CropLife AUSTRALIA

*Representing **the best** of the plant science industry*

CropLife Submission to Discussion Paper on Options for Regulating New Technologies

16 December 2016

1 INTRODUCTION

CropLife Australia (CropLife) is the national peak industry organisation representing the agricultural chemical and plant biotechnology sector in Australia. CropLife represents the innovators, developers, manufacturers and formulators of crop protection and agricultural biotechnology products. The plant science industry provides products to protect crops against pests, weeds and diseases, as well as developing crop biotechnology products that are key to the nation's agricultural productivity, sustainability and food security. The plant science industry is worth more than $17.6 billion a year to the Australian economy and directly employs thousands of people across the country. CropLife Australia is a member of CropLife Asia and part of the CropLife International Federation of 91 CropLife national associations globally.

CropLife recognises the importance of innovation in plant breeding. In most cases, plant breeding innovations are improvements and refinements of traditional breeding methods used to optimise plant health, nutritional quality and yield. Conventional breeding has a long history of delivering varieties that are safe for human and animal consumption.

The views presented in this submission reflect CropLife's concerns about the prospect of pre-market regulation of products developed using new technologies (termed plant breeding innovations by the plant science industry), based simply on the technique employed during the development of specific traits and not on the characteristics of the final product. Regulations, if needed, should be based on sound scientific principles and proportional to the degree to which the product is creating new potential risks to human health or the environment, and not solely triggered by the process used in the course of its development.

The Option supported by CropLife reflects its views that *plant varieties developed through the latest breeding methods should not be differentially regulated based on the techniques employed during their development if they are similar to or indistinguishable from varieties that could have been produced through earlier breeding methods*.

CropLife commends the Gene Technology Regulator for the high quality of the *Discussion Paper on Options for Regulating New Technologies* (Discussion Paper) and welcomes the opportunity to provide input addressing the specific consultation questions. This input is generally focussed on the use of new technologies in plant breeding, however, it is also applicable broadly to all types of organisms where such new technologies may be used.

2 SUPPORTED OPTION

CropLife strongly supports **Option 4** of the Discussion Paper, which proposes to exclude the products of oligo-directed mutagenesis (ODM), site-directed nuclease (SDN)-1 and SDN-2 from the scope of regulation.

Option 4 will have the effect of expressly clarifying the scope of the Gene Technology (GT) Regulations in relation to the products of new technologies in a manner that is consistent with the original scope and intent of the regulatory scheme, which excludes from regulation techniques with a history of safe use. This option is also consistent with the principle and central consideration that organisms created using gene technologies should be regulated in a manner that is commensurate with the biosafety risks they pose, and also goes some way to harmonising the regulation of gene technology under the *Gene Technology Act 2000* (Cth) with that of food produced using gene technology under the Food Standards Code.

The "cons" of the other three options are set out in the Discussion Paper and CropLife would like to emphasise important aspects of these. Option 1 is the least feasible of the options as it provides no legal clarity for stakeholders and would effectively render the regulatory scheme outdated and dysfunctional as technologies continue to evolve. Options 2 and 3 involve the regulation of technologies that are not included in Option 4 and they are not commensurate with risk. Claims regarding uncertainties associated with technologies simply because they were recently developed assumes a higher intrinsic risk compared to older technologies, when risk (if any) should be determined by the properties of resulting products and based on scientific evidence. Option 4, which is based on the properties of the modified organisms, is the most scientifically sound of the options.

The "cons" of Option 4 note that the current regulatory scheme is based on a process trigger, whereas this option is focussed on the product, thus this option challenges the current policy setting. The Discussion Paper also notes that this option would require changes to the GT Regulations to include specific techniques, rather than broad exclusions based on characteristics of the product. While it has long been the position of CropLife that any scientifically sound regulatory scheme should be based on the characteristics of the product, the Discussion Paper itself notes that a continuum of techniques now exists. It is evident that for the regulatory scheme to remain functional into the future, it must find a way to adapt to this continuum and CropLife strongly supports Option 4 as a first step in this direction, noting that the current regulations already specifically exclude certain mutagenesis techniques.

In addition to supporting Option 4, as invited by the Discussion Paper, CropLife proposes the Regulator consider amending the GT Regulations to expressly exclude cisgenic organisms from the scope of regulation. CropLife also supports the Regulator's intention to clarify that null segregants are not GMOs and therefore not subject to regulation.

In our scientific rationale that follows, the techniques included in the scope of Option 4, as well as cisgenesis, are compared against spontaneous mutation processes in eukaryotic organisms, induced mutagenesis techniques that are excluded from the scope of regulation (e.g. those listed in Schedule 1A of the GT Regulations) for which there is a history of safe use, currently regulated genetic engineering techniques (e.g. rDNA), as well as natural recombination and conventional breeding.

3 SCIENTIFIC RATIONALE

Option 4

The techniques included in the scope of Option4, namely mutagenesis techniques based on cellular DNA repair (SDN-1, SDN-2 and ODM techniques), have been used in plants for the targeted mutagenesis of endogenous genes to, for example, induce loss of function, modulate activity or alter function. This has been shown to result in agronomically useful traits such as herbicide tolerance, changed nutritional composition, and resistance to biotic (e.g. disease) and abiotic stresses.[1]

These types of techniques are often equated to established induced mutagenesis techniques (e.g. with the use of chemicals or radiation), as the SDN or oligomer essentially acts as a mutagenic substance, and they result in comparable genetic changes[2]. These techniques, however, differ in that they induce **targeted** genomic changes, meaning that the site of the genetic modification is known in advance and its effect can be predicted. [3,4] This requires prior knowledge of the gene or genes responsible for the desired trait. In contrast, induced mutagenesis techniques cause unknown, random, genome-wide changes.[5,6]

A concern with Option 4 raised in the Discussion Paper is that excluding these targeted mutagenesis techniques from regulation may not adequately manage potential risks their products may pose. Consideration of these risks should also take into account the effects of spontaneous, or naturally occurring mutation mechanisms, and that of the induced mutagenesis techniques that are excluded from regulation (Schedule 1A GT Regulations).

