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Biosafety by definition: an analysis of the New Zealand Environmental Protection Authority's reasons for not classifying organisms treated with double-stranded RNA as genetically modified or new organisms

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Abstract

The New Zealand Environmental Protection Authority (EPA) issued a Decision that makes the use of externally applied double-stranded (ds)RNA molecules on eukaryotic cells or organisms technically out of scope of legislation on new organisms, because in its view the treatment does not create new or genetically modified organisms. dsRNA molecules can be potent gene regulators in eukaryotes, causing what is known as RNA interference. RNA-based technology holds promise for addressing complex and persistent challenges in public health, agriculture and conservation but also raises the threat of unintended consequences. The Decision rests on their conclusion that dsRNA treatments do not modify genes or other genetic material and are therefore not heritable. The EPA conclusion is not consistent with the totality of peer-reviewed research on dsRNA or industry claims. The Decision applies to nearly all eukaryotes, however, the EPA relied upon knowledge of relatively few eukaryotes and its analysis neglected known exceptions. The Decision also has not taken into account the unique eukaryotic biodiversity of the country, much of which is still to be described. The regulator has potentially created precedent-setting definitions of previously undefined or alternatively defined key terms that trigger obligations under binding international agreements, in addition to domestic legislation. Finally, by placing no restriction on the source or means of modifying the dsRNA, the EPA removed regulatory oversight that could prevent the accidental release of viral genes or genomes. This article examines the scientific evidence, conclusions and recommendations of the EPA and also presents some additional options.

Introduction

In May 2018 the Decision-Making Committee of the New Zealand Environmental Protection Authority (EPA) published a 4-page announcement concluding that eukaryotic cells or organisms treated with double-stranded (ds)RNA are not new organisms (EPA, 2018a). This critical determination has implications for the regulation of new biotechnologies in New Zealand because there is growing interest in the development of dsRNA for use in medicine (Lam, 2012) and agriculture, such as for pest control (Sammons *et al.*, 2011; Van *et al.*, 2011; Whyard *et al.*, 2011; Huang *et al.*, 2018).

Environmental biotechnologies are regulated by the EPA under the 1996 Hazardous Substances and New Organisms (HSNO) Act (Hazardous Substances and New Organisms Act, 1996). The EPA can regulate on the basis that an organism that has been treated with dsRNA is a new organism, or instead categorize RNA as a chemical that could be a hazardous substance.

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38 RNA is not now and unlikely ever to be listed as a hazardous substance. This is deduced
39 from the observation that none of the terms RNA, dsRNA, ribonucleic acid, or siRNA return
40 anything in a search of the EPA's databases: "Approved hazardous substances with
41 controls", "Chemical Classification and Information Database", or "New Zealand Inventory
42 of Chemicals." Moreover, the Ministry of Primary Industries places RNA in the "Neglible
43 Risk Register" (MPI, 2018).

44 Therefore if dsRNA-treated organisms are to be regulated at all, they must be under the
45 country's biosecurity laws[*] directed at pathogens and pests, or as new organisms by the
46 HSNO Act. A new organism may be in a species or sub-species new to New Zealand and/or
47 be a genetically modified organism of any species (full definition in Supplemental Material).
48 Here I analyze the routine case where an organism that is not new (or considered to be a
49 biosecurity threat) is treated with dsRNA, and whether that treatment results in the
50 organism being a new organism under the HSNO Act, by creating a genetically modified
51 organism.

52 The HSNO Act says that a "genetically modified organism means, unless expressly provided
53 otherwise by regulations, any organism in which any of the genes or other genetic
54 material—(a) have been modified by *in vitro* techniques; or (b) are inherited or otherwise
55 derived, through any number of replications, from any genes or other genetic material
56 which has been modified by *in vitro* techniques" (Hazardous Substances and New
57 Organisms Act, 1996).

58 New Zealand is harmonized to the Cartagena Protocol on Biosafety (the Protocol) (CBD)
59 through its HSNO Act. The Protocol is an international treaty on the transboundary
60 movement of products of modern biotechnology, including living genetically modified
61 organisms. The Protocol does not apply to food and pharmaceutical products that are
62 unable to survive in the environment. The Protocol definition of a living modified organism
63 is "any living organism that possesses a novel combination of genetic material obtained
64 through the use of modern biotechnology." The Protocol definition of modern
65 biotechnology is "the application of: a. *In vitro* nucleic acid techniques, including
66 recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or
67 organelles, or b. Fusion of cells beyond the taxonomic family, that overcome natural
68 physiological reproductive or recombination barriers and that are not techniques used in
69 traditional breeding and selection."

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[*] Biosecurity is overseen by the Ministry of Primary Industries in New Zealand, and refers to "stopping pests and diseases at the border, before they get to New Zealand, and eradicating or managing the impact of those already here." **MPI**. Biosecurity. https://www.mpi.govt.nz/law-and-policy/legal-overviews/biosecurity/. Access date, 26 June 2018

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70 HSNO Act language is similar to—but consequentially different from—the Protocol. Both
71 make reference to *in vitro* techniques, but the Protocol emphasizes the use of nucleic acids
72 (eg dsRNA is a nucleic acid) whereas the HSNO Act emphasizes modification of genes and
73 other genetic material. dsRNA can be relevant to New Zealand law if its use modifies genes
74 or other genetic material, for example by being a type of mutagen or by becoming part of
75 the genome as in the use of transgenes. The HSNO Act defines neither genes nor genetic
76 material, so whether or not dsRNA treatments are in its scope is not made clear from
77 definitions.

78 The Convention on Biological Diversity provides some guidance by defining genetic
79 material (CBD). This is the parent treaty to the Protocol. New Zealand is a Party to both. The
80 Convention on Biological Diversity defines genetic material as "any material of plant,
81 animal, microbial or other origin containing functional units of heredity" and genetic
82 resources as "genetic material of actual or potential value." This is also how the
83 International Treaty on Plant Genetic Resources for Food and Agriculture defines both plant
84 genetic resources and plant genetic material (ITPGRFA).

85 Therefore, genetic resources are a special kind of genetic material, one that has actual or
86 potential value. Genetic resources are described as such things as organisms, seeds, zygotes
87 and cuttings (Europa; FAO). They include the nucleic acids such as DNA but are not
88 exclusive to them. None of these international or domestic legal instruments defines the
89 terms "genes" or "functional units of heredity." Nor do these instruments or domestic law
90 define the term "modify."

