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Submission on scientific and other matters made to the Decision-Making Committee for reconsideration of APP203395

This is document two of four provided to the DMC by Prof. Jack A. Heinemann, PhD.

Summary

The decision reached by the DMC in May 2018 failed in my view to demonstrate proper regard for the purpose and principles of the HSNO Act. The barriers that EPA relied upon to prevent the effects of *in vitro* techniques from modifying genes or other genetic material in cells or organisms are not reliable and do not exist in all eukaryotes that may be exposed. Furthermore, the effects on prokaryotes were not even considered despite the certainty of exposure when this technology is used outside of contained facilities.

The interpretations of key terms from the HSNO Act explicitly or implicitly used by staff and the DMC were inconsistent with previous use by EPA or disconnected without good reasons from formal uses that are in relevant international agreements. Moreover, the decision was taken in a short timeframe with limited input from other government agencies and no input from the public or specialist scientific community. While that may have been EPA's legal prerogative, as laid out in paragraph 18 (below) the decision-making process did not reflect the seriousness of the decision.

External treatments of cells and organisms with dsRNA is an important research technique and valuable biotechnology. In time external treatments of organisms with dsRNA in the out-of-doors may prove to be effective, provide benefits and may be done at acceptable risks to human health and the environment. Regulation on a case-by-case basis will help to accomplish this. However, ensuring New Zealand the benefits of responsible and safe biotechnological applications is not what this EPA decision will achieve.

The previous DMC erred in concluding that external treatments of eukaryotic organisms with dsRNA cannot cause heritable effects through modification of genes or other genetic material.[1] *It also erred by not considering whether* in vitro *techniques are involved. The EPA's use of the term* in vitro *techniques, among others, in its May 2018 determination was both inconsistent with previous uses and scientifically flawed leading to a decision not consistent with the purpose and principles of the HSNO Act. The key terms I will contest are:* in vitro *techniques, modify/modified and genetic material.*

Inheritance

1. As set out in document three of my submission,[2] the DMC and EPA staff relied upon an incomplete scientific analysis to conclude that the effects of external treatments of eukaryotic cells and organisms with dsRNA were not heritable. The limitations of the analysis were systematically identified in the new information provided to EPA (Heinemann 2019). As a result, the DMC must now recognise that external treatments of organisms with dsRNA can have heritable effects by modifying either genes or other

[1] Current scientific literature documents treatments of eukaryotes with externally applied dsRNA that cause heritable changes. See submission document three of four.

[2] Published as: Heinemann, J.A. Should dsRNA treatments applied in outdoor environments be regulated? Environ Int 2019;in press. https://www.sciencedirect.com/science/article/pii/S0160412019306038

genetic material and therefore in accordance with the purpose and principles of the HSNO Act external treatments of cells or organisms with dsRNA creates genetically modified organisms. In other words, heritability was an appropriate criterion but the DMC determination was incorrect.

2. Heritability was appropriate because it is part of how the HSNO Act defines a genetically modified organism. As recalled by the DMC, "genetically modified organism means, 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" (EPA 2018a).
   Satisfying either (a) or (b) is sufficient to determine that an organism is a genetically modified organism. If the *in vitro* technique, in this case treatment with dsRNA, results in modified genes or genetic material being passed on, that confirms that the technique is in scope of the HSNO Act certainly at least as per criterion (b). The definition does not require knowing the molecular biology of how the modified genes or genetic material are inherited.[3]

3. The DMC erred in its interpretation of the kind of modification that must occur from treatments. The DMC statement "that sequences derived from double-stranded RNA molecules have integrated into the genome of a eukaryotic cell or organism or has otherwise become inheritable in progeny of eukaryotic cells or organisms treated with externally applied dsRNA" (paragraph 4.9 of EPA 2018a) places the emphasis on propagation/inheritance of the nucleotide sequence of the dsRNA molecules used in the treatment. However, the Act does not say that the molecules or nucleotide sequences homologous to dsRNA must be heritable or preserved in offspring. To require the retention and propagation of particular nucleotide sequences would be inconsistent with other treatments that result in creating genetically modified organisms. For instance, external treatments with genome editing proteins result in the creation of genetically modified organisms without the nucleotide sequences of the genome editing protein being retained in the genes of the organism.[4] The editing protein only has to modify genes and then it may disappear. As set out in my third submission document, there are multiple ways that dsRNA can alter genes or other genetic material and then disappear.

In vitro

4. The key term from the HSNO Act that was not used in the determination by the DMC was "*in vitro* techniques" (paragraph 4.7 of EPA 2018a), and the definition of the terms "modify" and "modified" were not properly articulated. These terms equate the use of dsRNA treatments to other regulated forms of mutagenesis that do create genetically modified organisms and are not excluded by the regulations from being within the scope of the HSNO Act.