Spontaneous and induced mutagenesis

Mutations are generally classified as spontaneous or induced, and mutation theory establishes that all are derived from premutagenic damage to DNA. Premutagenic damage that leads to spontaneous mutations is produced by a variety of factors present in normally growing cells. The major causes of premutagenic damage include erroneous action of the replicative apparatus during DNA replication with an intact DNA template and the usual deoxynucleotides; misinsertion of a mutagenic nucleotide with loose specificity of base-pairing during DNA replication; and chemical reactions by endogenous mutagens, such as active oxygen species, and spontaneous decomposition of DNA bases.[7]

Spontaneous mutations are known to occur frequently and these bring about the genomic sequence changes that are the basis of evolution. Infidelities of the DNA replication and repair machinery can result in small local sequence changes, such as the deletion or insertion of one or a few adjacent nucleotides, or rearrangement of several neighbouring nucleotides. It is this mechanism that is exploited by the SDN technologies. In plants, estimates of mutation rates based on single nucleotide polymorphisms indicate more than ten spontaneous mutations per generation due to such mechanisms. Larger rearrangements of stretches of nucleotides may occur with the movement of transposable elements, which are known to be widespread in living organisms.[8] Transposition commonly results in gene gains, duplications and losses.[9]

1 Podevin, N, Davies, HV, Hartung, F, Nogue F, Casacuberta, JM (2013) Site-directed nucleases: A paradigm shift in predictable, knowledge-based plant breeding, *Trends in Biotechnology* 31: 375-383.

2 EFSA (European Food Safety Authority) (2015) Genetically modified organisms UNIT. Mandate Number: M-2015-0183; and see also: http://www.easac.eu/fileadmin/PDF_s/reports_statements/Easac_14_NBT.pdf

3 Hartung, F, Schiemann, J (2014) Precise plant breeding using new genome editing techniques: opportunities, safety and regulation in the EU, *The Plant Journal* 78: 742-752.

4 Podevin, N et al. (2013) *Trends in Biotechnology* 31: 375-383.

5 Hartung, F, Schiemann, J (2014) *The Plant Journal* 78: 742-752.

6 Podevin, N et al. (2013) *Trends in Biotechnology* 31: 375-383.

7 Maki, H (2002) Origins of spontaneous mutations: specificity and directionality of base-substitution, frameshift, and sequence-substitution mutageneses, *Annual Review of Genetics* 36: 279-303.

8 Arber, W (2010) Genetic Engineering Compared to Natural Genetic Variations, *New Biotechnology* 27: 517-521; Schnell, J, Steele, M, Bean, J, Neuspiel, M, Girard, C, Dormann N, Peason, C, Savoie, A, Bourbonniérre, L, Macdonald, P (2015) A Comparative Analysis of Insertional Effects in Genetically Engineered Plants: Considerations for Pre-market Assessment, *Transgenic Research* 24: 1-17.

9 Strauss SH, Sax JK (2016) Ending event-based regulation of GMO crops, *Nature Biotechnology* 34: 474-477;

Plants, in particular, have considerable capacity to undergo genetic change and several additional mechanisms underlying natural mutation have been described that contribute to a process of constant genome restructuring and reprogramming. In comparison, animal genomes are relatively stable and conserved.[10] Greater plasticity in plants is believed to contribute to the maintenance of adaptive phenotypes and facilitate longevity, and to be a natural consequence of their immobility.[11] The presence of duplicated forms of genes is common in plants, and multigene families have been found that have arisen from the duplication of larger genomic regions and whole genomes.[12] Of note, these mechanisms are considered to have a greater impact on genome sequence and function than gene insertion using genetic engineering.[13] Also notable is the lack of evidence that a naturally occurring random genetic change has resulted in a novel safety concern, e.g. due to the creation of new genes or alleles, changes in gene expression level, expression of novel proteins, or production of novel metabolites.[14]

The movement of transposable elements may have several potential impacts on the genome, in that they may contain one or more open reading frames, they may increase gene copy number, and/or may activate or inactivate genes. These changes can alter gene expression and the nature of proteins expressed, that may lead to modified or novel traits. In plants, transposable elements can account for substantial portions of the genome, e.g. 25% in rice and 57% in maize, and their insertion near genes have been associated with some important traits, e.g. the anthocyanin biosynthesis pathway in grapes that is responsible for berry colour.[15]

Spontaneous mutations may result in no effect on the phenotype of the organism, i.e. the mutations are neutral or silent, or they may modify a characteristic (in terms of level of gene expression), introduce a newly expressed characteristic, or cause the loss of a previously expressed characteristic. These effects may be selectively advantageous or disadvantageous, however evolutionary beneficial mutations are relatively rare.[16]