91 EPA received an application to determine if "eukaryotic cells treated with synthetic double
92 stranded RNA" were new organisms (Trought, 2018). As the regulatory authority, the EPA
93 makes two kinds of decisions (Fig. 1). The first kind is whether or not a regulated organism
94 or substance is safe to use or how it could be safe to use. This follows from a mandatory risk
95 assessment. The second kind, *which is the kind relevant to this article*, is whether an
96 organism is regulated. If it is not regulated, then a risk assessment by the Authority will not
97 be done regardless of whether or not a risk assessment would be useful. Therefore, the
98 focus of this article is not on what potential hazards may arise from the use of dsRNA, or
99 how to assess and mitigate putative hazards, which are covered elsewhere (eg Heinemann
100 *et al.*, 2013; FIFRA, 2014). The focus of this article is on the scientific information used by
101 the EPA to determine that dsRNA treatments do not result in the kinds of effects that make
102 an organism new or genetically modified.

103 Following a brief overview of RNAi, I will explore the scope of the Decision and then analyze
104 the major arguments and information sources used by the Decision-Making Committee and
105 EPA staff. The main reason for determining that treatments using dsRNA did not result in
106 new organisms was that externally applied (exo-)dsRNA is not inherited by the organism
107 (Fig. 1). The Committee identified several factors that prevented inheritance. These factors

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108 were that exo-dsRNA molecules could not enter the nucleus, they are not reverse
109 transcribed into DNA, and for both of these reasons they therefore could not integrate into
110 the DNA of the genome and modify it, and by implication only DNA and only the DNA in the
111 nucleus was heritable genetic material (paragraph 4.6 of Ref EPA, 2018a).

112

113 **The Science of RNAi**

114

115 RNA interference (RNAi) is a form of gene regulation in eukaryotes with many potential
116 biotechnological applications being discussed by regulators worldwide. (Heinemann *et al.*,
117 2013; FIFRA, 2014) RNAi pathways are found in nearly all eukaryotes (Agrawal *et al.*,
118 2003). RNAi is often referred to as gene silencing, but it also is known to sometimes cause
119 an increase in the expression of genes (Carthew and Sontheimer, 2009; Kim *et al.*, 2009).

120 RNAi results in what is called post-transcriptional gene silencing and transcriptional gene
121 silencing. Post-transcriptional gene silencing occurs through dsRNA-mediated
122 endonucleolytic cleavage or exonucleolytic destruction of the transcript or inhibition of
123 translation of the transcript (Carthew and Sontheimer, 2009; Rechavi, 2014). In some
124 organisms, dsRNA-mediated transcriptional gene silencing is caused by the modification of
125 histones and DNA, while in others it may only be modification of histones, resulting in
126 formation of heterochromatin and a decrease in transcription (Matzke and Birchler, 2005).

127 The nomenclature for dsRNAs is expansive, but the main classes include siRNA (short-
128 inhibitory RNA), miRNA (microRNA) and piwi-interacting RNAs (piRNA) (Carthew and
129 Sontheimer, 2009; Ghildiyal and Zamore, 2009). These types are foundation substrates in
130 biochemical pathways involving Argonaute proteins that cause RNAi.

131 The nomenclature should be used as an indicative guide to biogenesis of the dsRNA, but not
132 the activity of the active form. This is because regardless of their source, dsRNAs share the
133 same pathways in the cell (Ghildiyal and Zamore, 2009). "For example, siRNA is able to
134 mimic microRNA (miRNA) to inhibit translation or elicit the degradation of [messenger
135 RNAs] with partial sequence complementarity" (Zhou *et al.*, 2014).

136 All three active forms derive from longer dsRNAs. Cytoplasmic Dicer converts the longer
137 form of siRNA and miRNA into the active form of about 21-23 nucleotides. Argonaute
138 proteins bind to the RNA and carry out the regulatory functions (Carthew and Sontheimer,
139 2009). Drosha (or Dcl1) acts in the nucleus to process pri-miRNA into pre-miRNA, which
140 after transport to the cytoplasm is further processed to miRNA by Dicer (Kim *et al.*, 2009).

141 Once associated with the Argonaute proteins, one strand of the dsRNA molecule is degraded
142 and the other serves to guide the protein complex to its target. Some eukaryotic species
143 have Argonaute proteins that can bind either miRNA or siRNA, and some that specialize in

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144 one or the other, while other species have Argonaute proteins that distinguish between
145 miRNA and siRNA based on the structural features of the dsRNA.

146 It is the Argonaute proteins that determine the mechanism of silencing (Rechavi, 2014;
147 Rankin, 2015). Some Argonaute proteins, such as AGO2 in humans, have an endonuclease
148 activity called slicer. These complexes cleave the target messenger RNA molecule. Human
149 Argonaute proteins AGO1-4 and AGO1 of *Drosophila melanogaster* are examples that cause
150 translational inhibition or degradation of the target transcript through exonucleolytic
151 decay. Ago1 of *Schizosaccharomyces pombe* and AGO4 and AGO6 of *Arabidopsis thaliana* are
152 examples that cause transcriptional gene silencing through heterochromatin formation
153 (Kim *et al.*, 2009).

154 The dsRNA is sorted amongst competing Argonaute proteins according to the number of
155 mismatches and bulges, not because particular dsRNAs are genetically determined to
156 exclusively follow pathways dedicated to miRNA or siRNA (Ghildiyal and Zamore, 2009). In
157 *Drosophila*, AGO1 tends to favour duplexes with more bulges and mismatches and results in
158 translation inhibition while AGO2 prefers duplexes with near perfect complementarity and
159 results more often in messenger RNA cleavage (Ghildiyal and Zamore, 2009). However,
160 even these rules are different between animals such as *Drosophila* and plants (Ghildiyal and
161 Zamore, 2009) making it difficult to generalize for all eukaryotes. In short, intending a
162 particular dsRNA to be an siRNA does not mean that it will be.

163 The binding strength of the guide strand and target influences the outcome of the
164 interaction. The combination of near perfect antisense pairing between guide strand and
165 target involving an Argonaute with slicer activity results in strand cleavage by an
166 endonuclease activity (Massirer and Pasquinelli, 2013). The larger the number of
167 mismatches between the guide and target RNA, the more likely the silencing will be caused
168 by exonucleolytic decay or translational inhibition (Massirer and Pasquinelli, 2013).