5. The staff argued "that *in vitro* techniques are scientific techniques that occur outside a cell or organism in an artificial environment" (paragraph 3.10 of EPA 2018b). The courts have already ruled on a similar argument made by Scion in *The Sustainability Council of New Zealand Trust v. The Environmental Protection Authority*.[4] In that case Scion argued that the use of site-directed nuclease proteins was an *in vivo* technique because the enzymatic activity causing the modification of genes happened inside the cell.[5] The court rejected Scion's argument, saying in paragraph 26: "In this case the parties [which

[3] However this information can be essential for reducing some uncertainty during a risk assessment.

[4] https://forms.justice.govt.nz/search/Documents/pdf/jdo/76/alfresco/service/api/node/content/workspace/SpacesStore/1594ff52-8c2c-4bf5-8f15-29dbcecc6fa9/1594ff52-8c2c-4bf5-8f15-29dbcecc6fa9.pdf

[5] "[Scion] said that the genes in this procedure are not modified by *in vitro* techniques. Rather they are modified *in vivo* (within the treated cell) by the protein" (paragraph 21 of *The Sustainability Council of New Zealand Trust v. The*

included the EPA] are agreed that organisms resulting from the use of ZFN-1 and TALEs are organisms in which the genes or genetic material have been modified by *in vitro* techniques." ***In vitro* techniques in a process that causes the modification of genes or other genetic material are not erased because other reactions in the series occur *in vivo*.**

5.1. The March 2018 staff definition of *in vitro* techniques is ill advised because it could potentially make all existing genetically modified organisms not genetically modified organisms, thereby undermining the purpose and principles of the HSNO Act. For example, the use of DNA molecules assembled using *in vitro* techniques to modify genes is without doubt to use *in vitro* techniques to make a genetically modified organism. Nevertheless, the modification of genes occurs later, after the DNA enters a cell. There the DNA modifies genes when it is integrated into or recombined with DNA molecules in the nucleus or organelles *in vivo* (Figure 1). Clearly, that the last step occurs *in vivo* does not make genetic engineering an *in vivo* reaction. The *in vivo* reactions that result in modification of genes or other genetic materials may differ between materials (i.e. SDNs or recombinant DNA) but all these methods require *in vitro* techniques which include those needed to make the active molecules and/or to cause them to penetrate the cell or organism. dsRNA is no exception and multiple patents have been granted for assisting dsRNA uptake into cells and organisms (Deikman et al. 2017; Donohue et al. 2017; Mitter et al. 2015).

5.2. Treating cells or organisms with DNA molecules creates genetically modified organisms regardless of whether the treatment uses *in vitro* techniques inside an "artificial environment" or outside in a "natural environment". If this were not the case, then the HSNO Act would not apply to any genetic engineering activity by virtue of it being outside. It would make all my approvals from the EPA for development work unnecessary if I could just transform my research organisms with recombinant DNA molecules while standing on the banks of the Avon River, which is technically not difficult for me to do.

5.3. The High Court in its decision in *The Sustainability Council of New Zealand Trust v. The Environmental Protection Authority* ruled that treatments with site-directed nucleases such as ZFN or TALEN proteins create genetically modified organisms *regardless of whether the mRNA for such proteins or the proteins themselves were used. That was still true even if the mRNA did not integrate into the genome*. Moreover, the external treatment using dsRNA is not among the closed list of exemptions from the regulations. Experts serving the court, including EPA's expert, also agreed "that '*in vitro*' techniques are key words in the definition of genetically modified organisms in the Act" (paragraph 32).

