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CSIRO Submission 16/571

Response to the Office of the Gene Technology Regulator

Regarding OGTR Technical review of the Gene Technology Regulations 2001

Discussion paper: Options for regulating new technologies

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Introduction

This document contains responses to the eight questions presented in the Discussion Paper distributed by the OGTR. CSIRO undertakes research that includes internally funded discovery and technical development, industry and stakeholder funded discovery and development research and fully customer prescribed technical delivery or analytical work. As an industry linked innovation catalyst CSIRO seeks to ensure that opportunities for innovation are maintained. However as a trusted advisor to the Australian Government and the Australian people our aim is to remain impartial and to maintain public confidence in the safety and benefit of scientific developments. The development and appropriate regulation of gene technology presents challenges that are growing as the pace of change quickens. CSIRO maintains strong international networks and is involved in all of the key international fora at which the risks and benefits, opportunities and challenges of the most recent breakthroughs in biotechnology are discussed.

This Review of the Gene Technology Regulations has been the subject of consideration and consultation within the Business Units of CSIRO, particularly Agriculture & Food, Health & Biosecurity, and Land & Water which interact closely with customers and stakeholders that have significant interest in gene technology and biotechnology innovations. In relation to Gene Technology CSIRO has customers, clients and stakeholders principally in primary production industries and environment. Some gene technology activity also relates to synthetic biology with customers in the synthetic chemicals, fibres and biologicals industries. At present CSIRO does not undertake activities which would utilize gene technology directly in human health industries. This stakeholder profile provides the context for this response to the OGTR.

Executive Summary

In brief, the responses to the questions presented by OGTR are as follows:

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

CSIRO supports Options 3 and 4. Option 4 is preferred, but it is understood that Option 3 may be able to be enacted more rapidly. Significant delay enacting Option 4 would exacerbate the current uncertainty if Option 1 remains in force by default in the interim.

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

The Discussion Paper provides a comprehensive, if not exhaustive, list of risks and benefits and identifies the key issues that need to be resolved in this review.

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?

Option 2 sets an excessive level of regulation and restriction on activities producing organisms that could be generated by unregulated breeding processes, known to have very low risk.

Option 3 is preferable to Option 2. The small mutations induced are either equivalent to what could occur naturally or they replicate what already exists in other individuals/breeding lines of the species. There is no evidence to suggest this presents a higher risk than conventional breeding.

Option 4 is preferable to Option 2 or 3. Option 4 provides greater scope to utilize new breeding technologies to generate organisms that could be generated using conventional breeding processes. However, Option 4 requires clear interpretation of the regulations to ensure that this is adhered to. In microorganisms with pathogenic lines or subspecies, clear regulation is required to ensure that gene technology activities are regulated commensurate with risk.

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

Option 2 would maintain a restrictive regulatory burden likely to hinder innovation. It is likely to strongly discourage industry/stakeholder investment in innovation and translation to practice.

Option 3 would moderate the regulatory burden, enabling certain low risk activities to take place that could provide opportunity for a limited set of innovative processes.

Option 4 would set a regulatory burden commensurate with risk, enabling more sophisticated, but low risk, developments of value to health, primary industry and the environment. This option might present challenges in amending definitions in the current Gene Technology Regulations.

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

Improvements to clarity of definitions in Item 1 of Schedule 1 would be beneficial. With new breeding technologies SDN-2 and SDN-3 could be interpreted as falling within Schedule 1 Item 1 as this relies on interpretation of “foreign nucleic acid” and “non-homologous DNA”. A definition for “homologous DNA” would be highly beneficial.

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?

Yes. A GM organism that contains a gene drive element, particularly one based on RNA guided nucleases (but potentially any sequence specific nuclease), poses higher risks than conventional GMOs. Control of such risks requires a case-by-case assessment and could include various forms of containment, not just physical. Current regulations do not adequately address these risks.

7. What RNA interference techniques are you using, and are there RNA interference techniques that you believe have unclear regulatory status?

CSIRO research activities include both endogenously enacted and exogenously enacted RNA interference. When endogenous RNAi is achieved through integrated transgenes it is clear that the resulting organisms are GMOs. RNAi induced transiently from DNA based non-integrated genetic constructs, the organism is considered a GMO while it continues to contain the genetic construct(s), but not when organisms (parental or progeny) no longer contain the construct(s).

