniques: selection and propagation of somaclonal variants, embryo rescue, and cell fusion (including protoplast fusion)? If yes, the organism is not a GMO. However, you must check whether it meets the other new organism criteria so go to Pathway A item 6 onwards. If no, go to item 4.
Item 4	**Is the organism a result of mutagenesis treatments in use on or before 29 July 1998 (Regulation 3(1)(ba) of the Regulations)?** Is the organisms the result of mutagenesis that uses a chemical or radiation treatment that was in use on or before 29 July 1998? If yes, the organism is not a GMO. However, you must check whether it meets the other new organism criteria so go to Pathway A item 6 onwards. If no, go to item 5.
Item 5	**Does the organism result solely from artificial insemination techniques (Regulation 3(1)(c) of the Regulations)?** Is the organism solely the result of artificial insemination, superovulation, embryo transfer, or embryo splitting? If yes, the organism is not a GMO. However, you must check whether it meets the other new organism criteria so go to Pathway A item 6 onwards. If no, go to item 6.
Item 6	**Does the organism result from spontaneous deletions, rearrangements or amplifications (Regulation 3(1)(e) of the Regulations)?** Is the organism a result of spontaneous deletions, rearrangements, and amplifications within a single genome, including its extrachromosomal elements? If yes, the organism is not a GMO. However, you must check whether it meets the other new organism criteria so go to Pathway A item 6 onwards.

	If no, go to item 7.
Item 7	**Is the organism modified solely by the movement of nucleic acids using physiological processes, plasmid loss or spontaneous deletion (Regulation 3(1)(d) of the Regulations)?** Is the organism modified solely by the movement of nucleic acids using physiological processes, including conjugation, transduction, and transformation, or by plasmid loss or spontaneous deletion? If yes, go to item 8. If no, go to item 9.
Item 8	**Does the organism contain nucleic acid molecules produced using in vitro manipulation transferred using physiological processes, plasmid loss or spontaneous deletion (Regulation 3(2) of the Regulations)?** Are nucleic acid molecules produced using in vitro manipulation transferred using any of the techniques referred to in item 7? If yes, go to item 9. If no, the organism is not a GMO. However, you must check whether it meets the other new organism criteria so go to Pathway A item 6 onwards.
Item 9	**Has HSNO release approval without controls been given or has an organism of the same taxonomic classification with the same genetic modification been prescribed as a not new organism (Section 2A(2)(b))?** If a release approval has been given for an organism of the same taxonomic classification with the same genetic modification under Section 38 of the HSNO Act then the organism is not a new organism. If a release approval has been given for an organism of the same taxonomic classification with the same genetic modification under section 38I of the HSNO Act **without controls** then the organism is not a new organism, however, if this approval has been given **with controls** then it is a new organism. If an organism of the same taxonomic classification with the same genetic modification has been prescribed by regulations as not a new organism[5] then it is not a new organism. **If yes, the organism is not a new organism.** **If no, the organism is a new organism.**

[5] http://www.legislation.govt.nz/regulation/public/2009/0143/latest/whole.html#DLM2011201