
DuPont Pioneer (www.pioneer.com) is the world’s leading developer and supplier of advanced plant genetics, providing high-quality seeds to farmers in more than 90 countries. It provides agronomic support and services to help increase farmer productivity and profitability and strives to develop sustainable agricultural systems for people around the globe. Being the first to commercially introduce maize hybrids in the late 1920s, the entire history of the company is built on a commitment to scientific innovations. DuPont Pioneer scientists apply state-of-the-art conventional breeding and modern biotechnology tools to develop innovative products for our customers. Since 1975, DuPont Pioneer has offered canola, corn, sorghum, and forage seed products and seed technology solutions to meet the needs of Australian farmers, industry, and food manufacturers.

DuPont Pioneer appreciates the opportunity to provide input on the Office of Gene Technology Regulator (“OGTR”) “Technical Review of the Gene Technology Regulations 2001, Discussion paper: Options for regulating new technologies”. We appreciate OGTR’s willingness to be among the world leaders on the important topic of regulatory approach to products developed using new plant improvement technologies. Plant breeding innovations, such as oligo-directed mutagenesis and SDN-1 and SDN-2 enabled gene editing, can contribute to addressing global challenges related to food security, population growth, and climate change. However, practical application of these techniques in modern agriculture will inevitably be influenced by whether or not the regulatory regime treats their products in a manner commensurate with the potential risks they pose. DuPont Pioneer believes that any regulatory regime should be focused on the potential risks of a particular product regardless of the process used to develop it. It is the characteristics of the resulting plant, and not the method by which it was produced, that determines its safety.

The long history of safe use of plant varieties produced through domestication and conventional breeding demonstrates that the specific techniques used to develop them do not pose an inherent safety risk. Gene editing techniques can result in plants found in nature or similarly be produced with conventional breeding, albeit in a much more targeted and efficient fashion. Thus, the same regulatory regime should be consistently applied to all similar products regardless of the technique used in their development; if plants could be developed by a new plant improvement technique and by a conventional breeding technique, they should be regulated no differently.

Below please find DuPont Pioneer’s response to several questions invited for public comment.
Question 1: Which option/s do you support, and why?

DuPont Pioneer supports OGTR proposed Option 4, which most closely represents the current state of scientific knowledge and takes into consideration the baseline of safety established through the history of use of conventionally bred plant products.

Option 4 enables the same regulatory treatment of plants produced with new technologies and those that can similarly be obtained with various conventional breeding tools – such as use of plant’s own allelic variation, spontaneous mutations, or traditional induced mutagenesis. A few specific examples include:

- SDN-1 examples:
  - Targeted mutagenesis of FAD2 and FAD3 genes in soybean using TALENs\textsuperscript{1,2} resulting in a high oleic phenotype. FAD2 and FAD3 mutants, both spontaneous and X-ray induced, have been described in soybean as well as other plant species\textsuperscript{3}.
  - Natural (spontaneous) or transposon induced mutations in maize MS fertility genes have been a subject of discovery and classical genetic studies for decades\textsuperscript{4}. One of such mutations, in MS45 gene, is a component of DuPont Pioneer's Seed Production Technology (SPT) process\textsuperscript{5}. Most recently, targeted mutagenesis of MS genes in maize and several other monocots was achieved using CRISPR-Cas and meganuclease techniques\textsuperscript{6,7}.
  - TALEN-mediated mutation of three MLO genes in hexaploid wheat resulting in resistance to powdery mildew\textsuperscript{8}. The experiment was based on a prior knowledge about the loss-of-function mlo alleles existing in barley, Arabidopsis and tomato and shown to lead to resistance to fungal pathogens causing powdery mildew\textsuperscript{9,10,11}.

- SDN-2 and oligo-directed mutagenesis examples:
  - Various spontaneous and induced mutations in plant ALS (AHAS) genes leading to tolerance to sulfonylurea and imidazolinone herbicides have been described in several plant species\textsuperscript{12,13} and commercialized in a range of crops\textsuperscript{14}. Herbicide tolerance is conferred by specific amino acid changes in the ALS protein sequence. The same changes could be generated in maize and rice using CRISPR-Cas and TALEN mediated

\textsuperscript{1} Haun W. et al. (2014) Plant Biotechnology J. 12: 934.
\textsuperscript{8} Wang Y. et al. (2014) Nature Biotechnology 32(9): 947.
\textsuperscript{11} Bai Y. et al. (2008) Molecular Plant Microbe Interaction 21: 30.
\textsuperscript{13} Tan S. et al. (2005) Pest Management Science 61: 246.
\textsuperscript{14} https://agriculture.basf.com/en/Crop-Protection/Clearfield-Global.html
SDN-2 approach, and though oligo-directed mutagenesis approach in canola and predictably resulted in plant’s herbicide tolerance. Similar experiment was conducted in flax to generate two targeted amino acid changes in the native EPSPS gene resulting in glyphosate tolerance.

