

Responses to OGTR Discussion Paper – Technical Review of the Gene Technology Regulations

1. Which option/s do you support, and why?

Option 3 is supported.

We support the argument in Option 3 that recognizes that SDN-1 techniques that utilize site-directed mutagenesis and endogenous Non-Homologous End Joining (NHEJ) (or other template-free sequence modification techniques: e.g. Komor et al., 2016¹) are less disruptive in terms of DNA sequence changes than techniques such as chemical and radiation mutagenesis. Genome plasticity is a cornerstone of evolution and the foundation of novel trait development that underlies conventional breeding. Sequence mutations and rearrangements, such as exon shuffling, gene fusions and mobilisation, duplications, deletions and inversions continue to shape plant and animal genomes. Small and large genetic rearrangements can be spontaneous or induced by radiation, chemical mutagenesis or transposable element (TE) mobility. The application of mutagens such as ethyl methanesulfonate (EMS) or MNU (N-methyl-N-nitrosourea), which cause multiple, random, generally point mutations (single nucleotide polymorphisms, SNPs), and X-ray or gamma radiation genome disruption have been shown to cause multiple changes to the DNA sequence. A study by Morita et al. (2009)², for example, found that gamma radiation of barley induced multiple small mutations (1-16 bp), several large deletions (9.4-129.7 kb), several substitution mutations and two inversion mutations. Chemical mutagens, such as MNU, have been found to be even more efficient at inducing random nucleotide mutations than gamma radiation (Kurowska et al., 2012)³. Site-directed targeting of double-stranded DNA breaks and NHEJ typically introduce Insertion or Deletion mutations at precise locations in the genome. Naturally-occurring mutations and chemical and radiation mutagenesis techniques are unregulated because of their long history of safe use. Because the products of the routinely used 'natural' (GTTAC Communique of 6 June 2016 meeting) processes are recognized as safe, techniques such as CRISPR/Cas that offer the capacity to precisely target the location of a mutation should also be recognised as safe and similarly not subject to GMO regulation.

This argument emphasizes that it is the nature of the final organism or product rather than the method that is important. It cannot be reasonably argued that a food product incorporating germplasm with multiple, random radiation- or chemically-induced DNA mutations is safer than germplasm containing a single-site InDel mutation obtained through precise site-directed double-stranded DNA breaks and endogenous DNA repair mechanisms.

The rapid and widespread adoption of the facile site-directed mutagenesis techniques and the difficulty in discriminating between products derived from these techniques and 'natural' mutants mean that products incorporating changes derived from the use of these techniques have the potential to rapidly become widespread. Both established and start-up companies are taking advantage of the opportunities (e.g. Smyth 2015⁴). Following New Zealand's lead and

categorising products derived from the use of these techniques as GMOs will limit our capacity for research and the development of commercial outcomes. It would stymie Australian innovation at the same time as our competitors are actively developing products derived from the use of the technologies (www.agriculture.com/technology/how-gene-editing-will-change-agriculture).

2. Are there other risks and benefits of each option that are not identified in this document?

No, other than those mentioned in our response to question 1 above, the document is clearly explained and provides a good description of the pros and cons of each proposed option.

3. Is there any scientific evidence that any of options 2-4 would result in a level of regulation not commensurate with risks posed by gene technology?

Options 2 proposes to amend the GT Regulations so that dealings with all organisms developed using oligo-directed mutagenesis and all site-directed nuclease techniques are regulated under the GT Act.

Option 2 makes a discrimination that is not aligned with the risk. It would result in regulation of organisms that are not different from those that can occur spontaneously in nature or that can be generated through accepted 'natural' (GTTAC Communique of 6 June 2016 meeting) mutation techniques. Examples of these can be found in: Morita et al. 2009², Kurowska et al. 2012³, Chen et al. 1997⁵, Dooner and Weil 2007⁶, Magadum et al. 2013⁷, Horn et al. 2016⁸.

4. How might options 2-4 change the regulatory burden on you from the gene technology regulatory scheme?

It would depend on the type of information that would be required to show that the organism is not different to what might occur naturally or what might be induced through chemical or radiation mutagenesis. If it were only necessary to document the techniques used to induce DNA modifications and to show the absence of transgenic material and the nature of the modifications through sequencing, the regulatory burden would be alleviated sufficiently to allow our laboratories to develop the types of innovative products that the site-directed mutagenesis techniques have made possible.

As identified in "Cons", Option 4 would need a very clear distinction between SDN-2 and SDN-3, which would be challenging. Without this, IBCs would spend a lot of time agonising over whether specific cases are SDN-2 or SDN-3, and ultimately communicating with the OGTR for clarification.