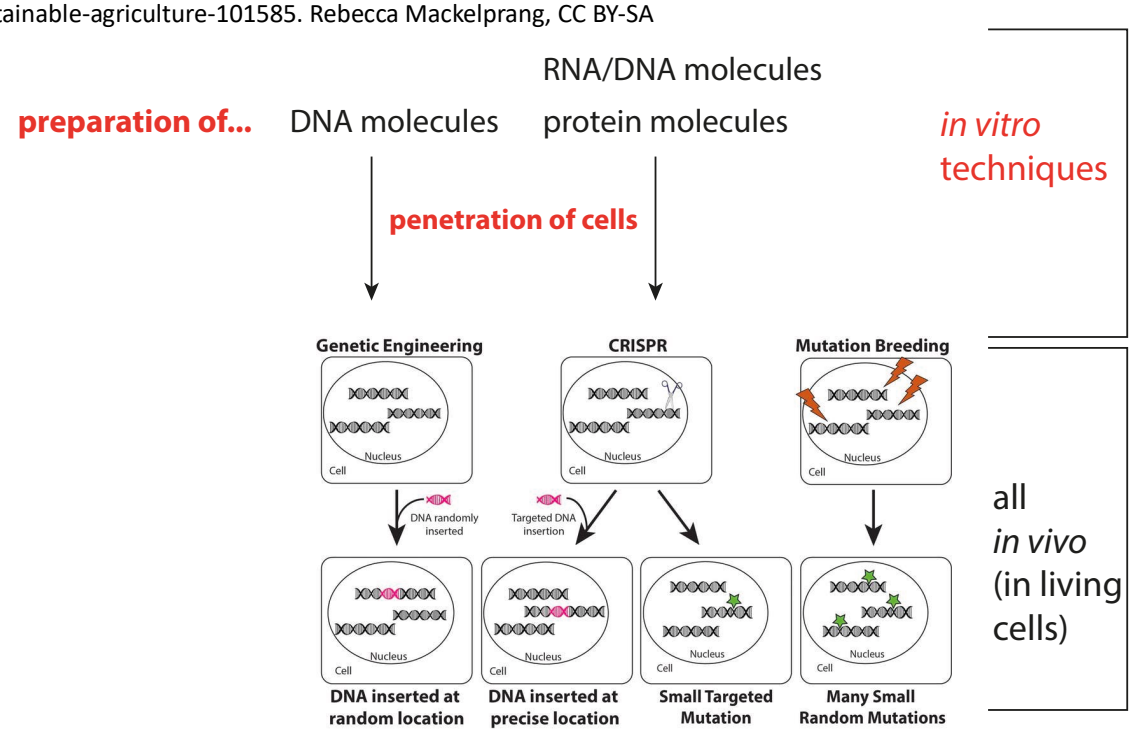
6. The reasoning used by staff in its March 2018 report was that: "3.11. Regardless of whether the dsRNA is created *in vitro* or *in vivo*, such molecules are not organisms, as they are unable to be replicated by the cell or any other mechanism, and the dsRNA molecules do not cause any permanent[6] or heritable

Environmental Protection Authority).

[6] Permanent is also a problematic word that neither appears in the HSNO Act nor is defined by staff. Nucleotide sequences are also not permanent or there would be no mutations that could arise from changing them. Evolution of species would not be possible if nucleotide sequences were permanent. Therefore, permanence cannot be the correct test. I presume that staff were looking to distinguish between the stability of a DNA sequence from the changes to genetic material that do not rely on the same kind of molecular stability. In which case the question becomes: *How many generations does a trait have to transmit to be considered heritable?* The answer is obvious in genetics, the science of inheritance which defines a trait as heritable because it transmits from one to the next generation. Likewise, the HSNO Act says "any number of replications", which includes as few as one. However, the number of generations a trait is transmitted could matter to risk assessment. It would depend on the trait, organism and relevant environment. Because it could matter to a risk assessment there is a need to subject organisms treated with dsRNA molecules to a risk assessment, which would only be done if the techniques were within scope of the HSNO Act.

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."

Figure 1. **A common depiction of genetic engineering and genome editing techniques**, adapted to show the respective *in vitro* (highlighted in red) and *in vivo* stages resulting in modified genes or other genetic material. All techniques understood to create genetically modified organisms involve reactions resulting in the modification of genes or other genetic material *in vivo*. dsRNA treatments do the same. Modified from https://theconversation.com/organic-farming-with-gene-editing-an-oxymoron-or-a-tool-for-sustainable-agriculture-101585. Rebecca Mackelprang, CC BY-SA



7. Firstly, whether or not a molecule is an organism is irrelevant. For example, DNA is not an organism but external treatments of organisms with it does create genetically modified organisms. Secondly, the DMC itself correctly rejected the staff argument that dsRNA molecules could not replicate in treated cells or organisms.[7]

8. Finally, each of the conditions used by the staff to disqualify external treatments using dsRNA as meeting the definitions of the Act are also wrong. Some molecules of DNA have no known means to replicate in some organisms but that does not mean they do not, nor does it mean that self-directed replication, as for example that occurs when a bacterium is transformed using a plasmid, is the only way through which they may persist and be passed on through genetic material. One alternative means is through recombination with or integration into other DNA molecules, as is the case for existing genetically modified commercial crop plants. RNA can do the same either via reverse transcription and recombination with DNA molecules or recombination with replicating molecules of RNA. As I recount in my third submission document, replication and/or recombination of RNA molecules can occur in fungi and has occurred to detectable levels between viruses in plants. dsRNA molecules do cause permanent[6] *and* heritable changes to genes and other genetic materials. Examples

[7] See paragraph 4.6 of the DMC decision: "The Committee acknowledged that while siRNA molecules replicate within treated eukaryote species (via RNA-dependent RNA polymerase amplification as part of the normal cellular RNA interference response)…". Unfortunately, the DMC instead invoked other equally flawed barriers to heritability.

include dsRNA-mediated DNA rearrangements and transition mutations. They may also alter traits determined by endogenously produced dsRNA-mediated processes in heritable material.[8]

9. In addition, the criteria for heritable modification were applied differently for dsRNA treatments than they are for other *in vitro* techniques. Treatments using other mutagens that create genetically modified organisms, although some are excluded by the regulations, involve chemicals or molecules that do not themselves persist or replicate in treated organism. The criteria used by staff would exempt from regulation any number of treatments that are currently regulated.[9]

