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Office of the Gene Technology Regulator
MDP 54
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Dear Sir/Madam

Re: Technical Review of the Gene Technology Regulations 2001

Thank you for the opportunity to provide feedback for the upcoming Technical Review of the Gene Technology Regulations 2001.

Please find enclosed a submission prepared on behalf of The University of Adelaide Institutional Biosafety Committee (IBC) for consideration by the Gene Technology Regulator.

Should you require any further information please contact me,

Yours sincerely

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Technical Review of the Gene Technology Regulations 2001

Thank you for the opportunity to provide feedback on the Technical Review of the Gene Technology Regulations 2001.

Members of the University of Adelaide Institutional Biosafety Committee (IBC) consulted researchers within their workplaces then met on the 24th November 2016 to discuss the proposed Options for regulating new technologies and define the IBCs position on proposed changes. Here we report our recommendations for consideration by the Gene Technology Regulator.

When discussing the Options, IBC members were adamant that we must not lose the public's trust by blurring the lines between genetic modification and random changes. We believe any Option that is implemented must have clear definitions and be amenable to compliance. Importantly, there must not be a public perception that researchers have the ability to bend the rules to avoid regulation lest the public lose faith in the gene technology regulatory process.

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

Option 3 was the model preferred by the Committee. The key reasons provided in favour of Option 3 were:

- It appears more likely to achieve compliance due to a clear distinction between random changes and modifications made to an organism's genome with intention. This would permit clear interpretation of genome editing regulations and require less value judgement on behalf of IBCs whether products are "different to conventional breeding".
- The sequence changes initiated through SDN-1 are indistinguishable from mutations generated by natural, chemical or high-energy means. Thus, the risks are unlikely to differ to those posed by naturally occurring mutations.
- Exclusion of SDN-1 from regulation would permit its widespread utility. The capacity to direct SDN-1 to specific regions of the DNA limits the chance of "off-target effects" that appear through chemical and radiation mutagenesis techniques.
- Changes to the current regulatory framework in order to adopt Option 3 would be minor.
- By providing some leniency with the exclusion of SDN-1 activities, this option would be in line with other international regulatory bodies.

The Committee's reasons for opposing Options 1, 2 and 4 were:

Option 1

- Was viewed as a non-viable option given the current uncertainty surrounding the regulation of these new technologies.

Option 2

- Will put us out of step with the majority of the world, with significant trade implications and obstruction of commercialisation opportunities

Option 4

- Is less objective and requires a significant amount of judgement on behalf of the IBCs. To regulate based on the size of the template for insertion does not appear to be a precise measure of risk.
- Has the potential for "template DNAs" to incorporate randomly into the genome resulting in undesired modifications.
- As it stands, the definition of Option 4 is too broad.
- It would rely on foreknowledge of whether the product of the genetic modification confers increased risk before work is conducted.

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

The IBC members and the stakeholders they represent agreed that the *Cons of Option 3* outlined in the *Discussion Paper: Options for regulating new technologies*, including trade implications and impedance of commercialisation applications, are valid shortcomings which must be considered in due course.

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 Committee did not identify any evidence to support this

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

The OGTR recognises that a precise distinction between excluded and regulated techniques must be defined in a manner that applies equally to all types of organisms. The opinion of the committee is that much of the classification burden will lie with IBCs and therefore extra administrative issues would be associated with Option 4. Issues may also arise when the University of Adelaide IBC authorises dealings previously approved by other IBCs, where the external IBC has a different interpretation of techniques to be excluded.

There was also equal concern that Option 3 would increase regulatory burden compared with Option 4, due to the high volume of CRISPR/CAS9 genome editing work that would require regulation.

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

Item 1 Schedule 1 is detailed on our website http://www.adelaide.edu.au/rb/oreci/gene-tech/gmo-dealings/#schedules and is consulted by our institution's researchers when deciding whether they are required to submit a Dealing Application to the IBC. If the item was changed, we would update our resources accordingly so researchers have access to current regulations.

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.

The IBC finds PC2 level containment for gene drive technology more than adequate and proposes that no additional regulations, such as required elevation to a licensable Dealing Not Involving Intentional Release, be imposed. Since Australia is an island, gene drive technology when applied to invasive animal species should be assessed liberally and not subjected to excessive precautionary regulation that would inhibit its development and adoption. We believe Gene Drives are an incredibly important technological tools for the future and PC2 containment level should facilitate rapid research into their functionality. If release into the environment promises to be useful, then the dealing should be elevated through DIR-level dealing regulation.

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.

RNA interference techniques currently being used within our Institution include transient induction of short interfering RNA (siRNA), stable expression of introduced, transgene-encoded short hairpin RNAs (hpRNA) and artificial microRNA (amiRNA).