For exogenous RNAi, the formulation and administration of double stranded RNA is currently reported to an Institutional Biosafety Committee. Since the RNA is not replicative, not integrated, and not heritable this should be excluded from regulation.

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.

Clarifying the regulatory status of null segregants (non-transgenic progeny of transgenic parent lines) as not being GMO; transient modification to not be GMO once the genetic material is no longer present; defining homologous DNA for distinction of SDN-2 and SDN-3; set definitions of organisms in relation to techniques, in particular microorganisms that may be pathogenic.

Response to specific questions raised by the OGTR

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

CSIRO supports Options 3 and 4, subject to the following considerations.

Option 3

Option 3 brings clarity in definition of the process being used and thus adheres to the policy framework of the Gene Technology Act. Although it recognises the properties of the resulting organisms, it is not defined by their properties and thus does not require an amendment of policy through review of the Gene Technology Act (which it is recognised is a separate process to that currently being undertaken).

Adopting Option 3 means that the terms “foreign nucleic acid” and “non-homologous DNA” from Schedule 1 Item 1 do not have to be further defined since it clarifies that no oligonucleotide or DNA sequence should be used in repair of the targeted DNA break. Option 3 avoids the need to consider the term “similar to or indistinguishable from the products of conventional breeding” as SDN-1 may be used to achieve this end but it is not restricted to that outcome. However, it does make achieving the end point of replicating natural variations in DNA target sites (genes) more difficult since there will be the need to screen (an unknown number of) organisms to identify those in which the desired variants are represented. Option 3 treats the breakage and repair of a double strand DNA as a natural event. Excluding from regulation any technique that generates a demonstrably site specific DNA break and unguided repair could assist in future-proofing the Regulations. Exempting any system using natural repair of a DNA breakage, regardless of how the break was induced, seems appropriate since this is one of the mechanisms that generates natural genetic variation in populations for all organisms. The end result of applying Option 3 is organisms that could spontaneously appear in any population that is maintained and appropriately monitored.

Option 3 does however explicitly restrict activities that use an oligonucleotide, or larger piece of DNA, to assist in the repair of an induced break through the process of homology-directed repair (HDR).

Option 4

Option 4 is less restrictive of innovative processes and will encourage investment in research and development that could provide significant benefit to Australian agriculture and biotechnology industries.

Adopting Option 4 would enable a process that can utilise homologous DNA to repair a break induced by SDN, under the description of SDN-2. The current definition describes “foreign nucleic acid” and “non-homologous DNA”. A clause which clearly defines “homologous” would better enable application of the Regulations. The definition could determine the degree of base substitutions allowed over a given interval for DNA to be classified as homologous as opposed to using the term “non-homologous” (with the implied status as “foreign”).

Option 4 would permit outcomes of activities that are "similar to or indistinguishable from the products of conventional breeding". Interpretation of this statement would be reinforced by the clear definition of homologous DNA. This would enable a straightforward process for scientists to submit plans to both Institutional Biosafety Committees and the OGTR to register the outcome of an applied technique and the organism generated as meeting the conditions of Schedule 1A and Schedule 1.

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

The benefit of ODN, SND-1 and SDN-2 have been characterised well in the Discussion Paper and their rapidity and specificity compared to the use of selective breeding in agriculture. The pressure on food production from population-driven increased demand and the impacts of climate change requires a more rapid response to be able to introduce improved traits for resilience to environmental conditions (e.g. drought, salinity, poor nutrition) and disease resilience under stress and intensive production. There is significant risk that Options 1 and 2 will inhibit the ability of industry to invest in these developments with the potential for future production losses that might have been avoidable. This risk remains with Option 3 since it is restrictive of more effective approaches to accumulating beneficial traits in given lines of production organism. The benefit of Option 4 is it will facilitate approaches to adaptation of production organisms to a wide variety of production environments and provide the ability to rapidly tailor those organisms in the face of changes, in timescales that cannot be achieved with selective breeding techniques even with the advances brought by DNA marker assisted selection.