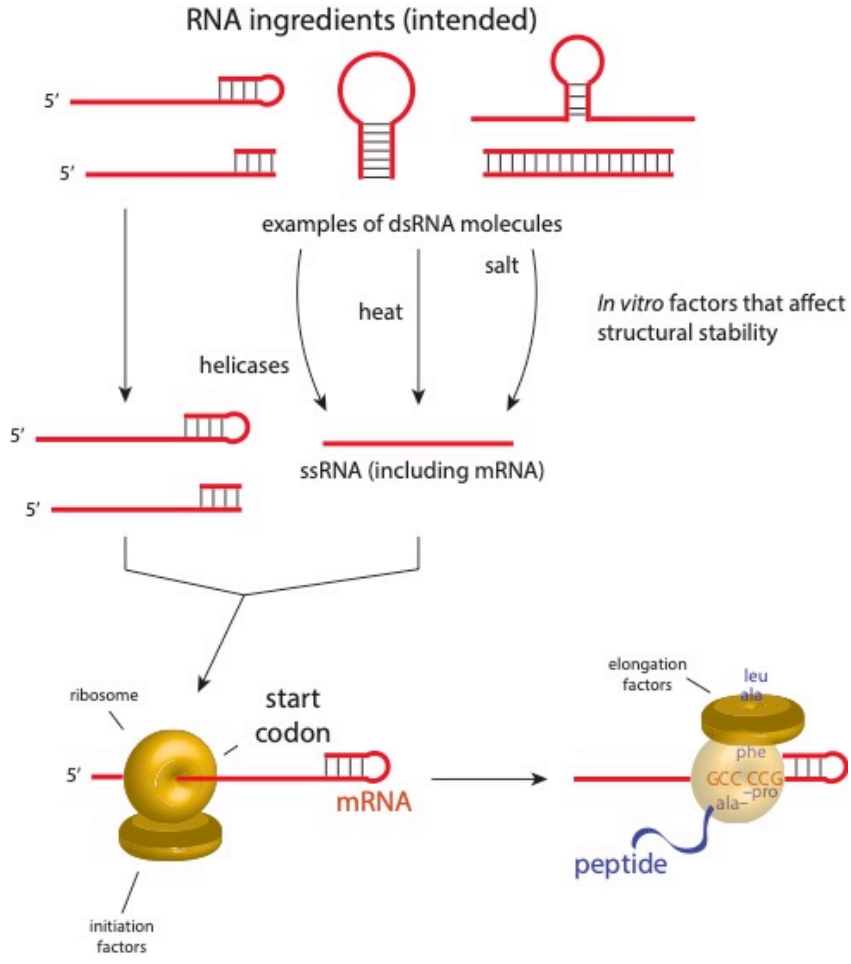
10. For example, it is possible to have dsRNA molecules (and/or treatments contaminated with single-stranded RNA molecules or denatured dsRNA molecules that are then single-stranded) that are not prevented from being translated (Figure 2). Consequently, as the decision is written, organisms that are not already genetically modified organisms could be treated with dsRNA molecules that may be translated to make ZFN or TALEN proteins. Unmistakably this would be in conflict with the High Court ruling in *The Sustainability Council of New Zealand Trust v. The Environmental Protection Authority* (see paragraph 5.3 above) because it would make them genetically modified organisms. Since the DMC decision makes no mention of this amongst the excluded conditions, the DMC appears to not have recognised that it had formulated a decision that exempts some treatments that are beyond its legal authority to exempt.

11. It is surprising that the DMC used heritability as its sole test rather than also consider whether or not the treatment was an *in vitro* technique (paragraph 4.7 of EPA 2018a). In document one of my submission,[10] I outlined how the applicant's and EPA staff's reference to "synthetic" or "artificially synthesised" dsRNA molecules produced using *in vitro* techniques were not included in the description of dsRNA treatments by the former DMC. Retaining these terms would have reinforced that *in vitro* techniques were involved. In addition, in the third document of this submission (first provided to EPA on 4 June[11]), I outlined examples of the *in vitro* techniques that may be used to both *create dsRNA* and to *cause its penetration into cells and organisms* where it can modify genes or other genetic material. The techniques used to cause penetration are sometimes even the same as used to introduce ZFN and TALEN proteins or RNA for the production of those proteins, into cells or organisms. Many are described in my fourth submission document.