169 While endo-siRNA, miRNA and piRNA may be born differently, they are not reliably
170 distinguished by the silencing biochemistry. Both miRNA and piRNA arise from
171 transcription of genomic DNA. Although this can also be true for siRNA, such as from
172 transgenes or transposons (endo-siRNAs), the term is also often reserved for exo-siRNAs
173 even if they have a hairpin structure. In general, miRNAs are not transcribed from the
174 protein coding region of a gene and may have more mismatches with their targets. The
175 converse is true for siRNAs. Thus, miRNA, piRNA and endo-siRNA all first appear in the
176 nucleus and exo-siRNA does not (Carthew and Sontheimer, 2009).

177 It is not possible to confidently extrapolate the outcome of exposure to exo-siRNA based on
178 similarity of nomenclature to endo-siRNA. Because of differences between organisms and
179 differentiated cell types, generalizations based even on the structure of the dsRNA molecule
180 often fail.

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181 As the nature and source of the dsRNA applied as exo-siRNA is undefined by the EPA in its
182 Decision, I will often use the term exo-dsRNA as a more generic description than exo-siRNA
183 in this analysis.

184

185 **The Decision**

186

187 The Committee's Decision in context is about the use of exo-dsRNA for the purpose of
188 causing RNAi. However, analysis of the Decision is made more complicated because the EPA
189 Decision-Making Committee described the application in various, and significantly different,
190 ways (Table S1) and different to the descriptions provided by either the applicant (Trought,
191 2018) or EPA staff (EPA, 2018b).

192 Moreover, the Decision does not preclude the use of dsRNA that might result in other kinds
193 of effects either inadvertently or on purpose. dsRNA (and RNA in general) can have effects
194 on organisms (eg Kalluri and Kanasaki, 2008; Kleinman *et al.*, 2008) other than RNAi,
195 including heritable effects that are not associated with RNAi. After all, RNA is itself the
196 material of genes, such as in RNA viruses and retroviruses. These viruses can have either
197 dsRNA or single-stranded RNA genomes. They replicate independently of human
198 intervention once inside a eukaryotic cell. Genetic information can pass from them to a DNA
199 genome, and back.

200 Research on dsRNA-mediated gene regulation has advanced rapidly, but there is much still
201 unknown about its biochemistry, even in the relatively few model organisms in which it has
202 been studied (Djupedal and Ekwall, 2009; Ghildiyal and Zamore, 2009). Already it is clear
203 that dsRNA-mediated gene regulation biochemistry is different between plants, animals,
204 and fungi (Ghildiyal and Zamore, 2009). Perhaps even more importantly, almost nothing is
205 known about RNAi pathways in species unique to New Zealand. According to the
206 Encyclopedia of New Zealand, Te Ara, "over 80% of the 2,500 species of native conifers,
207 flowering plants and ferns are found nowhere else." "The best guess of the numbers of land-
208 based native plants and animals is around 70,000 species. Insects and fungi dominate, each
209 having an estimated 20,000 species – many are not yet described" (Various).

210 *Other* exposures

211 The Decision could remove any need to notify the public of their potential exposures.
212 Potential unavoidable exposures of non-eukaryotic organisms, such as bacteria, to RNA that
213 could result in effects other than RNAi also were not evaluated in the documents released
214 by EPA. Small RNA molecules are gene regulatory agents in bacteria, but do not use the
215 biochemistry of RNAi (Papenfort and Vanderpool, 2015; Mars *et al.*, 2016). The intercellular
216 trafficking of regulatory RNA molecules indicates that exo-RNA is relevant to their biology
217 too (Sjöström *et al.*, 2015).

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218 Regulatory RNA in bacteria influence the transition from planktonic to biofilm growth
219 (Ashley *et al.*, 2017) and colonization of the intestine by pathogens (Han *et al.*, 2017). RNA
220 molecules serve as guides for the action of the nuclease Cas9 in the CRISPR/Cas9 system
221 (Marraffini and Sontheimer, 2010). Exo-dsRNA secreted by intestinal cells has been
222 implicated in adjusting the growth rate of different species of bacteria in the human gut (Liu
223 *et al.*, 2016). Nowhere in nature, and even rarely in the laboratory, would eukaryotic
224 organisms (as opposed to tissue culture cells) be free of prokaryotes.

225 *Kinds* of RNA Molecules and Treatments

226 The Committee did not address the physical description of the dsRNA in the approved
227 treatments. The applicant sought permission to use "synthetic" dsRNA, restricted as well to
228 those that would cause a temporary effect on the "activity of the complementary RNA"
229 (Trought, 2018). Although siRNAs tend to get processed down to <30 nucleotides, the
230 Decision is not restricted to externally applied dsRNA molecules of <30 nucleotides. The
231 dsRNA molecules possibly could be further chemically modified to mimic other classes or
232 RNAs such as piRNAs (Ghildiyal and Zamore, 2009) or to affect their longevity and stability
233 (Table 1). At least 128 different modifications have been reported so far in the literature
234 (Dar *et al.*, 2016; siRNAmod, 2018) and many synthesized siRNAs can be routinely ordered
235 with modifications (Bioland, 2018; Sigma, 2018).

236 Beyond modifications to the dsRNA molecules are the formulations or materials that might
237 be used to improve penetrance. The Decision imposed no restriction on method or material
238 for causing the dsRNA to be taken up by organisms.

239 The Decision makes it possible to use dsRNA made or amplified from natural sources, such
240 as cellular material, which could contain contaminating active RNA or retro viruses (Ngo *et*
241 *al.*, 2017). Without the requirement for the EPA to review any externally applied dsRNA,
242 treating a eukaryotic cell with either dsRNA corresponding to all or most of a messenger
243 RNA or most of an RNA virus genome would be allowed. Responsible use of dsRNA for
244 treating eukaryotes would unlikely include the purposeful amplification or modification of
245 RNA viruses. However, the Decision specifically removes EPA from responsibility for
246 protecting against inadvertent amplification of RNA viruses by saying "it was not necessary
247 to consider whether *in vitro* techniques were involved." This is surprising given the
248 accessibility of both genetic databases and recent revelations that a poxvirus was
249 assembled by purchasing the component DNA fragments through "the mail" and the
250 expectation that portable synthesizing equipment will be more common in the future
251 (Sharples, 2017). Even well intentioned molecular biologists, not to mention citizen
252 scientists, could use molecules of unknown potential to replicate in some eukaryotes.