An associated issue that is addressed obliquely in relation to Options 3 and 4 is that of "linkage drag". This is the associated loss of valuable allelic variants during a process of selective breeding for a particular trait or set of traits, even when employing well associated DNA markers. When using crossbreeding to transfer a favourable allele from one organism to another, variants can be transferred that might not be beneficial, thus disrupting selection for overall improvement of production traits. Traditionally this requires backcrosses or further selective crosses to recover original production characteristics in addition to retaining the desired new trait. By contrast the use of ODM, SDN-1 or SDN-2 enables introgression of the favourable allele directly into the desired background thus avoiding linkage drag. This saves time and resources and increases the value yield. In the case of animal breeding this also has the additional benefit of reducing the number of animals sacrificed and removed from breeding, improving the ethical values associated with breeding processes. Reducing the numbers of animals used to achieve a desired outcome is a pre-requisite in experimental science under the *Australian Code for the Care and Use of Animals for Scientific Purposes* laid down by the National Health and Medical Research Council (reference below). Under Options 1 and 2 there is a risk to our agricultural sector that linkage drag will continue to slow the pace of response to environment and market needs, with associated penalties in international competitiveness.

The benefit of trait introgression using ODM, SND-1 and SDN-2, is that those alleles being introduced are well understood and well characterised in the organisms from which they are being moved. This could reduce the risk that detrimental consequences might emerge in the resulting organisms as a result of unexpected interactions with linked traits moved by less precise

mechanisms. This may be of concern in animals where those consequences have negative health or welfare impacts or in plants where an unexpected weediness or invasiveness might emerge.

A risk with the use of a stable genetic background into which individual traits are introgressed step by step is the possible loss of genetic diversity. Genetic diversity is an evolutionary survival strategy that is built into the random reassortment of traits that is inherent in natural breeding processes. The challenge to the organisations seeking to use these new breeding technologies will be to maintain sufficient genetic diversity to provide a pool of organisms from which to select traits for introgression and use in commercial practice.

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?

The focus of the current regulatory system is to regulate organisms produced based on the method by which a change is generated rather than the change that has resulted. In contrast it is understanding any potential consequences of the change (to phenotype) that are used in assessment of the risk. Focus of regulation on the nature of the change and its impact rather than the mechanism by which it was achieved would align that regulation with the control and mitigation of any associated risk. This is a policy issue and is recognised as being outside the scope of the current review, since this is encompassed in the Gene Technology Act. The issue is raised only to highlight an opportunity in a future review of the Act, to improve alignment between level of risk and level of regulation. Constructing a combination of exclusions and assessments focusing on changes that meet a determined threshold may be a better foundation for regulation in line with risk.

Option 4 would set a reasonable regulatory burden with products generated utilizing new technologies that do not contain non-homologous DNA, not being subject to regulation. This would significantly increase the opportunities and likelihood for industry to invest in innovation as it would ensure products emerging from that investment could be readily released to generate production and/or environmental benefit. If those organisms provide food products, they would continue to be subject to review under the Food Safety Standards legislation to provide public confidence in the integrity of the food product.

This option would provide the opportunity for greater sophistication in the bringing together of traits known to be beneficial for production species that could be achieved using random trait mixing by traditional breeding followed by successive rounds of selection to achieve valuable combinations. An example of this is the introduction of the hornless or Polled trait into cattle that are horned but have been bred to achieve very high values of health, production and product quality in dairy production. The breeding of this trait from natural Polled, hornless animals, into elite dairy blood lines leads to loss of large numbers of valuable dairy traits. However the trait can be brought into the elite dairy blood lines by SDN based introgression (Carlson *et al*, 2016). Under Option 3 this would continue to be subject to Gene technology Regulations and would not be attractive to dairy producers. This segment of industry is represented by hundreds of producers each with their own blood lines, many built up over family generations to achieve best adapted genetics for best production in the local environment. Individual producers keen to adopt this breeding technology (through Option 4) would not be able to bear the regulatory burden that Option 3 would engender. This is in contrast to plant industries which obtain their seed stock from

larger companies who may be large enough to contemplate meeting the regulatory burden. However, even in these cases it is likely the regulatory burden of Option 3 would incur delays and cost that would inhibit application or force them to revert to alternative unregulated approaches.