12. In addition to how *in vitro* techniques were defined during this process, other undefined key terms were defined in ways that appear to have constructed the DMC's findings. In Decision paragraph 4.9 the DMC said that it required evidence of dsRNA integrating into the genome (i.e. according to Decision paragraph 4.6, to be chemically attached as part of the DNA polymer of chromosomes in the nucleus), or the sequence of nucleotides of a particular dsRNA had to in some other way become heritable, for the conclusion to be reevaluated (EPA 2018a). Implicit in the determination text was that the modification of genes or other genetic material in treated cells or organisms had to be the continued propagation of the dsRNA or a derivative cDNA, rather than the propagation of modifications made to genes or other genetic material by the dsRNA. In the third document of my submission[2] I provide overwhelming evidence of modification of genes or other genetic material that can happen following treatment of cells or organisms with dsRNA, different from that outlined by the former DMC.

[8] Heritable material as defined by the HSNO Act.
[9] Namely all those captured by the outcome of *The Sustainability Council of New Zealand Trust v. The Environmental Protection Authority*.
[10] Letter to Dr Stephen Cobb.
[11] Although I now have been made aware that EPA new of this new evidence since at least August 2018.

Figure 2. **dsRNA treatments that could result in availability of potentially translated RNA molecules *in vivo*.** Potential RNA ingredients of exo-dsRNA treatments are shown at the top (without implying size of the molecules). All of these are forms of dsRNA molecules which are allowed by the 1 May 2018 EPA decision. Some dsRNA molecules (left) may be translated directly with or without the classic 5' cap (for eukaryotes) or Shine-Dalgarno sequence (for prokaryotes) (Nakagawa et al. 2017; Shatsky et al. 2018). Alternatively, during preparation, storage or penetration of living tissue, they may denature (resulting in loss of base pairs illustrated by black lines) into ssRNA (right). The ssRNA molecules may be used as substrates for translation by ribosomes (bottom). If an RNA molecule also had the reading frame for a gene editor, then an exo-dsRNA treatment would result in an outcome already regulated. Note that translation of dsRNA molecules is a specifically identified and regulated risk in the proposed modifications to the Australian regulations discussed in paragraphs 18.2-18.5.



Genetic material

13. The previous DMC always associated DNA in chromosomes of the nucleus with the phrase “genes or other genetic material” in the Decision (see paragraphs 4.5-4.7 of EPA 2018a), and it specifically only mentioned alterations of DNA molecules in the chromosomes of a nucleus as necessary evidence for reconsideration of its Decision (paragraph 4.9). The DMC therefore wrongly restricted its interpretation of “genes or other genetic material” to exclude modifications to the known range of nucleic acids that act as genes in eukaryotes (e.g. replicating RNA elements in the cytoplasm of fungi), location of genes within cells including the cytoplasm of eukaryotes, and has the effect of equating “genes” with “genetic material”. These terms are not synonyms. Neither are they a more cumbersome way of saying, e.g.,

DNA and RNA.[12] Rather, the terms refer to very different things of relevance to the HSNO Act and risk assessment and have a long history of use. I encourage the new DMC to correct this mistake.

14. For the meaning of genes and other genetic material, the DMC used definitions from the Oxford English Dictionary. A dictionary of this type supplies definitions that are useful for most broad audiences, but are neither technically comprehensive nor fit-for-purpose in this technically demanding area of biotechnology. For example, the dictionary definition is useful to say that genes are found in chromosomes, but expert molecular biologists do not turn to the dictionary to generate lists of all non-chromosomal locations of genes, including the genome of mitochondria and chloroplasts or RNA elements found in the cytoplasm of fungi.

15. Reasonable sources for definitions on technical terms of central importance to the May 2018 determination can include international agreements in the area of biosafety, agriculture and conservation. Domestic legislation such as the HSNO Act are written to harmonise New Zealand to relevant international agreements and thus share a context for terminology. It is my view that the former DMC took upon itself considerable latitude to adopt definitions on terms for which there are neither national nor international consensus definitions and overlooked interpretations that could conflict with the determination that external treatment using dsRNA does not create a genetically modified organism.

16. In the Convention on Biological Diversity (CBD), Cartagena Protocol on Biosafety (CBD), the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), among others, the definition of genetic material is not articulated as the "DNA of the eukaryotic genome" (language used in paragraph 4.6 of EPA 2018a) in the nucleus of eukaryotic cells. Using the definitions from those instruments, modification of genetic material can result from changing the DNA of chromosomes in the nucleus, but also in other ways, such as by changing the replicating RNA elements in the cytoplasm of cells that have these, or modifying the histone proteins of chromosomes in cells that results in intergenerational transmission of associated traits.