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253 *Commercial* applications demonstrate heritability

254 Interestingly the EPA decision that exo-dsRNA treatments are not heritable through
255 modification of genes or other genetic material directly contradicts industry intellectual
256 property rights claims (Fillatti *et al.*, 2012; Crawford *et al.*, 2014; Deikman *et al.*, 2017). In
257 the patent "Methods and compositions for introducing nucleic acids into plants" including
258 dsRNA, the claim is for both treated organisms and their progeny:

259 "Several embodiments include *progeny seed or propagatable plant part* of such plants, and
260 commodity products produced from such plants…wherein the modification of the target
261 gene is non-heritable silencing of the target gene, *or heritable or epigenetic silencing of the*
262 *target gene*, or a change in the nucleotide sequence of the target gene; embodiments include
263 *the directly regenerated plant* exhibiting modification of the target gene and *plants of*
264 *subsequent generations grown from the directly regenerated plant* and exhibiting
265 modification of the target gene" (emphasis added to Huang *et al.*, 2018).

266 The type of patent used is a utility rather than plant variety patent and extends to the
267 ownership of organisms and future generations of organisms treated with exogenous
268 dsRNA similarly to how utility patents claim the use of genetically modified organisms.

269 "Several embodiments include a plant or a field of plants treated by a method, composition,
270 or apparatus described herein, wherein the plant exhibits a desirable phenotype (such as
271 improved yield, improved tolerance of biotic or abiotic stress, improved resistance to
272 disease, improved herbicide susceptibility, improved herbicide resistance, and modified
273 nutrient content) resulting from the treatment and when compared to an untreated plant.
274 Several embodiments include progeny seed or propagatable plant part of such plants, and
275 commodity products produced from such plants" (Huang *et al.*, 2018).

276 The maker of the dsRNA would apparently own an organism because it was exposed to the
277 dsRNA, potentially including entire fields of conventional crops or long-lived trees and their
278 seeds that have never been modified by insertion of DNA.

279

280 **Exo-dsRNA Is Not Confined to the Cytoplasm**

281

282 The Committee understood that exo-siRNA remains "solely as RNA molecules in the cell
283 cytoplasm outside the nucleus" (EPA, 2018a), consistent with advice received from staff
284 (paragraph 2.9 of Ref EPA, 2018b). Physical isolation of the genes and other genetic
285 material in the nucleus would be a biological barrier to inheritance of exo-dsRNA that was
286 confined to the cytoplasm (Fig. 1).

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287 However, no such barrier can be relied upon to keep dsRNA out of the nucleus. As discussed
288 in detail below, exo-dsRNA converted into siRNA is transported to the nucleus and causes
289 transcriptional gene silencing in at least some eukaryotes.

290 *Transport*

291 Processed exo-dsRNAs may be conducted to the nucleus in association with a variety of
292 proteins including Dicer and NRDE-3 (Various; Mao *et al.*, 2015). A decade ago researchers
293 reported that "NRDE-3 binds siRNAs generated by RNA-dependent RNA polymerases acting
294 on messenger RNA templates in the cytoplasm and redistributes to the nucleus" (Guang *et*
295 *al.*, 2008).

296 Djupedal and Ekwall (2009) writing about heterochromatin formation—which is specific to
297 the chromosomes in the nucleus—said that: "Exogenous siRNAs are thus capable of stable
298 and specific epigenetic regulation of target genes." Djupedal and Ekwall were cited in the
299 underlying research provided by staff to the Committee (eg paragraph 2.9 of Ref EPA,
300 2018b).

301 Carthew and Sontheimer (2009), also cited by EPA staff (eg paragraph 2.2 of Ref EPA,
302 2018b), said that miRNA and exogenous siRNA are biochemically interchangeable once in
303 the cytoplasm. Their biochemistries overlap, and no clear distinction can be made in the
304 kinds of silencing that they cause, further undermining certainty that externally applied
305 dsRNA could be relied upon to stay out of the nucleus.

306 Carthew and Sontheimer (2009) do make a distinction between miRNA and siRNA. They
307 mention that siRNAs but not miRNAs silence their own transcripts and when miRNA is
308 made in the cell, it is modified to prevent re-entry into the nucleus. However, as noted by
309 the authors, this distinction fails sometimes, and it does not apply to external dsRNA
310 (Carthew and Sontheimer, 2009).

311 *Nuclear* envelope

312 Cytoplasmic and nuclear contents are separated by the nuclear envelope and the
313 perinuclear space. However, each cell cycle the nuclear envelope breaks down in
314 eukaryotes with open mitosis, resulting in mixing with the cytoplasm (Gorlich and Kutay,
315 1999; Smoyer and Jaspersen, 2014). This cyclic breakdown provides the Argonaute protein-
316 associated RNA access to the chromosomes (Li, 2008). In animals at least, the nuclear
317 envelope can also rupture, resulting in mixing of content (Hatch and Hetzer, 2014). This
318 pathway is exploited by parvoviruses as part of the infection cycle.

319

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320 **Reverse transcription**

321

322 Among potential barriers to inheritance is that exo-siRNAs will not be reverse transcribed
323 (Fig. 1). Unfortunately, neither the Decision nor Staff Advice provided references or analysis
324 for the definitive existence of such a barrier.

325 Reverse transcriptase has the ability to synthesize a DNA molecule using an RNA molecule
326 as a co-factor (template), similar to how DNA itself replicates using a DNA strand as a co-
327 factor in DNA replication. Once a DNA strand has been synthesized by reverse transcriptase,
328 that strand can serve as a co-factor in the synthesis of a complementary strand to produce a
329 double-stranded DNA molecule.

330 A variety of enzymes commonly found in eukaryotes have reverse transcriptase activity
331 (Goic *et al.*, 2013). By some estimates, as much as 30% of the mammalian genome, and 10%
332 of the human, was created by the action of reverse transcriptase activity originating from
333 retroviruses (de Parseval *et al.*, 2003). Reverse transcriptases are also routinely used in
334 transcriptomics experiments, in the first step of amplification of the transcriptome,
335 including amplification of small RNAs even as small as siRNAs (Dard-Dascot *et al.*, 2018).

336 Reverse transcriptase requires a primer to initiate synthesis. A primer is another nucleic
337 acid polymer, usually RNA (such as a dsRNA molecule called a tRNA), that provides a 3´OH
338 group for strand extension. The primer may come from the secondary structure (eg a
339 hairpin structure), as is common in precursors of siRNA. Alternatively, the primer is a
340 second molecule that binds to the template strand. The primer gives the reverse
341 transcriptase reaction specificity because it binds by complementarity to a target sequence.
342 At least in the laboratory, it is possible for a reverse transcriptase reaction to proceed
343 without the addition of any particular primer molecule because there are sufficient
344 numbers of small RNA molecules naturally present in the cytoplasm to serve this purpose
345 (Frech and Peterhans, 1994).