Plant breeding has long exploited the genetic variation that results from spontaneous mutation mechanisms in selecting for important traits, and such spontaneous mutations may result in modified or new characteristics that are selected for and preserved in crop breeding, e.g. the semi-dwarf variation in cereal crops, which has contributed significantly to improved grain yield.[17] Limitations of spontaneous mutations in this context include their low frequency and that only a small number of such mutations lead to phenotypic characteristics of interest. Therefore, induced mutagenesis via physical (e.g. irradiation) or chemical treatments may be used to accelerate the process.[18] Such induced mutagenesis techniques result in random mutations, which may include deletions ranging in size from tens to millions of base pairs, and rearrangements that include inversions and chromosomal translocations.[19] A limitation of this approach is that large populations of mutagenized plants must be screened in order to select plants with desired changes to be included in breeding programs. Generations of crossing may then be needed to segregate away unwanted mutations that may impact on plant performance.[20]

Induced mutagenesis techniques commonly used for the development of new or improved traits in plant breeding are excluded from regulation (Schedule 1A GT Regulations) on the basis of their demonstrated history of safe use, with chemical and irradiation techniques in use for the

10 Murat, F, Van de Peer, Y, Salase J (2012) Decoding plant and animal genome plasticity from differential paleo-evolutionary patterns and processes, *Genome Biology and Evolution* 4: 917-928.

11 Borges, RM (2008) Plasticity comparisons between plants and animals, *Plant Signalling and Behaviour*, 3: 367-375.

12 Weber, N, Halpin, C, Hannah, LC, Jez, JM (2012) Editors choice: Crop Genome Plasticity and its relevance for food and feed safety of genetically engineered breeding stacks, *Plant Physiology* 160: 1842-1853.

13 Strauss SH, Sax JK (2016) *Nature Biotechnology* 34: 474-477; Schnell et al. (2015) *Transgenic Research* 24: 1-17.

14 Strauss SH, Sax JK (2016) *Nature Biotechnology* 34: 474-477.

15 Arber, W (2010) *New Biotechnology* 27: 517-521; Schnell et al (2015) *Transgenic Research* 24: 1-17.

16 Arber, W (2010) *New Biotechnology* 27: 517-521; Schnell et al. (2015) *Transgenic Research* 24: 1-17.

17 Xiong, J-S, Ding, J, Li, Y (2015) Genome-editing technologies and their potential application in horticultural crop breeding, *Horticultural Research* 2: 15019, doi:10.1038/hortres.2015.19.

18 Andersen, MM, Landes X, Xiang W, Anyshchenko A, Falhof, J, Østerberg, JT, Olsen, LI, Edenbrandt, AK, Vedel, SE, Thorsen, BJ, Sandøe P, Gamborg, C, Kappel, K, Palmgren, MG (2015) Feasibility of New Breeding Techniques for Organic Farming, Trends in Plant Science 20: 426-434; Xiong, J-S, Ding, J, Li, Y (2015) *Horticultural Research* 2: 15019, doi:10.1038/hortres.2015.19

19 Schnell et al. (2015) *Transgenic Research* 24: 1-17.

20 Podevin, N et al. (2013) *Trends in Biotechnology* 31: 375-383.

development of new crop varieties for at least 60 years.[21],[22] The FAO/IAEA Mutant Variety Database lists 3241 officially released cultivars in more than 200 plant species registered since 1950.[23] In the past 20 years, these techniques have been complemented by biotechnology, with the early techniques (e.g. rDNA) now also having a demonstrated history of safe use.

Targeted mutagenesis

The targeted mutagenesis techniques of SDN-1 and SDN-2 employ a site-directed nuclease (SDN) to create a double--stranded break at a defined site in the genome, and exploit the natural cellular mechanisms for DNA repair.

For SDN-1, deletions, insertions and rearrangements are often observed at repair sites, and these are analogous and indistinguishable at the DNA sequence level from deletions, insertions and rearrangements that are obtained using induced mutagenesis techniques, and observed in sites flanking transposon movement or DNA insertions in genetically engineered plants.[24] The exact sequence of mutated organisms cannot be predicted but their phenotypes can be screened for the presence of the intended change.[25]

For SDN-2, the outcomes are more predictable than for SDN-1 due to the use of a template to direct repair of the DNA double-stranded break. The repair template is introduced to the cell at the same time as the SDN and results in the precise change defined by the repair template.[26]

The ODM mutagenesis technique differs to the SDN techniques in that it does not employ a nuclease to create DNA double-stranded breaks at target sites in the genome, and it uses a short oligonucleotide to direct DNA repair. In both ODM and SDN-2, the oligonucleotide/repair template is identical to the corresponding site in the genome with the exception of the nucleotide changes intended to be incorporated during repair.[27]

An argument raised to support claims of risk associated with these technologies is the possibility for unintended, off-target effects. However, the site-directed nature of SDN-1, SDN-2 and ODM techniques reduces these effects in comparison with tools that induce random mutagenesis. Furthermore, in plants, while off-target effects have been reported with SDN techniques, their frequency is considered to be well below that which occurs with other mutagenesis techniques, and comparable to that which occurs in cross breeding.[28] The ODM and SDN techniques require careful target design, which depends on the availability of precise genome sequence and knowledge of gene function. Further, their precision/specificity and efficiency must be optimised through experimentation, and optimal methods for delivery into the relevant target cells need to be determined. Thus, these techniques are not as technically simple to employ in any eukaryote as the general media suggests. Though the general finding to date has been that often no off-target target effects are observed; when they have been observed they are typically similar to or less than the generation to generation variability or variability among individuals in the genome of a species.[29]

21 Hartung, F, Schiemann, J (2014) *The Plant Journal* 78: 742-752

22 Ahloowalia, B. S., Maluszynski, M., & Nichterlein, K. (2004). Global impact of mutation-derived varieties. *Euphytica* 135(2), 187-204.