- Targeted replacement (swap) of unfavorable allele in a variety of interest with the favorable allele of the same gene from another variety is another potential application of SDN-2 technique. In this instance the homology directed repair involves a DNA template sequence that encodes the favorable allele. The favorable allele is brought into the recipient line at its native genomic location and replaces the current allele. Such an outcome is similarly achievable through conventional breeding by introducing the desired gene allele through a series of breeding crosses.

The 2001 Explanatory Statement to Schedule 1 of the Gene Technology Regulations 2001 (the “GT Regulation”) elaborates on two risk considerations based upon which organisms listed in Schedule 1 have been excluded from the GT Regulation. It identifies organisms resulting from certain technologies where the “process mimics natural mutation processes” and, accordingly, use of such technologies “give rise to organisms that can occur in nature, and as such do not pose a particular biosafety risk to the environment or human health and safety”. Further, “Organisms that result from exchange of DNA within the same species (and where no genetic material from any other species is introduced) are not, therefore considered to be GMOs for the purposes of the regulatory scheme” due to the similarity to inherent cellular processes. Examples of oligo-directed mutagenesis, SDN-1, and SDN-2 developed organisms provided above illustrate that these organisms meet these criteria and thus pose a particular biosafety risk and should have the same regulatory treatment as organisms listed in Schedule 1 (i.e., considered to be not genetically modified).

Further, exclusion of SDN-2 from GMO regulation under Option 4 would be in alignment with Section 10 Definitions of the Gene Technology Act 2000, where:

“**genetically modified organism** means:

(a) an organism that has been modified by gene technology...”

whereas:

“**gene technology** means any technique for the modification of genes or other genetic material, but does not include...(b) homologous recombination...”

SDN-2 technique activates a plant’s endogenous homology-directed repair (i.e., homologous recombination) mechanism to promote the target gene edit.

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17 http://www.cibus.com/technology.php
Option 4 will be also in alignment with the current **Item 1 of Schedule 1 (regulation 5)** that describes "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)" as organisms that are not genetically modified. Oligo-mediated mutagenesis, SDN-1, and SDN-2 are used to develop organisms that do not contain any foreign, non-homologous DNA sequences from another species. Absence of foreign DNA sequences (i.e., the SDN process components) can be confirmed through molecular assays if those components are delivered on plasmid vectors. RNA and protein based delivery methods, those not involving introduction of heritable genetic material, have also emerged and can be used to develop similar organisms\(^{21,22,23}\).

Thus, DuPont Pioneer supports Option 4 as being based on current scientific knowledge and in alignment with the risk criteria explained in the GT Regulations that certain organisms are not GMOs and do not pose any unique biosafety risks to the environment or human health and safety due the processes used in their development. Accordingly, consistent with OGTR’s current policies, DuPont Pioneer proposes that OGTR updates Schedule 1A of Section 5 and/or Item 1 of Schedule 1 to clarify that organisms obtained with a use of oligo-directed mutagenesis and SDN-1 and SDN-2 targeted mutagenesis that do not contain any foreign, non-homologous DNA sequences from another species are similarly not GMOs and do not present a unique biosafety risk.

**Question 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?

DuPont Pioneers believes that Options 2 and 3 will result in a level of regulation not commensurate with risk. Most considerations for these options provided by OGTR as reasons for regulation are not unique to the new techniques, but instead are similarly, or even to a greater extent, relevant to conventional breeding techniques that have an established history of safe use and are out of scope of the GT Regulation.

Specifically, the following reasons outlined as “Pros” of **Option 2** are not scientifically justified as to pose a technique-specific risk unique to oligo-directed mutagenesis and SDN-1 and SDN-2 targeted mutagenesis and therefore requiring an increased level of regulatory scrutiny for products developed with these new techniques:

- With regard to the potential for unintentional interference of the gene altered by the new technique with native genes and biochemical pathways, the potential for such interference depends on the function of the altered gene itself and is no different if the gene has been altered by a new technique or by any other technique, and in particular, techniques in


common practice today in conventional breeding (spontaneous mutations, chemical or irradiation mutagenesis) which have a long history of safe use. Thus, this reasoning is not a unique feature of the new technique to be in scope for considerations.

- With regard to the purported unknown precision of oligo-directed mutagenesis and SDNs and possible unintended changes in the genome, it is important to consider that spontaneous mutations in plants occur all the time and at an impressive rate. For example, single nucleotide polymorphisms (SNP) are estimated to account for 1.3% of base pairs in maize. Similarly, in one study, indels (small DNA insertions or deletions) were detected in 43% of 502 examined maize loci. Any potential imprecision, or so called off-target cutting, of oligo-directed mutagenesis, SDN-1 and SDN-2 techniques is expected to be limited to a very similar DNA sequence due to the mode of action of oligo-directed repair and programmable SDNs. Thus, potential off-target cutting caused by the new techniques would be likely at a frequency substantially lower than the existing rate of spontaneous mutations in plants. The rate of spontaneous mutations can be further increased by classical (chemical, irradiation) mutagenesis for the purpose of breeding. Unlike oligo-directed mutagenesis and SDNs, unintended mutations caused by classical mutagenesis are random and not possible to predict. As acknowledged by the European Food Safety Authority (EFSA), the frequency of mutations is predicted to be higher after mutation breeding.

