Submission on the Technical Review of the Gene Technology Regulations 2001 Discussion Paper:

Options for Regulating New Technologies

To: Regulations Review

Office of the Gene Technology Regulator

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About Cibus Global Ltd.

Cibus Global, Ltd. develops products for agricultural, industrial, and human health markets worldwide. Cibus' primary expertise is the delivery of highly precise gene editing which can be used to combat genetic disorders in human and animal health, to produce improved strains of microbes for use in industrial biotechnology and to help accelerate plant breeding. In the crop plant sector Cibus is combining genome sequencing, bioinformatics, cell culture, precise gene editing and plant breeding to develop new crop varieties with improves tolerance to diseases, herbicides and environmental stresses, and with improved yield and quality characteristics. The new varieties are no different from those which could be developed using cross-breeding but the whole process can be years or even decade faster and for this reason Cibus calls the process the Rapid Trait Development System (***RTDS***™).

Cibus Global, Ltd. was co-founded by Dr. Peter Beetham and Dr. Keith Walker in 2001 and is based in San Diego, California with subsidiaries in Europe and North America, and a research and development center in San Diego, California.

Dr. Beetham completed his PhD at the Queensland University of Technology and, during the mid-1990s he was responsible for some of the earliest applications of ODM to plants and some of the earliest publications (Beetham 1999). Cibus is the leading company in the application of oligonucleotide mutagenesis (ODM) and has used the technology successfully in several crops.

In 2004, USDA APHIS evaluated the use of ODM in Cibus ***RTDS*** and concluded it to constitute a modern and improved form of mutagenesis which, like other forms of mutagenesis, is excluded from US regulations designed for the products of transgenic technology. With products now approaching global markets, Cibus is in dialogue with regulatory authorities worldwide.

Over the next decade Cibus is aiming to develop products for all major crops with the most advanced programmes including rapeseed, rice, potato and flax or linseed. The Cibus development pipeline includes advanced development products that could play a significant role in helping to manage weed resistance in Australia in addition to improving harvestable yield and combating plant pathogens. Cibus will partner with Australian companies to make these products available to Australian growers.

Consultation questions

For over twenty years, scientists have been investigating the utility of oligonucleotides as agents of precise mutagenesis to overcome the shortfalls of older forms of mutagenesis which generate multiple random mutations throughout the genome.

Cibus scientists have been at the forefront of this research throughout this period. The following response to the consultation questions are focused on the regulatory status of ODM technology as it is used by Cibus for the development of agricultural plant based products.

Oligonucleotide-directed mutagenesis (ODM) is a base pair-specific precision mutagenesis technology that has been successfully employed since the 1990's in bacterial, fungal, mammalian and plant systems (Aarts et al., 2006; Beetham et al., 1999; Gocal et al., 2015; Moerschell et al., 1998; Yoon et al., 1999; Zhu et al., 1999). ODM uses chemically synthesized oligonucleotides as chemical mutagens to guide plant cells to make one or a few predetermined nucleotide changes to their own DNA.

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

Cibus 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. Of the options provided, this appears the one most consistent with the spirit, scope and intent of the Australian regulatory scheme in that none of these techniques involves the moving or rearranging of genes; they all mimic natural processes and work through natural mechanisms; and the regulation is commensurate with the risks they pose, especially when viewed in the context of techniques currently excluded from regulation.

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?

Cibus agrees with the Gene Technology Technical Advisory Committee in its advice to the Regulator that SDN-1, SDN-2 and oligo-directed mutagenesis are unlikely to pose risks that are different to naturally mutated organisms. In 2011, a working group of experts from all EU Member States also concluded that ODM was an improved for of mutagenesis that was likely to lead to fewer unintended effects than chemical or radiation methods.

ODM development began in the mid-1990s and over the course of twenty years of evaluation, development and application, scientists have built a solid scientific understanding of how the technique works and how oligonucleotides should be designed to ensure precision and avoid unintentional off-target changes to the genome.

Off-target effects are theoretically possible by oligonucleotide recombination or by off-target mis-match repair.

Recombination can be avoided by using short, single-stranded oligonucleotides which are incompatible with all current models of DNA recombination in plants. The protected ends of the

oligonucleotide, which are intended to extend the half-life of the oligonucleotide in the cell, are also incompatible with recombination.

Oligonucleotide mismatch repair requires the oligonucleotide to be entirely homologous with the targeted gene location with the exception of the nucleotide(s) targeted for change. A single additional mismatch can reduce conversion efficiency by an order of magnitude with additional mismatches quickly reducing conversion efficiency to zero.

In order to have the possibility for off-target mismatch repair, off-target locations are needed with a high level of homology to the oligonucleotide. For sequences beyond 25 nucleotides in length the probability of homologous off-target regions is extremely low. Crop reference genomes can be used to identify off target areas with the closest match to the oligonucleotide sequence. These sites can be sequenced before and after exposure to oligonucleotides to confirm that no mismatch repair has taken place at these locations.