    16.1. The international community defines genetic resources as a special kind of genetic material, one that has actual or potential value. The Convention on Biological Diversity defines genetic material as "any material of plant, animal, microbial or other origin containing functional units of

[12] Parliament chose the words "genes" and "other genetic material", terminology that predates the HSNO Act and was used by the United Kingdom (e.g. COUNCIL DIRECTIVE of 23 April 1990 on the deliberate release into the environment of genetically modified organisms (90/220/EEC)), not the words "DNA" and "DNA/RNA". New Zealand lawmakers had two opportunities to say "DNA and RNA" if that is what they wanted to say instead of "genes and other genetic material". The first opportunity would have been in the definition of a genetically modified organisms and the second opportunity was in the definition of genetic elements. The wording of genetic elements is newer than the definition of genetically modified organisms. It was introduced in the 2002 New Organisms and Other Matters bill. I participated in the process through a submission and appearance before the select committee of the day. My submission articulated that genetic modification of organisms did not require retention of the modifying nucleic acids and I even used RNA interference as one example. (It was already well known by 2002 that external treatments with dsRNA caused heritable changes. I reviewed that evidence at the request of then EPA staff in a report I provided in circa 2007.) The term genetic element preserved this aspect of heritable modification without defining the molecular causation. The existing definition of genetically modified organism was consistent and therefore required no adjustment. Indeed, "genetic material" and "heritable material" are operationally synonymous terms in the HSNO Act.
I acknowledge that in some contexts those speaking about genetic material or heritable material mean genes or DNA. It is common in molecular biology to not distinguish between genes, genetic material and heritable material. However, these words do have different meanings in context (see footnote 13). For example, the HSNO Act does not define heritable material as a synonym of DNA or genes. While there is no definition of genetic material in the HSNO Act, it's used to refer to units of heredity as small as genes to as large as seeds etc, mirroring how the same term is used in UK legislation (see above).

heredity" (CBD). The ITPGRFA defines both plant genetic resources and plant genetic material as "any material of plant origin, *including reproductive and vegetative propagating material*, containing functional units of heredity" (emphasis added to ITPGRFA). Genetic resources are described as such things as *organisms, seeds, zygotes and cuttings* (Europa ; FAO ; Heinemann et al. 2018). At the 22nd Session of the Conference of FAO (held in 1983) it was decided that the "'base collection of plant genetic resources' means a collection of *seed stock or vegetative propagating material (ranging from tissue cultures to whole plants)*…" (emphasis added to UNFAO 1983). **Therefore, and for some considerable time, *genetic material* has been a term to mean organisms, seeds, zygotes and cuttings, not just DNA in chromosomes found in cell nuclei.**[13] Treatments that modify organisms, seeds, zygotes and cuttings using *in vitro* techniques create genetically modified organisms. Failure to embrace this larger meaning of "genes or other genetic material" focused the DMC and EPA staff onto too narrow a range of dsRNA treatment effects.

16.2. The DMC departed from the international convention I discuss above when it said in paragraph 4.5: "The Committee interpreted this to mean whether any genes or genetic material **of** a eukaryotic cell or organism (including *in vitro*-cultured cell lines, germline cells of whole animals or plants, etc.) would be modified by the treatment with externally applied double-stranded RNA molecules" (emphasis in bold added). Herein it unmistakably fails to equate genetic material with the cultured cell lines, germline cells or whole animals or plants and instead as something less than these things and not obviously different from genes themselves. Notably, as discussed in paragraph 16.4, genetic material may be modified by inclusion of genetic elements.

16.3. Neither these international instruments nor domestic law define the term "modify." The term "modify" as used by international agencies such as the UN Food and Agriculture Organisation includes the "*chemical* modifications of DNA and chromatin, for instance, affecting the degree of chromatin compaction or the accessibility of regulatory sequences to transcription factors" which may be "meiotically and mitotically inherited" (emphasis added to CGRFA 2015), not just changes in sequences of nucleotides in DNA molecules in chromosomes found in nuclei, or covalent linkage between DNA and RNA molecules. As discussed in the third document of my submission, modifications of the kind described by UN FAO can result from an external treatment with dsRNAs. Moreover, dsRNA can cause heritable effects, such as through transition mutations and DNA rearrangements, without needing to propagate along with the modifications that it makes. Treating cells with dsRNA resulting in RNA-dependent DNA methylation, one RNA interference pathway, is a

[13] (A) "Referring to genetic material as 'any material of plant origin, including reproductive and vegetative propagating material, containing functional units of heredity' has a long tradition in management of GRFA [genetic resources for food and agriculture], and is consistent with classical applied genetics because the predominant tool is breeding. The agricultural genetics literature from the 1940s explicitly equated 'genetic material' with that which could recreate the plant (seeds or propagules) (Weiss, 1943)" (Heinemann et al. 2018). (B) "(a) 'plant genetic resources' means the reproductive or vegetative propagating material of the following categories of plants: • (i) cultivated varieties (cultivars) in current use and newly developed varieties; (ii) obsolete cultivars; (iii) primitive cultivars (land races); (iv) wild and weed species, near relatives of cultivated varieties; (v) special genetic stocks (including elite and current breeders' lines and mutants);" (UNFAO 1983). (C) "Genetic resources (GRs) refer to genetic material of actual or potential value. Genetic material is any material of plant, animal, microbial or other origin containing functional units of heredity. Examples include material of plant, animal, or microbial origin, such as medicinal plants, agricultural crops and animal breeds." Source: World Intellectual Property Organization (WIPO) https://www.wipo.int/tk/en/genetic/. (D) "…plant genetic material is to be understood as any plant material containing genetic information which is capable of self-reproduction or of being reproduced in a biological system" (Leskien and Flitner 1997). Note that quote (D) makes explicit that genetic material has the qualities of both heritable material (capable of self-replication) and genes (being reproduced in a biological system) as defined in the HSNO Act.

mutagenesis technique not excluded by the regulations.