346 It is uncertain whether all exo-dsRNA molecules could be substrates for reverse
347 transcriptase, but it is unlikely that none could be. RNA from viruses can be captured by
348 reverse transcriptase for conversion into DNA molecules and integration into chromosomal
349 DNA, as well as by Dicer for production of siRNA (Goic *et al.*, 2013). It has long been known
350 that RNA elements can be converted into DNA by the action of reverse transcriptase in
351 eukaryotes. For example, a DNA virus, that infects animals, evolved via recombination
352 between a DNA virus, that infects plants, and an RNA virus, that infects animals (Gibbs and
353 Weiller, 1999). The process involved reverse transcriptase from a third virus acting on the
354 animal RNA virus to convert an RNA genome into DNA.

355 Significantly, an enzyme from bacteria has been discovered that is able to reverse
356 transcribe from RNA templates and create short DNA fragments that were subsequently

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357 recovered in the chromosome (Silas *et al.*, 2016). The possibility that DNA molecules are
358 generated *in vivo* using exo-dsRNA constructs is made even more plausible by this
359 discovery because the bacterial enzyme is most closely related to the reverse transcriptase
360 of retrotransposons found in eukaryotes.

361 Thus, under the right conditions reverse transcriptase is able to use exo-siRNA as a
362 substrate. The Decision places no size or structural constraints on the exo-dsRNA that can
363 be used and therefore does not preclude conversion to DNA.

364

365 **Other DNA modifications caused by dsRNA**

366

367 dsRNA can cause at least three other kinds of changes to DNA in the chromosomes of the
368 nucleus of a cell independent of being reverse transcribed: DNA deletions; (Matzke and
369 Birchler, 2005) changes in chromosome copy numbers; (Khurana *et al.*, 2018) and
370 modification of nucleotides (Matzke and Birchler, 2005).

371 *Deletion*

372 The eukaryote *Tetrahymenia thermophila* has an "RNAi-mediated process that directly
373 alters DNA sequence organization" (Mochizuki and Gorovsky, 2004). Approximately 12,000
374 DNA sequences, comprising 46 mega-bases, are deleted. (Noto and Mochizuki, 2017) DNA
375 fragments removed from *Paramecium tetraurelia* chromosomes by a dsRNA-guided
376 mechanism are ligated together to form an extra-chromosomal element that is transcribed
377 and processed into more dsRNAs (Rechavi and Lev, 2017). While this process has been
378 described for endogenous dsRNAs, the example further demonstrates the difficulty in
379 making generalizations about dsRNA effects on DNA.

380 dsRNA also causes heritable changes in DNA rearrangements in the eukaryote *Oxytricha*
381 *trifallax.* These organisms have two nuclei in each cell. The somatic macronucleus contains
382 the genes being actively transcribed in somatic cells. During development of the
383 macronucleus, 95% of the germline genome is destroyed resulting in extensive
384 fragmentation followed by permutations and inversions (Nowacki *et al.*, 2008). RNA guides
385 the rearrangement process. Exo-dsRNA that targeted these guides prevented reassembly of
386 DNA fragments in the macronucleus (Nowacki *et al.*, 2008).

387 *Copy* number

388 The number of copies of chromosomes in the macronucleus in the cells of *O. trifallax* is
389 regulated by dsRNA. The number of duplicates of chromosomes in the MAC was shown to
390 increase from exposure to exo-dsRNA (Khurana *et al.*, 2018). The exposure did not
391 noticeably alter gene expression, but the effects on chromosome number were dependent
392 on Dicer and RdRP activity. Using antibodies that recognize DNA:RNA hybrid molecules,

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393 siRNAs were shown to directly associate with chromatin. Moreover, the exo-dsRNA effect
394 on the copy number of the DNA chromosomes was heritable (Nowacki *et al.*, 2010).

395 *Modification*

396 Modification of genetic material is caused by more than just changes to the primary
397 sequence of DNA molecules through integration, deletion or mutagenesis. Transcriptional
398 gene silencing is caused by chemical modifications in the form of methyl groups added to
399 nucleotides and histones by RNA-directed DNA methylation, promoting heterochromatin
400 formation (Djupedal and Ekwall, 2009). Methylation of DNA also influences RNA splicing
401 patterns in insects, altering protein structure and diversity (Brevik *et al.*, 2018).

402 Finally, methylation can also change mutation frequency because methylated cytosines
403 deaminate to thymine, causing transition mutations. T:G mismatches are 10 times less likely
404 to be repaired than other mismatches (Holliday and Grigg, 1993). In both people and plants
405 methylation tends to occur more in genes with naturally lower numbers of C residues,
406 presumably because of historical deleterious transition mutations at these loci (Zilberman,
407 2017). The outcome of the use of exo-dsRNA could be targeted mutagenesis in the
408 eukaryotes that have RNA-directed DNA methylation pathways.

409 The modification of histones and nucleotides in genes passes through mitosis and meiosis
410 (CGRFA, 2015). Once methylation has occurred, it can be propagated independently of
411 further stimulation by exogenous dsRNA. As Djupedal and Ekwall (2009), who also were
412 cited by EPA staff, say: "It is easy to visualize how DNA methylation is inherited from
413 mother cell to daughter cell considering that DNA replication is semi-conservative and the
414 newly synthesized strand may be methylated with the 'old' strand as template. Likewise,
415 half of the histones are partitioned to each DNA helix during S-phase, and may thereby
416 guide histone modifications to newly incorporated histones. This would provide means for
417 maintenance of the chromatin setting over cell divisions." This mechanism has been shown
418 for both sexual and asexual reproduction of eukaryotes.

419 The examples above would fall well within the parameters of evidence that dsRNA causes
420 modification of genes or other genetic material that is "capable of being inherited by the
421 progeny of the organism, or…capable of causing a characteristic or trait that can be
422 inherited" (EPA, 2018b).

423

424 **Genes are not confined to the nucleus**

425

426 Even if it were the case that exo-dsRNA was confined to the cytoplasm, eukaryotes have
427 genes there too. Cytoplasmic organelles called mitochondria and chloroplasts have DNA

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428 genomes. Separate from them, some eukaryotes have self-replicating DNA and RNA
429 elements in the cytoplasm.

430 The eukaryotes *Kluyveromyces lactis, Pichia acacia* and *Debaryomyces robertsiae* host
431 cytoplasmic linear DNA plasmids (Wickner, 1986; Wickner and Edskes, 2015). Large
432 versions of these "virus-like elements" have all the genes necessary for replication and
433 maintaince, and may provide some of these functions for additional smaller versions (Kast
434 *et al.*, 2015).