5. How do you use item 1 of Schedule 1, and would it impact you if this item was changed?

Item 1, Schedule 1: A mutant organism in which the mutational event did not involve the introduction of any foreign nucleic acid (that is, non-homologous DNA, usually from another species). This item defines for our laboratory the difference between a GM and a non-GM organism. Typically, we interpret Item 1, Schedule 1 as meaning an organism that has spontaneously occurring mutations or mutations that have been induced by chemical or radiation mutagenesis. If it were updated to include the products of site-directed mutagenesis it would allow us to take advantage of these techniques to develop new products for agriculture, horticulture and other industries. It would also allow us to introduce these new technologies into the teaching environment.

6. Might contained laboratory research on GM gene drive organisms pose different risks to other contained research with GMOs, and how could these risks be managed? Supporting information and science-based arguments should be provided where possible.

Organisms created with gene drive cassettes modify the germline genomes of their offspring by inserting a replicated gene in a site-directed manner. Because transmission of the gene drive is through sexual reproduction, gene drives are unlikely to create safety concerns to laboratory personnel beyond those of corresponding genes without the “drive” features.

Therefore, the changed risk for laboratory research using gene drives in organisms is associated with an unintentional release, with the potential for uncontrolled breeding with external populations (wild or cultivated organisms). In general, there are 3 such cases, differing by the degree of reproductive fitness benefit that they confer on the organism.

1. The gene drive gives the organism a selective advantage, in which case the organism would be covered by existing legislation and classified as a DNIR. If an unintentional release occurred, such a gene present as cargo in a gene drive would represent a greater likelihood of establishing itself in the external population than an ordinary gene, and therefore represent a greater health/environmental/economic risk than the latter.

2. The gene drive gives the organism a large selective disadvantage, in which case the gene drive would be unlikely to spread through the external population, and would be no greater risk than the release of existing GMOs covered by NLRDs or exempt dealings. This would be the case for GMOs in which the reproductive fitness cost of the gene drive to the organism outweighs the ability of the gene drive to replicate itself to occupy both alleles at every generation.

3. The gene drive gives the organism neither advantage nor disadvantage, or the degree of disadvantage is not large, in which case there is a chance that an unintentional release would enable the gene drive to enter the external population and become fixed within a few generations.

In practice, accurately quantifying the reproductive fitness benefits for a gene drive GMO under conditions of unintentional release would be challenging, and therefore it would be difficult to discriminate between cases 2 and 3. Moreover, there is the potential for mutations in the guide

sequence or in genes that interact with the target sequence to change the effect of the gene drive on reproductive fitness after an initial assessment has been done.

In cases 1 and 3 the health, environmental and economic consequences of an unintentional release and subsequent entry of gene drives into an external population would be determined by the identities of the organism and gene drive involved. For many organisms that are used as biological models in laboratory research and are of low commercial value, the consequences would be minimal. However, the use of gene drives in some organisms warrants closer scrutiny than in others. These include:

1. Organisms used for food or other natural products, which could have reduced commercial value if their GM status affected that, especially such organisms that proliferate rapidly or disperse widely.
2. Organisms that are vectors for serious diseases of humans or other organisms, which could have detrimental health consequences if the gene drive inadvertently conferred a greater ability to spread the disease.
3. Australian native organisms, in which a gene drive that confers reduced reproductive fitness could cause a population crash and possibly extinction. This, in turn, could have serious flow-on environmental consequences.

For these reasons it will be important to consider the specifics of each proposed dealing so as to ensure that containment addresses the specific risk of GMO escape. Consideration might be given to requiring higher standards of containment and genome target site specificity for laboratory research using gene drives in organisms outlined in points 1-3 above. Various strategies have been proposed for controlling gene drive releases, either as a response or pre-emptively, as described in Esvelt et al. 2014⁹ and Oye et al. 2014¹⁰.

7. What RNA interference techniques are you using, and are there RNA interference techniques that you believe have unclear regulatory status? Please provide details of the techniques and science-based arguments for whether these techniques pose risks to human health or the environment.

We use RNA interference techniques that are generally derived from stably incorporated T-DNA sequences and therefore these RNAi plants are defined as GMOs and they remain clearly within the regulations. RNA oligonucleotides and plasmid and viral vectors expressing shRNA or miRNA sequences are also used.

8. Do you have proposals for amendments to any other technical or scientific aspects of the GT Regulations? All proposals should be supported by a rationale and a science-based argument.

No.

References

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