It is clear that with respect to industry Option 4 is preferable. However the Polled trait introgression by SDN does highlight a significant issue. This trait involves a 200 bp insertion combined with an 11 bp deletion. This would classify as SDN-3 and would not be covered under Option 4 and therefore subject to the Gene Technology Regulations. The DNA that is introduced is not "foreign" to cattle and could be considered "homologous DNA" for the species as it could be introduced by traditional breeding. Though not specifically addressed in Option 4 there is an ***undefined*** boundary between oligonucleotide DNA for repair and "long template" DNA – SDN-3 – though the Discussion Paper defines SDN-3 as repair with "a long template to insert new sequences, with similar outcomes to inserting transgenes". It is possible that this could be addressed by a definition of "homologous DNA". This issue may be seen as pertaining to policy rather than current regulations, in which case this could be addressed in the forthcoming review of the Gene Technology Act. Cattle have already been produced using this technique (Carlson *et al*, 2016). They have shown normal growth and good health and are currently alive and well. Analysis of their genome shows that the predicted introgression has occurred and that there are no other genetic changes detectable in a 20 fold sequencing coverage of all regions of the genome for which an "off-target" event could be predicted.

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

Option 2 would increase the regulatory burden both for conducting research and for the commercialisation of any innovations that arise. If the innovation is significant and the financial returns are high enough, some larger commercial partners may attempt to translate the innovation into practice. However, this pre-supposes the research can be conducted to demonstrate the potential commercial value. This option would certainly result in an innovation penalty and the reduction in opportunity and the commercial and/or environmental gains that could be generated by Australian business. This would be seen in both plant and animal agriculture, especially the latter.

Whichever option is adopted complex issues may arise in the area of international trade should trading partners adopt different approaches to regulation.

Option 3 would enable certain research activities by removing one barrier within the regulatory process. The use of SND-1 techniques would allow a subset of research activities to both acquire basic and translational research funding with industry enabled to translate these outcomes into industry practice. However the use of SND-1 to recreate known variations or to induce new variations in genes within a line of plants or animals will involve a significant degree of randomness and the need for potentially extensive and costly screening for progeny with the desired traits. In the case of animals this is an important consideration in relation to the NH&MRC *Australian Code the Care and Use of Animals for Scientific Purposes*.

This calls into question the distinction between SDN-1, SND-2 and SDN-3, their definitions and difference between Option 3 and Option 4. Outcomes from SND-1, a point mutation or a single

base pair deletion, may be indistinguishable from outcomes generated by SDN-2, using an oligonucleotide incorporating the change to repair the break. This highlights the question of process versus outcome. It concerns detection and characterisation of agricultural organisms (plants and animals) and their product derivatives that are involved in trade, and has the potential to impact on industry competition and industry investment in the Australian innovation system. If it is not possible to distinguish these in pursuance of the Australian regulatory process how will it be possible to determine the nature of materials imported into Australia or against which our agricultural products must compete in an open international market?

Option 4 would enable much greater sophistication in the generation of organisms to include specific beneficial traits. In the case of animals this has an additional benefit in relation to the ethics of animal experimentation which require all efforts to be made to reduce the numbers of animals used to undertake experimental or technical procedures. There is a strong argument in favour of Option 4 when it is used to regenerate existent genotypes.

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

> *"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)."*

In plant biology it is routine to use mutant strains of organisms such as wheat and barley in pre-breeding activities to develop novel traits. Item 1 of Schedule 1 is currently applied to outputs of these experiments when it is clear that the end products do not contain foreign nucleic acid. In animal biology these experimental approaches are not possible. Until recently it was not possible to construct a modification of the animal genome in a manner that did not introduce non-homologous DNA, selective markers and component of DNA constructs would inevitably contain these (for example generating transgenic poultry, Tyack *et al*, 2013). However this situation has now changed substantially with the efficiency of the gene editing techniques of CRISPR/Cas9 and TALEN to cause specific targeted breaks in genomic DNA to encourage homology directed repair. This has presented the opportunity to introduce DNA into an animal that is homologous, from the same species and does not carry any foreign sequence.

Therefore in animals and plants it is now possible to create an organism that involves the introduction of homologous DNA with a mutation that is not "foreign nucleic acid".

SDN-2 could introduce DNA that is not "foreign DNA", but under Option 3 this would still be considered subject to the Gene Technology Regulations. Under Option 3 the same event could be generated at random requiring a lengthy and expensive screening procedure to result in an outcome indistinguishable from an SDN-2 process. This would generate uncertainty as to how regulation could be enforced with a likelihood of creating barriers to industry investment and thus to innovation.

It is recognised that to accept introduction of nucleic acid that is homologous but containing small changes creates uncertainty. If those changes are known to exist in the species then Schedule 1 Item 6 can be used. This can be resolved for repair with an oligonucleotide or large template by

defining the term “homologous” with a rate of allowable substitution based on the gene and/or species under consideration.