23 https://mvd.iaea.org/#!Home, accessed 21 November 2016; Podevin, N et al. (2013) *Trends in Biotechnology* 31:375-383.

24 Schnell et al. (2015) *Transgenic Research* 24: 1-17.

25 Jones, HD (2015) Future of breeding by genome editing is in the hands of regulators, *GM Crops & Food* 6: 223-232.

26 Sprink, T, Eriksson, D, Schiemann, J, Hartung, F (2016) Regulatory Hurdles for Genome Editing: Process- vs Product-Based Approaches in Different Regulatory Contexts, *Plant Cell Reports* 35: 1493-1506; Jones, HD (2015) GM Crops & Food 6: 223-232.

27 Sprink et al (2016) *Plant Cell Reports* 35: 1493-1506; Jones, HD (2015) *GM Crops & Food* 6: 223-232.

28 European Food Safety Authority Panel on Genetically Modified Organisms (2012) Scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function, *EFSA Journal* 10: 2943; Podevin, N et al. (2013) *Trends in Biotechnology* 31: 375-383.

29 See e.g. Cho, SW, Kim, S, Kim Y, Kweon, J, Kim HS, Bae, S, Kim, J-S (2014) Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases, *Genome Research* 24: 132-141; Shen, B, Zhang, W, Zhang, J, Zhou, J, Wang, J, Chen, L, Wang, L, Hodgkins, A, Iyer, V, Huang, X, Skarnes, WC (2014) Efficient genome modification by CRISPR-Cas9 nickase with minimal off-target effects, *Nature Methods* 11: 399-404; Cho, SW, Kim, S, Kim, JM, Kim, J-S (2013) Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease, *Nature Biotechnology* 31: 230-232; Gaj, T, Gersbach, CA, Carlos FB (2013) ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering, *Trends in Biotechnology* 31: 397-405; Hwang, WY, Fu, Y, Reyon, D, Maeder, ML, Tsai, SQ, Sander, JD, Peterson, RT, Yeh J-RJ, Joung, JK (2013) Efficient genome editing in zebrafish using a CRISPR-Cas system, *Nature Biotechnology* 31: 227-229.

In plants, off-target effects may be greatly limited by downstream selection to remove undesired phenotypes. [30] In plants, while it is theoretically possible, unintended effects arising from cross-breeding do not correspond to increased impacts on safety, e.g. production of a new toxin or allergen, as there are no documented cases of this occurring.[31]

The targeted nature of SDN-1, SDN-2 and ODM techniques differentiates them from transgenesis which is characterised by the random integration of recombinant DNA. Targeted integration of transgenes is possible with the SDN-3 technique, which like SDN-1 and SDN-2 induces DNA double-stranded breaks at specific locations.

Differentiating SDN-1, SDN-2 and ODM from SDN-3

The SDN-1, SDN-2 and ODM techniques are distinguished from established techniques for genetic modification (e.g. rDNA technology) and SDN-3 techniques in one critical way: the changes to the DNA sequence induced by these methods brings about changes in **endogenous gene sequence and function** that are in principle **possible to create using conventional methods** (i.e. crossing/breeding, mutagenesis). Furthermore, the genes or genetic material altered by these techniques and the respective organisms that carry these changes may likely be **indistinguishable at the DNA sequence level from organisms which can be obtained with alternative mutagenesis or breeding methods.** In contrast, established techniques for genetic modification or SDN-3-type techniques may result in the **integration of a novel functional gene(s)** that encodes for the production of an introduced protein(s) or other expressed substance. SDN-3 may also potentially be used to introduce gene stacks at a specific single locus.[32]

As described in the Discussion Paper, a difference between SDN-2 and SDN-3 is the extent of the genetic modification introduced using the repair template. We emphasise, however, the key distinguishing feature of the intended outcome: SDN-2 aims to make a site-specific modification to **endogenous gene function**, whereas SDN-3 intends targeted **insertion of a specific gene construct with promoter, coding region and terminator sequences**. For example, SDN-2 appeals to plant breeders due to the potential to modulate the function of existing alleles directly in elite breeding lines and avoid the impracticalities of generations of crossbreeding to introduce the desired trait.[33] With SDN-3, the inserted gene may not already be present in the genome sequence of the host organism or its sexually compatible breeding pool (transgenesis), or it may be present in its sexually compatible breeding pool (cisgenesis). Where SDN-3 is used for transgenesis, the resulting product **could not be achieved using conventional methods** (i.e. crossing/breeding, mutagenesis), and this differentiates transgenesis from cisgenesis (see section on cisgenesis below).

Another consideration, as noted in the Discussion Paper, is that organisms created using ODM, SDN-1 and SDN-2 may not be easily identifiable, even with DNA sequencing, as the genetic changes may not be distinguishable from spontaneous or induced mutations. The Discussion Paper notes that this is an issue for enforcement and compliance with the regulatory scheme.[34] It is, however, also clear that the regulation of such products would be disproportionate to the risks they pose.

Use of Successive Rounds of SDN-2 or ODM

The Discussion Paper refers to the use of successive rounds of SDN-2 to achieve substantial modifications that are more comparable to SDN-3 in terms of the risk posed by resulting organisms. While this approach could be considered both technically and practically challenging for the development of a commercial product given the time and cost involved compared to only using SDN-3, these products can still be differentiated from SDN-3.

30 Wolt, JD, Wang, K, Yang, B (2016) The Regulatory Status of Genome-edited Crops, *Plant Biotechnology Journal* 14: 510-518.