The products of ODM will contain one or a few nucleotide changes of a kind that occurs in nature every day. Scientists have demonstrated that these kinds of nucleotide changes arise naturally during the normal reproductive process and are passed on through generations (Ossowski, et al, 2010). The exact nucleotide changes generated by ODM are very likely to be found in nature given enough time or if sufficiently large populations could be sequenced. A nucleotide change guided by ODM is indistinguishable from the same change occurring spontaneously and is only distinguishable from the same change induced by chemical mutagenesis by any unintended mutations remaining in the chemical induce plant after back-crossing.

Given that the risks posed by ODM are no greater than those posed by older forms of chemical mutagenesis it seems apparent that the level of regulation should be no greater.

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

The adoption of Option 2 and Option 3 without consideration for ODM under Schedule 1A by the OGTR would significantly impact the timelines for commercialisation of Cibus products developed using ODM and significantly impact the viability of such products in the Australian market.

Additionally, an apparent GM classification for a non-transgenic crop that was classed as non-GM or non-LMO in other producing markets would lead to an unfortunate level of confusion in export markets that may be best avoided.

In contrast the adoption of Option 4, especially as it applies to current and future products would provide an incentive to ensure our development portfolio continues to include the Australian market.

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

Cibus concurs with the OGTR that item 1 of Schedule 1 of the GT Regulations can be a source of uncertainty and therefore welcomes the opportunity to comment on the potential impact of any proposed changes.

Cibus is of the view that in relation to its application of ODM technology**,** the adoption of Option 4 is consistent with the principles underlying Schedule 1 Item 1 of the regulations which lists the following techniques as not requiring regulation, in particular Item 4 which refers to 'Chemical-induced mutagenesis':

Item	Description of technique
1	Somatic cell nuclear transfer, if the transfer does not involve genetically modified material
2	Electromagnetic radiation-induced mutagenesis.
3	Particle radiation-induced mutagenesis
4	**Chemical-induced mutagenesis**
5	Fusion of animal cells, or human cells, if the fused cells are unable to form a viable whole animal or human.
6	Protoplast fusion, including fusion of plant protoplasts
7	Embryo rescue
8	*In vitro* fertilisation
9	Zygote implantation
10	A natural process, if the process does not involve genetically modified material Examples of natural processes include conjugation, transduction, transformation and transposon mutagenesis

Cibus contends that within the context of Option 4 the use of ODM technology as the platform for ***RTDS*** is consistent with the 2001 Explanatory Statement noted in the Discussion Paper which elaborates on the idea of risks from gene technology in relation to the list of organisms that are not GMOs in Schedule 1:

- "organisms resulting from such technology [chemical and radiation mutagenesis] are not considered to be GMOs for the purposes of the legislation because the process mimics natural mutation processes and the organisms have not had genes inserted or deleted by virtue of gene technology."
- the techniques "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".

Cibus is of the view that based on this explanatory note the current Oligonucleotide Directed Mutagenesis (ODM) is not Gene Technology under Schedule 1A Item 4 for the following reasons:-

- Whilst the term 'chemical' is not defined in the regulation, it is a widely used and relatively well-defined term.
- Any molecule could be described as a chemical, a more restrictive definition limits 'chemicals' to molecules that are artificially synthesised rather than the product of a biological process.
- Under either definition the oligonucleotide design used by Cibus is a chemical. It is not the product of a biological process, it is chemically (i.e. artificially) synthesised. It has no biological activity, other than to be a uniquely precise mutagen
- The mutations induced by ODM are identical to those arising in nature and identical to those arising from other forms of mutagenesis but without the unintended mutations arising from the random nature of older chemical and radiation mutagens
- Furthermore, the ODM process mimics natural mutational processes intimately to the extent that the oligonucleotide guides the cells natural enzymes to make the nucleotide change.
- Additionally, the oligonucleotide is introduced into the plant cell as a synthetic chemical rather than using a plasmid or viral vector and no genes are inserted or deleted from the host plant genome.

Cibus further contends that Crop varieties produced using Oligonucleotide Directed Mutagenesis are not genetically modified organisms based on Schedule 1, Item 1:-

- An oligonucleotide is not foreign nucleic acid, it does not come from another species, it is chemically synthesised and has no biological origin.
- The nucleotide sequence within the oligonucleotide can only ever be homologous. It is specifically designed to be homologous and will not function otherwise
- Additionally, the word 'introduction' is not defined however, the context of Item 1 suggests the introduction (or incorporation) into the genome. The mutational event is initiated by the alignment of the oligonucleotide alongside the homologous region of the target gene. The oligonucleotide is not introduced or incorporated into the host genome. Only the targeted mutational event is inherited.
- Whilst the oligonucleotide contains a short nucleotide sequence, it is single stranded, it cannot replicate and has no biological activity other than being a uniquely specific mutagen.

Therefore, based on this reasoning, it seems that ODM as a process is consistent with the wording of Schedule 1 Item 1 as currently written. However, further definition of key terms would be valuable in reducing ambiguity.

In the context of the wording of Schedule 1 Item 1 it seems reasonably apparent that 'introduction' is intended to convey 'introduction into the genome'; that 'foreign nucleic acid' is genetic material from a different organism; and that 'foreign nucleic acid (that is non-homologous DNA, usually from another species)' is intended to communicate that the genetic material being introduced into the genome is materially different from that which is already present.

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