435 The yeast and filamentous fungi are host to self-replicating dsRNA agents located in the
436 cytoplasm (Wickner, 1986). These RNA elements range in size from 1.5 kilobase-pairs to
437 over 76 kbp. Moreover, these elements have acquired genes from other organisms and
438 other dsRNA elements through RNA-RNA recombination, making it possible for them to
439 acquire sequences directly from exo-dsRNAs (Ramírez *et al.*, 2017).

440 Presumptive exclusion of dsRNAs from the nucleus does not prevent interaction with these
441 cytoplasmic genes. Neither the EPA staff nor the Decision-Making Committee addressed the
442 broader diversity of genes or other genetic materials in eukaryotes.

443

444 **dsRNA is heritable**

445

446 dsRNA molecules themselves can be amplified by RdRP acting on the target messenger
447 RNA. Staff viewed this as a self-limiting reaction. (paragraph 2.14 of Ref EPA, 2018b). The
448 description of the process by staff was based on the assumption that the only source of
449 renewal of both the primary siRNA and secondary siRNAs is from primed RdRP activity.
450 However, RdRP has an unprimed activity as well and this mechanism can generate
451 secondary siRNA (Maida and Masutomi, 2011). Further, the staff have erroneously
452 categorized all secondary siRNAs as having 5´ triphosphates. It is only RNA molecules
453 synthesized by unprimed synthesis that have 5´ triphosphates, and then will have them
454 only in the 5´ most terminal siRNA molecules after Dicer cleavage (Maida and Masutomi,
455 2011). Moreover, the staff statement is at odds with the ability of primary exo-dsRNA to
456 generate secondary siRNAs that act on other genes (Simmer *et al.*, 2010). Finally, it ignores
457 the contribution that secondary siRNAs generated from exo-siRNAs make to transcriptional
458 gene silencing and perpetuation of the effect, and off-target silencing, through interactions
459 in the nucleus (Zhou *et al.*, 2014).

460 Returning to the central point which is that while RNAi can be self-limiting, (Houri-Zeevi
461 and Rechavi, 2017) it does not in all cases self-extinguish. It has been shown to result in
462 transmission between cells usually for around 3-5 generations, but has been observed to
463 transmit for up to 80 generations (Houri-Zeevi and Rechavi, 2017). Secondary small RNAs
464 can prime tertiary small RNAs in the germline cells of the nematode *Caenorhabditis elegans*

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465 “and therefore set in motion a feed-forward process that could theoretically preserve
466 transgenerational inheritance ad infinitum” (Rechavi and Lev, 2017).

467 Critically, where transgenerational effects of exo-dsRNA have been studied at all, there is
468 evidence that the self-limiting behavior of RNAi can be an active process, (Houri-Ze’evi *et*
469 *al.*, 2016) not the outcome of dilution as hypothesized in the evidence relied upon by the
470 EPA staff (paragraph 2.6 of Ref EPA, 2018b). This could mean that there are other
471 eukaryotic organisms in the vast repository native to New Zealand that lack this second tier
472 of biochemistry modulating the response, or natural mutants that lack it. Interestingly,
473 mutations in these limiting pathways in *C. elegans* cause hypersensitivity to exo-dsRNA
474 stimulation (Houri-Zeevi and Rechavi, 2017).

475 The limiting mechanisms are also not assurances that the transience of the effect is shorter
476 than ability of the effect to cause harm. Moreover, the limiting response can be reduced by
477 repeat exposures to the exo-dsRNA (Houri-Zeevi and Rechavi, 2017). Repeat exposures are
478 possible under the EPA Decision. According to the HSNO Act, an organism is modified when
479 its genes or other genetic material have been modified, not only when they are transmitted
480 to offspring. This is important to consider in particular for long-lived genetic resources or
481 other species of conservation value, such as trees.

482 *Unintended* Heritable Changes

483 The common biochemistry accessed by exo-dsRNA and endo-dsRNA creates competition
484 between them (Waldron, 2016). Traits made stable and heritable by endo-dsRNA may be
485 destabilized through competition with exo-dsRNA. If the outcome of the competition for
486 Argonaute or other proteins is an alternative heritable pattern of gene expression, then this
487 too is a heritable effect of treatment with exo-dsRNA.

488 Exposing the eukaryote *C. elegans* to exo-dsRNA downregulated the production of endo-
489 dsRNAs that are necessary for the inheritance of endo-dsRNA effects (Houri-Ze’evi *et al.*,
490 2016). This effect was not specific to the sequence of the genes controlled by particular
491 endo-dsRNA, but to production of proteins necessary for intergenerational transmission of
492 RNAi caused by endo-dsRNAs.

493 A critical feature of this observation is that any attempt to determine the longevity of exo-
494 dsRNA-mediated RNAi must define how often an organism will be exposed to exo-dsRNA.
495 This is because the “‘transgenerational timer’ is being reset by initiation of new RNAi
496 responses, and therefore ‘second triggers’ extend the inheritance of ancestral silencing”
497 (Houri-Ze’evi *et al.*, 2016). Exposure frequencies will determine the duration of the effect
498 both in time and number of generations.

499

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500 **Conclusion**

501

502 The EPA Decision defines the use of dsRNA applied externally to eukaryotes as out of scope
503 of their legislation. The Decision has important implications because all native and
504 endogenous eukaryotes, even those yet to be discovered, as well as those described as
505 exotics, with the exception of organisms banned by biosecurity laws, come under the
506 jurisdiction of the HSNO Act.

507 A significant concern is that the Decision might extend to the unregulated use of RNA
508 genomes of viruses or modified viruses. The Committee:

509 - put no constraints on the size of the dsRNA molecules.
510 - constrained treatment to organisms that are not excluded by the Biosecurity Act,
511 *but did not constrain the source of the dsRNA to be used*.
512 - decided that *in vitro* techniques did not have to be considered, removing any
513 obligation to notify the use of *in vitro* conversion or synthesis of RNA genomes
514 into dsRNA molecules.
515 - did not describe what it meant by external treatments, leaving chemical and
516 biological vectors (eg ingestion of micelles) of any description possible.