31 Schnell et al. (2015) *Transgenic Research* 24: 1-17.

32 Podevin et al. (2013) *Trends in Biotechnology* 31: 375-383.

33 Jones, HD (2015) *GM Crops & Food* 6: 223-232.

34 It is for similar enforcement and compliance reasons that high processed oils and sugars that do not contact any detectable GM DNA or protein are exempted from Australia mandatory labelling requirements for GM foods.

As noted above, SDN-2 aims to **modify endogenous gene function**, whereas SDN-3 intends to **integrate a novel genetic construct into the genome**. Where repeated rounds of SDN-2 results in modifying the function of an endogenous gene it will remain SDN-2. Where the outcome is equivalent to the insertion of a gene that is outside of the sexually compatible breeding pool, this is consistent with transgenesis (SDN-3). Where the outcome is equivalent to the insertion of a gene that is within the sexually compatible breeding pool, this will be consistent with cisgenesis, which CropLife suggests should be excluded from regulation.

Additionally, established breeding techniques could be used to achieve the same result as successive rounds of SDN-2 or ODM. For example, mutagenic techniques may be used to generate multiple mutations, which are then combined by crossing. This has been done with barley developed in Australia by creating a breeding stack of a gamma-radiated mutant, an EMS-derived mutant and a spontaneous mutant from Ethiopia.[35]

Cisgenesis

In plants, cisgenesis may involve the use of established techniques for genetic modification methods, i.e. random integration of recombinant DNA molecules into the genome, or SDN-3 techniques for site-specific integration of a gene. Cisgenic organisms are characterised by the use of donor DNA that originates from the species itself or a cross-compatible species, i.e. the wider sexually compatible gene pool for the species, and the resulting organisms could in principle be developed using conventional breeding techniques.[36] In crops, the use of cisgenesis has been reported for improving pathogen resistance, e.g. scab resistance in apple and late blight resistance in potato.[37] Transgenesis is differentiated from cisgenesis in that it allows for the integration of DNA from an unrelated, cross-incompatible species.[38]

In cisgenesis, the introduced DNA is a naturally occurring fragment of genomic DNA that contains the gene of interest with its associated regulatory sequences, i.e. promoter, coding region including its introns, terminator sequences, and 5' and 3' untranslated regions in the normal-sense orientation.[39] This genomic DNA, the protein(s) it encodes, and the phenotype it confers already exist in nature and are not novel to the germplasm pool. The use of cisgenesis requires prior knowledge of the gene sequence, its position, and its function in the genome of origin. When the cisgene is integrated into the genome of the recipient, it is expected to show comparable fitness, toxicity/allergenicity, and potential effects on non-target organisms to organisms developed using conventional methods.[40] It is also possible that the expression of the cisgene may fall outside the range of expression variation observed in conventional varieties, however such an outcome is also possible via conventional breeding.[41] This contrasts with transgenesis for the introduction of a novel trait that does not occur in the species and cannot be introduced using conventional breeding methods.[42]

In agriculture, wild relatives of domesticated crops and landraces have long been used in intra- and inter-specific hybridisation. The primary advantage of cisgenesis over conventional breeding methods is improved efficiency and ability to respond to agricultural challenges.[43] This arises as a result of more targeted access to:

35 Tanner, G. J., Blundell, M. J., Colgrave, M. L. and Howitt, C. A. (2016), Creation of the first ultra-low gluten barley (Hordeum vulgare L.) for coeliac and gluten-intolerant populations. *Plant Biotechnology Journal*, 14: 1139–1150. doi:10.1111/pbi.12482

36 Cardi, T (2016) Cisgenesis and genome editing: Combining concepts and efforts for a smarter use of genetic resources in crop breeding, *Plant Breeding* 135: 139-147.

37 Cardi, T (2016) *Plant Breeding* 135: 139-147.

38 Araki, M, Ishii, T (2015) Towards social acceptance of plant breeding by genome editing, *Trends in Plant Science* 20:145-149.

39 Holme, IB, Wendt, T, Holm PB, Intragenesis and cisgenesis as alternatives to transgenic crop development, *Plant Biotechnology Journal* 11: 395-407.

40 Cardi, T (2016) *Plant Breeding* 135: 139-147; Schouten, HJ, Krens FA, Jacobsen E (2006) Cisgenic plants are similar to traditionally bred plants, *EMBO Reports* 7: 750-753.

41 European Food Safety Authority Panel on Genetically Modified Organisms (2012) Scientific opinion addressing the safety assessment of plants developed through cisgenesis and intragenesis, *EFSA Journal* 10: 2561.

42 Schouten, HJ, Krens, FA (2006) Do cisgenic plants warrant less stringent oversight? *Nature Biotechnology*, 24:753.

43 Cardi, T (2016) *Plant Breeding* 135: 139-147.

i. Specific beneficial traits that are present in the crossable breeders' pool and wild relatives but not in crop plants. This will help widen the available genetic variation and allow breeders greater ability to utilise genetic potential present in wild relatives. Exotic breeding lines and wild relatives have broader genetic variability that allow adaptation to changing environmental conditions via natural evolutionary processes.[44]

ii. Specific beneficial traits without the disadvantages of unwanted traits associated with linkage drag. Overcoming linkage drag requires successive generations of backcrossing, however, this is not always possible and depends on the chromosomal positon of the desired trait.[45]

iii. Previously inaccessible beneficial traits. In some cases, beneficial traits are positioned in chromosome regions that have very low recombination frequencies, which means that the chance of transferring the specific trait via conventional breeding to a breeding line is very low or impossible.