16.4. The HSNO Act does not restrict its scope of *in vitro* techniques or heritable changes to those that alter nucleotide order in molecules of DNA. If it did, there would be no need for the definition of a genetic element. Genetic elements are defined as –

(a) "heritable material; and

(b) any genes, nucleic acids, or other molecules from the organism that can, without human intervention, replicate in a biological system and transfer a character or trait to another organism or to subsequent generations of the organism."

Heritable material means "viable biological material, including gametes and spores, arising from the organism that can, without human intervention, regenerate the organism or reproduce a new generation of the same species of the organism." Genetic elements are both things such as seeds and things such as nucleic acids. The HSNO Act recognises that genetic elements are a source of risk and therefore must be taken into account. It would be incongruous to exclude genetic elements created through the use of *in vitro* technologies from what is considered to be a regulated item under the HSNO Act.

16.5. As set out in my third submission document, methylation patterns established by external dsRNA treatments replicate without human intervention and transfer a trait to subsequent generations. Silencing mediated by persistent rounds of siRNA generation is also transmitted and does not require human intervention to do so. Heritability of traits, by whatever means, due to modifications using *in vitro* techniques is a special feature of the risks to be regulated and why genetically modified organisms are included as new organisms in the HSNO Act.

Precaution with consultation

17. I encourage the new DMC to review its consultation practices. It may be worthwhile to also consult the Defence Technology Agency. The new DMC also should more thoroughly support the government agencies with which it already consults. Each of these agencies should receive the EPA's staff report well in advance of a decision by the DMC, providing time for them to consult with their own staff and chief scientists.

17.1. The 2018 determination followed invitations to the Ministry of Primary Industries and the Department of Conservation to comment on the use of externally applied dsRNA. The former never responded; the latter was not aware of any biosecurity risks.

17.2. In the matter of DOC, EPA had a responsibility to have evaluated risks to biosecurity to inform that agency rather than just determine that the use of dsRNA was out of scope of legislation. If it had, it might have realised that the decision could result in the release of whole or partial viruses. In the matter of MPI's unresponsiveness, I believe that the EPA should have confirmed that MPI did not wish to reply, felt competent to reply and had *adequate time to reply*. If MPI felt that it did not have access to the necessary expert advice, it is reasonable to expect that EPA would work with MPI to find it.

18. The short timeline and aborted and limited consultation undertaken by EPA in 2018 was also out of step with international practice. In other countries, a decision by regulators of this magnitude has involved convening expert scientific advice, public comment and review by government.

18.1. The white paper produced following a meeting of the Scientific Advisory Panel (SAP) of the US Federal Insecticide, Fungicide, Rodenticide Act and the United States Environmental Protection Agency concluded that the technology deserves risk assessment. The US Environmental Protection Agency "consulted with the SAP on scientific issues that might be unique to RNAi and how they could fit under the existing risk assessment framework." The SAP agreed with the US EPA "regarding inadequacies of the current environmental fate and non-target effects testing frameworks for dsRNA PIPs [plant incorporated protectants] *and exogenously applied dsRNA products*. **Uncertainties in the potential modes of action in non-target species, potential for chronic and sublethal effects, and potential unintended consequences in the various life stages of non-target organisms are sufficient justification to question whether the current Agency framework for ecological effects testing is applicable to dsRNA PIPs**" (emphasis added to FIFRA 2014).

18.2. Presently in Australia there is before Parliament a proposal to change the definition of a genetically modified organism (OGTR 2018). These reconsiderations also included external treatments of organisms with dsRNA. Rather than attempt to deregulate dsRNA techniques with a three-person committee that acted on a short internal report, Australia conducted a comprehensive process that included multiple agencies (i.e. Office of the Gene Technology Regulator, Food Standards Australia New Zealand, Legislative and Governance Forum on Gene Technology, Department of Health) involving extensive public consultations, followed by a parliamentary process that serves as a final review because the proposed deregulation still could be disallowed. While some kinds of treatments with dsRNA may become excluded from Australia's regulations, allowed treatments would be far more limited than would be allowed under the 1 May 2018 decision by EPA.

18.3. In this submission I will not evaluate the scientific validity of the arguments for allowing certain treatments in Australia.[14] And in any case, the recommendations may never be implemented. I will, however, explain how less expansive the Australian proposal is compared to the New Zealand decision. I would also point out that by including external treatments of organisms with dsRNA in a proposal to discuss their exemption from regulations, *they had not arrived at the conclusion that treatments do not create genetically modified organisms under still current* (as of this writing) *regulations*.