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.

Yes. Gene drive experiments will pose significantly different risks than other GMO research.

A number of CSIRO staff have co-authored published manuscripts that consider the risks presented by generating GM organisms that contain synthetic gene drive systems (typically based on CRISPR/Cas9 modified systems) (Akbari *et al*, 2015; Webber *et al*, 2015). There is a great deal of international debate about the risks, ethics and controls associated with gene drive work. At present there are likely to be few projects initiated or funded to undertake this type of gene technology. Although this may change in the future, it is unlikely there will be large numbers of gene drive experiments undertaken in Australia.

Gene drive experiments involve transgenic organisms being generated and are self-evidently subject to the Gene Technology Regulations. The objective is to demonstrate that a genetic trait associated with a gene drive cassette will be inherited by all progeny when breeding with a non-gene drive mate (Esvelt *et al*, eLife 2014). There have been only a small number of experiments published so far showing evidence of the process. The risks that this technology presents will be determined by a number of factors; what the trait associated with the gene drive is, how efficiently the gene drive replicates (does it achieve doubling), what other biological factors of the target host play a role in the gene drive function, fitness of the carrier animal or reproductive success of a carrier animal. Therefore the level of risk that gene drive experiments pose is potentially highly variable, hard to predict and thus needs to be assessed on a case-by-case basis. It is highly likely that a review of such experiments would require at least one and potentially two (or more) of the following controls suggested by Akbari *et al* (2015):

1. Physical: triple nested barrier containment (PC3/AC3)
2. Ecological: Performed in a geographic location isolated from natural populations and unfavourable to the animal should it escape (e.g. *Anopheles* in Boston, *Aedes* in Geelong)
3. Reproductive: Using a laboratory strain of the animal restricted in its ability to reproduce with native populations (e.g. Drosophila with compound autosomes)
4. Molecular/genetic: sgRNA and Cas9 genetically separated (different loci or chromosomes; gene drive designed to target something unique to laboratory strain (e.g. artificial target)

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.

Summary

Endogenous (transgenic) RNAi is permanent and inherited: covered by Gene Technology Regulations. Exogenous (synthesised, or purified, and delivered) RNAi is transient and not inherited: not covered by Gene Technology Regulations.

Explanatory notes

RNAi falls into two clear categories based on how it is achieved technically. Endogenously generated RNAi uses the insertion of a genetic construct into the genome of an organism to achieve expression of a short hairpin RNA or long double stranded RNA transcript. Such transcripts must then be appropriately processed by cellular machinery to enter the pathways that alter the level of expression of a target gene (or genes) in order to achieve a desired phenotypic effect. Generally this involves the introduction of a genetic cassette including promoters, coding sequences (often small, ~ 100 base pairs) and terminators. As these cassettes most often contain sequences not native to the organism into which they are introduced, or at best are rearrangements of the DNA of a host genome (cisgenic), they are achieved by significant genetic modification of the host. This clearly results in a genetically modified organism and is subject to the Gene Technology Regulations.

The second approach is exogenously delivered RNAi. This is achieved by the application of RNA molecules from outside of the cells or body of the target organism. The material may be short double stranded or hairpin structured RNA (siRNA or shRNA; as short as 20-22 base pairs) or longer double stranded RNA in the range of a few hundred base pairs (dsRNA; typically 400 bp) which may have been manufactured by chemical synthesis, by in vitro biological synthesis (using enzymes and DNA templates) or by in vivo biological synthesis (for example made with bacteria). In vitro and in vivo synthesis is generally used for the longer RNA and the manufactured RNAi molecules are purified by some means before final use. The short or long RNAs are often fragile in biological systems and, because of their high level of surface electrical charge, require some additional chemicals (usually lipids or polymers) to transit across the cell membrane to gain entry to the cytoplasm of the cell where they have their biological function.

Exogenous RNAi has an effect that is transitory, lasting only as long as the small RNA remains stable and functional. As RNA is a transactional molecule, within cells there are systems present designed to breakdown and remove RNA. Most importantly there is no evidence that RNAs used for RNAi are capable of being reverse transcribed and integrated into the genomic DNA of the organism in which they have been administered. Furthermore such an integration event, even if it were possible, would require that the integration happen in a genomic location able to allow expression of a functional transcript thereafter to have any biological effect. In the animal kingdom delivery of RNAi to the brain is extremely difficult, due to impasse at the blood brain barrier. There is equivalent impasse at the blood reproductive system barrier (Sui & Cheng, 2012)

– this means that the RNA cannot access the germline cells of the body. All of these considerations mean that the effects of exogenously applied RNAi are transient and are not incorporated or inherited by offspring of organisms treated with exogenous RNAi. Similar effects, of repressed gene expression can be induced by a number of different stimuli but are not consider for regulation as they are transient in effect and the effect is not inherited. The use of exogenously applied RNAi should not fall within the scope of the Gene Technology Regulations.