Cisgenesis also allows for overcoming the inability to introgress valuable traits via conventional breeding in commercial species that are clonally propagated or sterile.

Plants created by cisgenesis are considered to be analogous to that which can be created using conventional plant breeding methods as the transfer of the same genetic material would be possible. This is consistent with the conclusion reached by the GMO Panel of the European Food Safety Authority that similar hazards can be associated with cisgenic and conventionally bred plants.[46] The types of changes that may occur in the genome due to cellular DNA repair mechanisms during conventional breeding are also expected to occur at the integration site in cisgenic plants, but only at that locus.[47] Changes that may occur with the insertion include rearrangements or translocations in the flanking regions, which could impact on genes and open reading frames. However, as detailed above, such changes are reported to occur spontaneously in plants, and are especially common in regions where transposons are active, and with the use of induced mutagenesis techniques, and these result in larger changes than that created at integration sites for cisgenes (or transgenes). [48]

Null Segregants

CropLife also supports the Regulator's intention to clarify that null segregants are not GMOs. There is no scientific basis for organisms that are derived from GMOs (that would be regulated under the Act) that no longer contain the DNA insert that was integrated into the genome of that GMO to be regulated under the Act. Such organisms have lost the transgenic event (insert) due to normal segregation following conventional breeding with an organism that did not contain the transgenic event. These organisms do not contain any elements of the transgenic event and cannot be identified as being a GMO, or derived from one, using detection tools for the detection of the original transgenic event. These organisms are therefore indistinguishable from that obtained through conventional breeding methods and should be excluded from regulation in the same manner. We note that it is for the same reasons that the products of ODM, SDN-1 and SDN-2 should also not be regulated.

44 Andersen, MM et al (2015) *Trends in Plant Science* 20: 426-434.
45 Andersen, MM et al (2015) *Trends in Plant Science* 20: 426-434.
46 European Food Safety Authority Panel on Genetically Modified Organisms (2012) *EFSA Journal* 10: 2561.
47 European Food Safety Authority Panel on Genetically Modified Organisms (2012*) EFSA Journal* 10: 2561.
48 Schouten, HJ, Krens FA, Jacobsen E (2006) *EMBO Reports* 7: 750-753

4 ADDITIONAL COMMENTARY

In the Discussion Paper, the Regulator calls for commentary in a number of different areas, some of which are addressed in this submission. Additional commentary on the areas of RNA interference and the importance of consistency in regulation between Australian Government agencies is provided below.

RNA interference

RNA interference (RNAi) is a natural eukaryotic cellular mechanism used to silence or modulate the expression of genes that is triggered by the presence of double-stranded RNA (dsRNA).[49] RNAi-based methods involving the topical application of double-stranded RNA (dsRNA) for the purpose of silencing or modulating the expression of specific endogenous genes in a target organism should not be included in the scope of gene technology regulation. This is a non-transgenic approach with potential crop protection applications, including the management of insect pests, and the protection of beneficial domesticated insects such as the honeybee from disease.[50] It requires mechanisms for efficient delivery and uptake of dsRNA by target organisms but these **do not involve the creation of GMOs**.[51] The delivery of dsRNA to cells of target crop pests to induce RNAi and silencing of genes impacting on fitness or mortality have been reported, e.g. in nematodes, and lepidopteran and coleopteran insect, however, optimal strategies for efficient delivery and uptake remain under investigation.[52] Products based on this technology should be regulated in accordance with existing schemes for biological plant protection products.

Consistency of Regulation between Australian Government Agencies

CropLife believes it is important that new technologies are regulated as consistently as possible between Australian Government regulatory agencies. This is a matter of good regulatory practice and serves to avoid a situation whereby, for example, a product is regulated as a GMO in regard to its release into the environment, but not as a GM food, and vice versa.

Food Standards Australia New Zealand (FSANZ) has held two scientific workshops that considered a range of new technologies for the purpose of providing advice on the regulation of derived GM food.[53] A summary of the workshop outcomes is outlined below:

TECHNIQUE	REGULATED AS GM FOOD
Targeted transgene addition or replacement (c.f. SDN-3)	Yes
Cisgenesis / Intragenesis	Yes – but should only require a simplified food safety assessment
Oligo-directed mutagenesis	No
Targeted mutagenesis (c.f. SDN-1; SDN-2)	No – so long as not used to introduce a new gene

49 Baum, JA, Bogaert, T, Clinton, W, Heck, GR, Feldmann, P, Ilagan, O, Johnson, S, Plaetinck, G, Munyikwa, T, Pleau, M, Vaughn, T, Roberts, J (2007) Control of coleopteran insect pests through RNA interference, *Nature Biotechnology* 25: 1322-1326.

50 Scott, JG, Michel, K, Bartholomay, LC, Siegfried, BD, Hunter, WB, Smagghe, G, Zhu, KY, Douglas, AE (2013) Towards the elements of successful insect RNAi, *Journal of Insect Physiology* 59: 1212-1221.

51 Robinson, KE, Worrall, EA, Mitter, N (2014) Double stranded RNA expression and its topical application for non-transgenic resistance to plant viruses, *Journal of Plant Biochemistry and Biotechnology* 23: 231-237.

52 Killiny, N, Hajeri, S, Tiwari, S, Gowdam S, Stelinski, L (2014) Double-stranded RNA uptake through topical application, mediates silencing of five CYP4 genes and suppresses insecticide resistance in *Diaphorina citri*, PLOS ONE 9: e110536; Burand, JP, Hunter, WB (2013) RNAi: Future in insect management, *Journal of Invertebrate Pathology* 112:S68-S74; Baum, JA et al (2007) *Nature Biotechnology* 25: 1322-1326.