517 *Heritability*

518 The EPA was certain that exo-dsRNA molecules could not be inherited by eukaryotes and
519 this was the primary rationale for the determination that eukaryotes treated with them
520 were not new or genetically modified organisms for the purposes of the HSNO Act.
521 Prohibiting inheritance were various biological barriers (Fig. 1):

522 - exo-dsRNA does not mix with material in the nucleus of the cell. This, however, was
523 shown to be false. Moreover, the EPA failed to account for replicating RNA elements
524 in the cytoplasm of some eukaryotes, and the literature on RNA-RNA recombination.
525 - exo-siRNA is not reverse transcribed. This was shown to be plausible for some
526 dsRNA molecules but demonstrably false for others.
527 - exo-dsRNA is not inheritable because it does not modify the DNA genome. This was
528 shown to be false. First, exo-dsRNA may replicate independently of the DNA genome
529 using RdRP-based amplification, as can other RNA-based elements in eukaryotes
530 that are clearly genetic material. Second, exo-dsRNAs can modify DNA in
531 chromosomes in some cell types or species. Modifications include heritable
532 methylation of nucleotides and histones, DNA deletions and rearrangements, and
533 changes in chromosome copy number.

534 In contrast to the EPA, the industry developing dsRNA treatments for broad scale
535 environmental applications is convinced that the treatments result in heritable changes. For

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536 example, an exo-dsRNA treatment was used to effect a color change in petunia flowers that
537 resulted in subsequent generations of the plant that retained the modified trait. Those
538 progeny were used to illustrate the multi-generational claim of ownership made by the
539 patent holder (see paragraph 0173 of Ref. Huang *et al.*, 2018).

540 *Terminology*

541 The common understandings of terms not already defined in the HSNO Act served in this
542 instance to reinforce the conclusion that dsRNA did not modify genes or other genetic
543 material (EPA, 2018b). For the meaning of genes and other genetic material, definitions
544 were taken from the Oxford English Dictionary. A dictionary provides for its broad audience
545 by supplying definitions that are useful for most applications readers may have, but are not
546 technically comprehensive. For example, the dictionary definition is useful to say that
547 chromosomes are genes and genetic materials, but experts do not turn to the dictionary to
548 generate lists of all non-chromosomal genetic materials.

549 Reasonable sources for definitions on technical terms of central importance can include
550 relevant international agreements in the area of biosafety, agriculture and conservation.
551 These are also of practical value because they underpin international rules of trade and
552 protection of organisms and biological material. In carefully negotiated and legally binding
553 international instruments, it can be as deliberate to choose to not define particular terms as
554 it is to define others. In the agreements described earlier, genetic material is not defined as
555 specifically and exclusively the DNA of chromosomes in the nucleus of cells. Using the
556 definitions from those instruments, modification of genetic material can result from
557 changing the DNA of chromosomes in the nucleus, but also in other ways, such as by
558 changing the replicating RNA elements in the cytoplasm of cells that have these, or the
559 histone proteins of chromosomes in cells that will pass on an associated trait.

560 In Decision paragraph 4.9 the Committee said that it required evidence of dsRNA
561 integrating into the genome (ie, according to Decision paragraph 4.6, to be chemically
562 attached to the DNA of chromosomes in the nucleus), or the dsRNA itself had to in some
563 other way become inheritable, for the conclusion to be reevaluated. Implicit in the Decision
564 text was that the modification had to be the continued propagation of the dsRNA, rather
565 than the changes it made to the genetic material of an organism. Certainly if the dsRNA
566 were propagated that would satisfy international definitions of modification, which also can
567 mean a change to the primary order of nucleotides in a DNA molecule as would result from
568 linkage to a dsRNA molecule, if that could occur. However, the terms used by international
569 instruments are also consistent with what agencies such as the UN Food and Agriculture
570 Organization include, such as the "*chemical* modifications of DNA and chromatin, for
571 instance, affecting the degree of chromatin compaction or the accessibility of regulatory
572 sequences to transcription factors" (emphasis added to Ref CGRFA, 2015). As discussed
573 above, that is a kind of modification that can result from a treatment with exo-dsRNAs

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574 without reliance on continued transcription (Rechavi, 2014). dsRNA can cause heritable
575 effects without needing to propagate along with the modifications that it makes.

576 *Other* options

577 The EPA had other options. One would have been to decide for various reasons (eg that
578 RNA was genetic material in its own right as in some viruses, or was a nucleic acid as
579 referred to by the Protocol, or that the EPA had insufficient information about the diversity
580 of eukaryotic responses to dsRNA to extrapolate further) that eukaryotes treated with
581 dsRNA would be regarded as new organisms unless further information were to come to
582 light to show the opposite. Specifically, EPA could require further evidence that molecules
583 derived from double-stranded RNA molecules *cannot* modify genes or other genetic
584 material or *cannot* otherwise be passed to progeny of eukaryotic cells or organisms treated
585 with externally applied dsRNA.

586 Had the EPA decided that eukaryotes treated with dsRNA were, at least for now, new or
587 genetically modified organisms, it could have completed a risk assessment with the
588 outcome possibly being that cells and organisms treated with external dsRNA in the
589 laboratory were low risk, requiring the minimum biocontainment infrastructure. EPA could
590 have decided this for the whole country, not requiring applications for further risk
591 assessments and thus minimized costs to researchers and developers.

592 Alternatively, EPA could have extended approval to eukaryotic organisms held in a variety
593 of containment facilities, tying the approval to physical containment conditions appropriate
594 to the type of organism. Such facilities and requirements are already commonplace because
595 of work with recombinant DNA.

596 Likewise, EPA could have reduced compliance costs for those using exo-dsRNA in contained
597 facilities by limiting the approval to synthetically produced dsRNA molecules, as requested
598 in the original application, prohibiting dsRNA derived from pathogens such as RNA viruses.
599 Work using dsRNA derived from viruses would then require additional risk assessment.

600 It is important to emphasize that RNA effects are still rapidly being described even in model
601 research organisms. "Among some animal groups [in New Zealand], new species are being
602 discovered faster than scientists can cope with them" (Various) much less test them for
603 dsRNA responses. The clear statements that there is likely to be much more to discover
604 about dsRNA effects as more species are studied, statements made in the references used to
605 develop advice from staff (EPA, 2018b), were not mentioned in the advice provided to the
606 Committee. The narrow treatment by EPA of how dsRNA could modify genes or genetic
607 material is surprising given the nation's pride in its native biodiversity.

608 Biosafety risk assessment is a technical specialty wherein the complexity of the biological
609 world must be fully considered and uncertainty in the extent of our knowledge humbly
610 recognized. In the future, it might be determined that some or all uses of externally applied

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611 dsRNA create no unmanageable risks to human health, the environment, or to society. This
612 would be a welcome finding because there is potential for dsRNA-based products to be at
613 least short-term remedies for some problems. Coming to this position hopefully will be an
614 evidence-based and precautionary process. Only that kind of process has the ability to build
615 trust in responsible providers of biotechnology and agencies that serve to protect the
616 public's interest in the environment. Taking shortcuts will inevitably create delays.