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.

Null-segregants

CSIRO supports any amendments to the Gene Technology Regulations that give clarity to the classification of "null segregants" as not categorised as GMOs and not subject to the Gene Technology Regulations since by definition they do not contain any transgenic foreign genetic material that was present in one or both of the parent lines. This is based on the complete segregation of chromosomes during meiosis and the ability to clearly identify the presence of the transgene(s) in the parent and their absence in the null segregant progeny. Since null segregants cannot be differentiated from a non-GM organism or a non-GM parental line there is no scientific basis to regard them as being subject to the Gene Technology Regulations. However, the processes to produce a null-segregant will involve at least one GM-parent line. These processes are clearly subject to the Gene Technology Regulations.

Transient modification

CSIRO supports the concept that "organisms that are genetically modified in a transient manner (e.g. using agro-infiltration) are GMOs while the genetic modification or trait is present, and are no longer GMOs once both the trait and genetic modification are no longer present". In parity with the arguments relating to null segregants, after transient modification is finished, and in the absence of detectable foreign genetic material, the organism cannot be differentiated from the non-GM parental line. Thus during an agro-infiltration process the presence of foreign genetic material mean that the undertaking is subject to the Gene Technology Regulations. However, after the procedure an organism that does not retain the additional genetic material and has not incorporated this material in its germline should not continue to be subject to the Gene Technology Regulations.

Distinction of SND-2 and SND-3 – definition of homologous DNA

The Discussion Paper characterises that "the difference between SDN-2 and SDN-3 lies in the extent of the nucleotide sequence difference between the native target sequence and the repair template", "it is generally accepted that SDN-2 involves changes to one or a few nucleotides, whereas SDN-3 involves inserting a new gene or other genetic elements." SDN-2 is characterised as using an oligonucleotide to repair the SND induced DNA break whereas SDN-3 uses a larger

(DNA) template. If this contains a gene not found in the host species then the outcome is a GMO and is regulated. This definition of SDN-3 is extended from the ZFN-3 described by Lusser *et al* (2012) which implies the possibility of introduction of a "new gene" but does not require it to be new. It is possible that a larger DNA template could be substantially homologous to the host target that has been broken for repair or is homologous to DNA found at the same genetic locus, as that being repaired, but originating from another breeding line of the same species (thus homologous for that genetic locus in that species). In these cases the outcome is the transplantation of a homologous genetic locus between members of the same species. Thus the Discussion Paper states **"*a precise distinction between these techniques*** [SND-2 and SDN-3] ***is required*** - it is a challenging task to provide convincing, risk-based arguments to support a clear legal distinction between these techniques, especially so when it must apply equally to all types of organisms." Oligo or long DNA templates could be defined by the level of homology that they present in relation to equivalent genetic loci within the species and could be used to circumvent the arbitrary definition of "oligonucleotide or long template" and the issue of introducing a "new gene" such as the ZFN-3 technique. This may continue to present too great a challenge to revisions to the current regulation.

Definitions of organisms in relation to techniques

The Discussion Paper raises a legitimate concern regarding the use of the SDN-1 and SDN-2 techniques in association with certain organisms such that "for pests or disease-causing organisms, for example pathogenic microorganisms, small sequence changes might give rise to significant risks". It is recognised that this is a significant issue. Since the major opportunities for application of Option 3 and Option 4 are with the agricultural and environmental sectors for which the definition of organism is plant or animal would it be possible to set definitions that exclude these organisms under those options whilst including microorganisms in regulation to mitigate risks associated with potential pathogens. The number of exceptions of microorganisms for which these techniques will be required to reduce barriers to technological developments for industry benefit will be much smaller. With this suggestion there is recognition of the Discussion Paper statement that "blanket exclusions may not be commensurate with the level of risk posed by these techniques - alternatively, drafting exclusions that do not apply to all organisms is potentially complex."

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