53 http://www.foodstandards.gov.au/consumer/gmfood/Pages/New-plant-breeding-techniques-in-the-spotlight.aspx

For the targeted mutagenesis techniques of ODM, SDN-1 and SDN-2, it was recognised that the genomic changes were typically small and definable with predictable outcomes, and these outcomes were comparable to that possible with mutagenesis. Thus, food derived from plants developed using these techniques should not be regarded as GM food. This scientific interpretation is consistent with our submission supporting the exclusion of the products of ODM, SDN-1 and SDN-2 from the scope of the OGTR's regulatory scheme.

For cisgenesis, it was recognised that derived food would be similar to that produced using standard transgenic techniques, but the transferred genes will be derived from the same or a closely related species, which is likely to be commonly used as food and have a history of safe use. For this reason, a reduced food safety assessment required was considered proportional to the risk posed. This view is consistent with the conclusion reached by the GMO Panel of the European Food Safety Authority that similar hazards can be associated with cisgenic and conventionally bred plants.[54] In the context of environmental release, however, CropLife believes that organisms developed using cisgenesis pose equivalent biosafety risks to those developed using conventional methods.

CropLife recognises that the OGTR and FSANZ operate under separate pieces of legislation and that they regulate different products of biotechnologies for different risks, therefore there may be unavoidable areas of divergence (differentiation of regulation of cisgenic organisms may be one example). We strongly believe, however, that efforts should be made to harmonise the way in which new technologies are regulated as far as possible, consistent with Australian Government's high-level policy priority of minimising regulatory 'red tape'. CropLife believes this is achievable as an outcome of the current technical review of the regulations.

54 European Food Safety Authority Panel on Genetically Modified Organisms (2012) *EFSA Journal* 10: 2561.

5 CONCLUSION

In this submission, CropLife has presented scientific arguments in support of Option 4 as provided in the Discussion Paper. This option is supported as it reflects the long-held position of the plant science industry that regulation should be scientifically sound, evidence-based and proportionate to the risks presented by novel characteristics of the product, and not the technology used to create it. It is noted that this presents a challenge for the Australian regulatory scheme that has a process-trigger. While this scheme has provided one of the most robust, science-based regulatory systems in the world for established techniques for genetic modification, biotechnology has evolved and the scheme no longer provides legal clarity for the continuum of techniques and applications that exist today.

The application of Option 4 would require the addition of specific techniques and organisms in Schedules 1 and 1A in the GT Regulations to exclude them from the scope of regulation. This would, however, be a short-term fix and the challenge of biotechnological advancement can be expected to continue. The comparability of products of the new technologies discussed in this submission with that of other unregulated techniques demonstrates that it may be impossible to determine the techniques used. This highlights the overarching need for the regulatory scheme to takes a broader approach than just process in order to accommodate established technologies as well as new and emerging technologies over the longer-term. CropLife would like to see this overarching need canvassed in the anticipated review of the *Gene Technology Act 2000* (Cth) in 2017.

6 WORKS CITED

Note that the numbering of citations in this section is not intended to align with the numbering of footnotes in the main body of the submission.

1. Podevin, N, Davies, HV, Hartung, F, Nogue F, Casacuberta, JM (2013) Site-directed nucleases: A paradigm shift in predictable, knowledge-based plant breeding, *Trends in Biotechnology* 31: 375-383.
2. EFSA (European Food Safety Authority) (2015) Genetically modified organisms UNIT. Mandate Number: M-2015-0183.
3. http://www.easac.eu/fileadmin/PDF_s/reports_statements/Easac_14_NBT.pdf
4. Hartung, F, Schiemann, J (2014) Precise plant breeding using new genome editing techniques: opportunities, safety and regulation in the EU, *The Plant Journal* 78: 742-752.
5. Maki, H (2002) Origins of spontaneous mutations: specificity and directionality of base-substitution, frameshift, and sequence-substitution mutageneses, *Annual Review of Genetics* 36: 279-303.
6. Arber, W (2010) Genetic Engineering Compared to Natural Genetic Variations, *New Biotechnology* 27:517-521.
7. Schnell, J, Steele, M, Bean, J, Neuspiel, M, Girard, C, Dormann N, Peason, C, Savoie, A, Bourbonniérre, L, Macdonald, P (2015) A Comparative Analysis of Insertional Effects in Genetically Engineered Plants: Considerations for Pre-market Assessment, *Transgenic Research* 24: 1-17.
8. Strauss SH, Sax JK (2016) Ending event-based regulation of GMO crops, *Nature Biotechnology* 34: 474-477.
9. Murat, F, Van de Peer, Y, Salase J (2012) Decoding plant and animal genome plasticity from differential paleo-evolutionary patterns and processes, *Genome Biology and Evolution* 4: 917-928.
10. Borges, RM (2008) Plasticity comparisons between plants and animals, *Plant Signalling and Behaviour*, 3:367-375.
11. Weber, N, Halpin, C, Hannah, LC, Jez, JM (2012) Editors choice: Crop Genome Plasticity and its relevance for food and feed safety of genetically engineered breeding stacks, *Plant Physiology* 160: 1842-1853.
12. Xiong, J-S, Ding, J, Li, Y (2015) Genome-editing technologies and their potential application in horticultural crop breeding, *Horticultural Research* 2: 15019, doi:10.1038/hortres.2015.19.
13. Andersen, MM, Landes X, Xiang W, Anyshchenko A, Falhof, J, Østerberg, JT, Olsen, LI, Edenbrandt, AK, Vedel, SE, Thorsen, BJ, Sandøe P, Gamborg, C, Kappel, K, Palmgren, MG (2015) Feasibility of New Breeding Techniques for Organic Farming, *Trends in Plant Science* 20: 426-434.
14. Ahloowalia, B. S., Maluszynski, M., & Nichterlein, K. (2004). Global impact of mutation-derived varieties. *Euphytica*, 135(2), 187-204.
15. https://mvd.iaea.org/#!Home, accessed 21 November 2016.
16. Jones, HD (2015) Future of breeding by genome editing is in the hands of regulators, *GM Crops & Food* 6:223-232.
17. Sprink, T, Eriksson, D, Schiemann, J, Hartung, F (2016) Regulatory Hurdles for Genome Editing: Process-vs Product-Based Approaches in Different Regulatory Contexts, *Plant Cell Reports* 35: 1493-1506.
18. European Food Safety Authority Panel on Genetically Modified Organisms (2012) Scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function, *EFSA Journal* 10: 2943
19. Cho, SW, Kim, S, Kim Y, Kweon, J, Kim HS, Bae, S, Kim, J-S (2014) Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases, *Genome Research* 24: 132-141.
20. Shen, B, Zhang, W, Zhang, J, Zhou, J, Wang, J, Chen, L, Wang, L, Hodgkins, A, Iyer, V, Huang, X, Skarnes, WC (2014) Efficient genome modification by CRISPR-Cas9 nickase with minimal off-target effects, *Nature Methods* 11: 399-404.
21. Cho, SW, Kim, S, Kim, JM, Kim, J-S (2013) Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease, *Nature Biotechnology* 31: 230-232.
22. Gaj, T, Gersbach, CA, Carlos FB (2013) ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering, *Trends in Biotechnology* 31: 397-405.
23. Hwang, WY, Fu, Y, Reyon, D, Maeder, ML, Tsai, SQ, Sander, JD, Peterson, RT, Yeh J-RJ, Joung, JK (2013) Efficient genome editing in zebrafish using a CRISPR-Cas system, *Nature Biotechnology* 31:227-229.