With that, traditional mutagenesis is a universally deployed tool in modern breeding, with over 3200 mutants registered in the FAO/EAAE mutant variety database and no verified reports raising safety concerns. This justifiably establishes the safety baseline for plant breeding. Thus, the potential for unintended changes in the genome is not a unique feature of the new technique to be in scope of considerations. Any potential imprecision of the new techniques is expected to be significantly less than the rates of spontaneous mutations or classical mutagenesis for which there is an established history of safe use.

Thus, DuPont Pioneer strongly agrees with the primary “Con” identified by OGTR for Option 2 that oligo-directed mutagenesis, SDN-1, and SDN-2 techniques give rise to changes identical to those that occur from processes that are excluded from regulation because they have a long history of safe use. Capturing oligo-directed mutagenesis and SDN techniques under regulation will therefore not be commensurate with the potential risks posed by products developed with these technologies.

Adopting Option 3 will unjustifiably result in a different regulatory regime of plant varieties produced with SDN-1 and varieties produced with oligo-directed mutagenesis or SDN-2 techniques. As evident from the list of examples in our comment to Question 1, all three techniques can be used to generate plants indistinguishable from what can be produced using conventional breeding techniques or found in nature. These examples illustrate that oligo-

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27 https://mvd.iaea.org/
directed mutagenesis and SDN-2 techniques are not expected to produce organisms carrying any novel inherent risks that would justify their differential regulatory treatment from products of traditional mutagenesis or SDN-1 technique.

DuPont Pioneer strongly supports the OGTR’s recognition that unjustifiable regulatory treatment could discourage developers from using certain new techniques and impede commercialization of the resulting products. Potentially, this could also lead to limiting the use of oligo-directed mutagenesis and SDN-2 for trait discovery and evaluation purposes only, while subsequently attempting to re-create the same product with traditional mutagenesis. This would be a superficial, time and resource consuming, and unguaranteed results approach, yet resulting in the same end-product having different regulatory treatment solely based on the process used.

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

DuPont Pioneer is an early adopter of the CRISPR-Cas technology for development of agricultural products. Our first CRISPR-Cas enabled commercial product, next generation waxy maize, produced with SDN-1 technology is currently in development. A series of other potential applications of CRISPR-Cas in DuPont Pioneer core crops are being envisioned, for example those that can improve plant’s disease resistance, drought tolerance, or increase nutritional value. Additionally, DuPont Pioneer has publicly stated it welcomes the opportunity to collaborate with others to realize the full potential of the CRISPR-Cas advanced breeding technology. Our company has a long history of collaboration and broadly advancing science. DuPont Pioneer is open to entering further collaborations which would contribute to developing the technology across all crops and geographies for the greater good. The Options outlined by OGTR will impact DuPont Pioneer’s and any other third party’s use of plant breeding innovations such as CRISPR-Cas based on the potential regulatory burden associated with each Option.

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

DuPont Pioneer is a leader in the field of CRISPR-Cas advanced breeding technology for the development of agricultural products. Amendment of item 1 of Schedule 1 to additionally include organisms produced by targeted mutagenesis approach (oligo-directed mutagenesis, SDN-1, and SDN-2) and not containing any foreign, non-homologous DNA sequences from another species would enable development of innovative and sustainable solutions for growers.

29 https://www.pioneer.com/home/site/about/news-media/news-releases/template CONTENT/guid.1DB8FB71-1117-9A56-E0B6-3EA6F85A9EA92
similar to those realized through common plant breeding practices, but with even greater quality and accuracy and with more efficient development timelines.

DuPont Pioneer also welcomes OGTR’s initiative to provide regulatory clarity for other types of the new techniques, namely, techniques where organisms are genetically modified in a transient manner, as well as null segregants. As stated in the discussion paper, “The legislation can be clearly interpreted” for considering these techniques to generate products outside the scope of the current GMO legislation. DuPont Pioneer thanks OGTR for this clarification and supports the amendment of the GT Regulations to formally recognize this policy.

In closing, DuPont Pioneer appreciates OGTR’s commitment to a clear regulatory policy which will enable the Australian plant industry to operate in a predictable and science-based regulatory environment. As a science company, DuPont Pioneer supports a regulatory regime which is based on current scientific knowledge, proportional to risk, and promotes innovation. Scientifically unjustified and unnecessarily onerous regulatory requirements will stifle innovation, preventing beneficial products from entering the market, and likely narrow application of new technologies to a handful of high margin commodity crops while significantly limiting their potential adoption by small and medium organizations across wide range of crops.

DuPont Pioneer thanks OGTR for the opportunity to comment.

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