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Part 5 of the HSNO Act
Assessment of hazardous
substances and new organisms

EPA

s26
Determination of new organism
or hazardous substance

s25
Restriction of import, manufacture,
development, field testing, or release

not reverse transcribed
no access to nucleus
do not integrate
into genes
not
inherited

Not a new organism.
Not regulated by EPA.

Figure 1: Context of the EPA Decision. The EPA has two different activities described by sections 25 and 26 of the HSNO Act. (Left) Under s26, EPA decided that eukaryotes treated with exo-dsRNA were not new or genetically modified organisms because exo-dsRNA is not inheritable. That conclusion is pictured as the center of an onion (center left), further protected by several additional layers that all contribute to increasing certainty in the conclusion. The layers are, from outermost, that exo-dsRNA: has no access to the nucleus and genes or other genetic material therein, cannot be reverse transcribed into DNA, and therefore cannot modify genes or other genetic material in the nucleus through integration, and it is not the genes or other genetic material of a eukaryote. (Right) If EPA decided that

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820 treatment of eukaryotic cells or organisms with dsRNA modified genes or genetic material
821 by *in vitro* techniques, then s25 would apply.
822

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Table 1. Common siRNA *in vitro* chemical modifications

2´ O-Methyl phosphoramidites	2´-O-Me-rA, 2´-O-Me-rC, 2´-O-Me-rG, 2´-O-Me-rU	Increase stability, longer lasting RNAi effects
2´ Fluoro phosphoramidites	2´-FluoC, 2´-FluoU	
5´ modifications	5´-Amino, 5´-Biotin, 5´-Cholesterol, 5´-Phophorylation and 5´-Thio	Various reasons, e.g. cholesterol for improved penetration through membranes.
3´ modification	3´-amino	
Table content amalgamated from several sources. (Dar *et al.*, 2016; Bioland, 2018; Sigma, 2018)		

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828 **Supplemental Materials**

829

830 Relevant Hazardous Substances and New Organisms Act definitions (Hazardous Substances
831 and New Organisms Act, 1996).
832 2A Meaning of term new organism
833 (1) A new organism is—
834 (a) an organism belonging to a species that was not present in New Zealand
835 immediately before 29 July 1998:
836 (b) an organism belonging to a species, subspecies, infrasubspecies, variety,
837 strain, or cultivar prescribed as a risk species, where that organism was
838 not present in New Zealand at the time of promulgation of the relevant
839 regulation:
840 (c) an organism for which a containment approval has been given under this
841 Act:
842 (ca) an organism for which a conditional release approval has been given:
843 (cb) a qualifying organism approved for release with controls:
844 (d) a genetically modified organism:
845 (e) an organism that belongs to a species, subspecies, infrasubspecies, variety,
846 strain, or cultivar that has been eradicated from New Zealand.
847 (2) An organism is not a new organism if—
848 (a) the organism is not a genetically modified organism and—
849 (i) an approval is granted under section 35 or 38 to release an organism
850 of the same taxonomic classification; or
851 (ii) the organism is a qualifying organism and an approval has been
852 granted under section 38I to release an organism of the same taxonomic
853 classification without controls; or
854 (iii) an organism of the same taxonomic classification has been prescribed
855 as not a new organism; or
856 (b) the organism is a genetically modified organism and—
857 (i) an approval is granted under section 38 to release an organism of
858 the same taxonomic classification with the same genetic modification;
859 or
860 (ii) the organism is a qualifying organism and an approval has been
861 granted under section 38I to release an organism of the same taxonomic
862 classification with the same genetic modification without
863 controls; or
864 (iii) an organism of the same taxonomic classification with the same
865 genetic modification has been prescribed as not a new organism;
866 or
867 (c) the new organism was deemed to be a new organism under section 255
868 and other organisms of the same taxonomic classification were lawfully
869 present in New Zealand before the commencement of that section and in
870 a place that was not registered as a circus or zoo under the Zoological
871 Gardens Regulations 1977.
872 (2A) A new organism does not cease to be a new organism because—

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873 (a) it is subject to a conditional release approval; or
874 (b) it is a qualifying organism approved for release with controls; or
875 (c) it is an incidentally imported new organism.
876 (3) Despite the provisions of this section, an organism present in New Zealand before 29
877 July 1998 in contravention of the Animals Act 1967 or the Plants Act
878 1970 is a new organism.
879 (4) Subsection (3) does not apply to the organism known as rabbit haemorrhagic
880 disease virus, or rabbit calicivirus.
881
882

Table S1. Purpose of the application

<table>
<tr><th>Source</th><th>Description‡</th><th>Notes</th></tr>
<tr><td>Application “To obtain a determination of whether an organism is a new organism” APP203395 (Trought, 2018).</td><td>Eukaryotic cells that have been transiently transfected with synthetic molecules of double stranded RNA to inhibit (temporarily) the <u>activity of the complementary RNA</u>.</td><td rowspan="4">Application for eukaryotic cells (which may be tissue culture) becomes a determination for all eukaryotic organisms.

Application for use of synthetic/artificial dsRNA molecules contrasts with determination for all dsRNA molecules of undisclosed source or size.

Application originally limited to an activity on the mRNA target that is temporary to any form of expression suppression to any RNAi treatment outcome in the determination.</td></tr>
<tr><td>EPA Staff Report “Determining whether eukaryotic cell lines treated with double-stranded RNA are genetically modified organisms” (EPA, 2018b).</td><td>[the applicant] seeks a determination…on whether eukaryotic cells treated with artificially synthesised dsRNA to transiently <u>suppress the expression of user-selected genes</u> are new organisms for the purpose of the Act.</td></tr>
<tr><td>EPA Decision “Purpose of the Application” page 1 (EPA, 2018a).</td><td>“eukaryotic cell lines that have been treated with externally applied double-stranded RNA molecules for the purpose of <u>inducing a transient small interfering RNA (siRNA) response</u> are new organisms.”</td></tr>
<tr><td>EPA Decision section 2 (EPA, 2018a).</td><td>“eukaryotes treated with double-stranded RNA molecules were</td></tr>
</table>

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	considered genetically modified organisms.”	
‡Highlighted terms are inferred as homologous in the different passages.		

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890 double-stranded RNA are genetically modified organisms. Environmental Protection
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