24. Wolt, JD, Wang, K, Yang, B (2016) The Regulatory Status of Genome-edited Crops, *Plant Biotechnology Journal* 14: 510-518.

25. Tanner, G. J., Blundell, M. J., Colgrave, M. L. and Howitt, C. A. (2016), Creation of the first ultra-low gluten barley (Hordeum vulgare L.) for coeliac and gluten-intolerant populations. *Plant Biotechnology Journal*, 14:1139–1150.

26. Cardi, T (2016) Cisgenesis and genome editing: Combining concepts and efforts for a smarter use of genetic resources in crop breeding, *Plant Breeding* 135: 139-147.

27. Araki, M, Ishii, T (2015) Towards social acceptance of plant breeding by genome editing, *Trends in Plant Science* 20: 145-149.

28. Holme, IB, Wendt, T, Holm PB, Intragenesis and cisgenesis as alternatives to transgenic crop development, *Plant Biotechnology Journal* 11: 395-407.

29. Schouten, HJ, Krens FA, Jacobsen E (2006) Cisgenic plants are similar to traditionally bred plants, *EMBO Reports* 7: 750-753.

30. European Food Safety Authority Panel on Genetically Modified Organisms (2012) Scientific opinion addressing the safety assessment of plants developed through cisgenesis and intragenesis, *EFSA Journal* 10: 2561.

31. Schouten, HJ, Krens, FA (2006) Do cisgenic plants warrant less stringent oversight? *Nature Biotechnology*, 24: 753.

32. Andersen, MM, Landes X, Xiang W, Anyshchenko A, Falhof, J, Østerberg, JT, Olsen, LI, Edenbrandt, AK, Vedel, SE, Thorsen, BJ, Sandøe P, Gamborg, C, Kappel, K, Palmgren, MG (2015) Feasibility of New Breeding Techniques for Organic Farming, *Trends in Plant Science* 20: 426-434.

33. Baum, JA, Bogaert, T, Clinton, W, Heck, GR, Feldmann, P, Ilagan, O, Johnson, S, Plaetinck, G, Munyikwa, T, Pleau, M, Vaughn, T, Roberts, J (2007) Control of coleopteran insect pests through RNA interference, *Nature Biotechnology* 25: 1322-1326.

34. Scott, JG, Michel, K, Bartholomay, LC, Siegfried, BD, Hunter, WB, Smagghe, G, Zhu, KY, Douglas, AE (2013) Towards the elements of successful insect RNAi, *Journal of Insect Physiology* 59: 1212-1221.

35. Robinson, KE, Worrall, EA, Mitter, N (2014) Double stranded RNA expression and its topical application for non-transgenic resistance to plant viruses, *Journal of Plant Biochemistry and Biotechnology* 23: 231-237.

36. Killiny, N, Hajeri, S, Tiwari, S, Gowdam S, Stelinski, L (2014) Double-stranded RNA uptake through topical application, mediates silencing of five CYP4 genes and suppresses insecticide resistance in Diaphorina citri, *PLOS ONE* 9: e110536.

37. Burand, JP, Hunter, WB (2013) RNAi: Future in insect management, *Journal of Invertebrate Pathology* 112:S68-S74.

38. http://www.foodstandards.gov.au/consumer/gmfood/Pages/New-plant-breeding-techniques-in-the-spotlight.aspx