18.4. The regulatory amendment specifically constrains the dsRNA molecules used in any kind of treatment in the following ways. "This item provides that techniques involving applying RNA to an organism to temporarily induce RNA interference are not gene technology, provided that:

- the RNA cannot be translated into a polypeptide
- the organism's genome sequence cannot be altered as a result, and
- an infectious agent cannot be produced."[15]

As demonstrated in my third submission document, to meet these conditions would require testing and forethought that is well beyond the requirements set by the New Zealand EPA decision. For example:

[14] I do not know whether the Australian Office of the Gene Technology Regulator was aware, for example, of replicating RNA molecules in the genomes of some organisms, the effects of dsRNA on prokaryotes, or the longevity of heritable effects including by means of replication separate from methylation. Moreover, Australia has different international obligations to New Zealand. Notably, it has not ratified the Cartagena Protocol on Biosafety.

[15] EXPLANATORY STATEMENT Select Legislative Instrument 2019 No. XX *Gene Technology Act 2000* https://www.legislation.gov.au/Details/F2019L00573/Download on 15 July 2019.

18.4.1. the dsRNA would have to be limited in size and sequence characteristics to ensure no peptides could be produced in any exposed species (Figure 2).

18.4.2. the *in vitro* techniques used to isolate, amplify and purify the dsRNA would have to be of a quality that ensured that no viral genomes in whole or in part were contaminating the intended dsRNA formulation.

18.4.3. the species or cells treated would have to be limited to those that did not have RNA-dependent DNA polymerase activity.

18.5. *None of the above qualifications, indeed no qualifications of any kind on the dsRNA, was placed on the kinds of treatments allowed by EPA in its May 2018 decision*, which is remarkable both in absolute terms and in comparison with other jurisdictions. Open air treatments, as forecast by the EPA (EPA 2018b), would cause simultaneous multispecies exposures including potential human exposures. There is so little knowledge of the potential effects. Only in the last couple of months has it even been reported that human skin cells react to external dsRNA exposure (Liao et al. 2019).[16]

18.6. The 1 May 2018 decision as far as I can tell propelled New Zealand further than possibly all other countries in the world in its breath and underestimation of this new technology and its anticipated powerful effects on the organisms intended, and not intended, to be exposed to it. Even in countries that have far more experience with the release and management of genetically modified organisms, such as Australia and the United States, there is no equivalent of the New Zealand EPA decision on external treatments of organisms with dsRNA.

In a nutshell.

19. The use of dsRNA as an external treatment of cells or organisms is an *in vitro* technique as understood in the context of the HSNO Act[4]. Similar to other regulated *in vitro* treatments, such as the use of gene editing nucleases, dsRNA treatments modify genes or other genetic material causing heritable effects with sometimes the dsRNA or a derivative homologous nucleic acid not persisting in the treated cell or organism. Cells or organisms that have been treated with dsRNA "have been modified by *in vitro* techniques" and if they reproduce then their descendants have genes or other genetic material "inherited or otherwise derived, through any number of replications, from any genes or other genetic material which has been modified by *in vitro* techniques". Therefore, both clauses (a) and (b) of the HSNO Act definition of a genetically modified organism are satisfied even though satisfying either of them would be sufficient to conclude that external treatments of cells or organisms with dsRNA creates genetically modified organisms.

    The process used to decide the contrary was extraordinary by international standards. It was unbefitting the seriousness of the decision as demonstrated by the investigative and consultative processes being used in Australia, Europe and the United States. Moreover, the historical, legislative and international treaty context of the words adopted by the HSNO Act did not figure in the EPA's interpretations. Terms such as "genetic material", "heritable material" and "genetic element" are used in a variety of ways in different contexts as well as having informal, jargon or formal meanings. Therefore, in some contexts these terms are synonyms and in others they mean very different things. There was no apparent attempt in either the staff report or the DMC report to place these terms into context of the purpose and principles of the new organisms provisions of the HSNO Act. Doing so reveals an underlying logic

[16] "[T]he authors treated early-passaged volar keratinocytes with polyinosinic:polycytidylic acid (poly[I:C]) (a synthetic analogue of dsRNA), and they found that poly(I:C) suppresses KRT9 expression."

and cohesion of word choice, making apparent the risk special to gene technologies and the potential dispersal pathways of modified organisms and genes. Case-by-case risk assessments, of external dsRNA treatments of cells or organisms, performed by the EPA is justified and appropriate in the context of New Zealand society's precautionary approach to gene biotechnology. To accomplish this, the 1 May 2018 decision should be retracted.

Submitted by

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[17] This submission is provided in accordance with the University of Canterbury Critic and Conscience of Society and Academic Freedom Policy (2018) as the author's expert opinion and not as statements of the opinion of the University of Canterbury.

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