We do not believe organisms that have been subjected only to RNA interference are GMOs. In the context of genome modification we only regard RNA to be important when it acts via a DNA intermediate. For example, inducing stable expression of shRNA by lentiviral transduction of a shRNA-expressing construct would constitute generation of a GMO and should be regulated. However, transfection of siRNAs would not generate a GMO and should not be regulated.

RNAi (i.e. hpRNA and amiRNA) techniques in plants that currently require stable integration of a DNA into a plant should still be considered GMOs. If "null-segregants" from these plants contain heritable changes in DNA, but no transgene, then they are no different from null-segregant plants generated by SDN-1. Hence these plants should not be regulated.

We believe this distinction between regulated and unregulated technologies is in line with the risks they pose. RNAi techniques themselves pose no significant risk to human health or the environment. The risks associated with using lentiviruses for stable integration of sequences into human genomes is well established (Pauwels et al. 2009).

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.

The IBC proposes three additional amendments to the GT regulations:

1. That **zebrafish (*Danio rerio*)** be included in the laboratory animals listed in Schedule 3 Part 1.1(a) for which PC1 level containment is considered sufficient, unless the nature of its genetic elements warrants a higher level of containment. Our reasons for requesting this are:
    a. Unlike mice, rats or rabbits, zebrafish (that are commonly sold in Australia for keeping as pets by the general public) have not become, and do not appear able to become, feral in the Australian environment and are not able to cross-breed with Australian native species. There is therefore a negligible risk that any genetically modified zebrafish will escape into the Australian environment
    b. Relaxation of the restrictions on GMO work with zebrafish could mean that the aquaria that house them may not be subject to meaningless and expensive design and use conditions.

2. That the following techniques, when applied to plants, should not be classified as genetic modification
    a. Generation of null-segregants
    b. Transient modification by techniques such as agroinfiltration

    Non-GM-GMO grafted plants, however, should remain classified as GMOs.

3. That ***Drosophila melanogaster*** be included in the laboratory animals listed in Schedule 3 Part 1.1(a) for which PC1 level containment is considered sufficient, unless the nature of its genetic elements warrants a higher level of containment. Our reasons for requesting this are:
    The current technical review of the Gene Technology Regulations 2001 states that amendments to the regulations could be considered *if our scientific understanding of the risks posed has changed*.

    This suggested amendment is based on an improved understanding of the very low level risks associated with GM *Drosophila melanogaster* strains. In the 15 years since the 2001 Gene Technology Regulations were put in place, there have been no reports of incidents with adverse effects on human health or the environment associated with the use of genetic modifications in *Drosophila melanogaster*. This is in spite of the fact that *Drosophila* research has burgeoned in recent years (currently

~4000 papers/year on *Drosophila*) with thousands of scientists around the world routinely using GM flies.

The PC2 containment levels in Australia are out of step with other modern countries conducting research with GM flies (e.g. US, UK, Europe), where work is conducted at a PC1 level, unless the nature of the genetic modifications has an inherent higher risk and warrants PC2 (see below).

Data supporting the proposed amendment are given below:

a) *Drosophila melanogaster* is a harmless Australian species

Drosophila melanogaster, the vinegar fly, is an experimental species that has been used for genetic research since 1909. There is a very low level of inherent risk to environment, crops, and human health with this species because:

1. It is already an established species in Australia, and is found throughout the world.
2. It is not a disease vector like mosquitoes, tse-tse, etc.
3. It does not bite or sting.
4. It is not a crop pest. Although *D. melanogaster* is often called a fruit fly it is not a true fruit-fly such as the family Tephritidae, and does not affect any crop. It should also be distinguished from the spotted winged Drosophila species *Drosophila suzukii* which is a fruit pest species.

In May 2001 a report on the use of genetically modified animals was prepared by the Royal Society, London. The section on Drosophila melanogaster from page 11 states:

> *"Methods for reproducibly creating stable, heritable GM insects were developed almost 20 years ago, using the well-known genetic model insect Drosophila melanogaster.* ***It is generally considered harmless as it is neither a significant agricultural pest nor a disease vector and no adverse consequences to human health or the environment of this large-scale genetic engineering have been reported.*** *Many thousands of different GM strains of Drosophila have subsequently been produced in laboratories around the world, and there are far more GM strains of Drosophila than there are of all other GM insects combined. It has become the paramount model organism for studying animal development and genetics." ('Section 4.2 Medical research: creating GM animals to understand gene function. The use of genetically modified animals. The Royal Society. London UK. May 2001. Available at https://royalsociety.org/~/media/Royal_Society_Content/policy/publications/2001/10026.pdf')*

b) Genetic modifications commonly used in GM fly research do not pose risks to health or the environment.

The vast majority of transgenic fly stocks involve innocuous genetic elements commonly found in non-pathogenic species.

These include:

- FLP, GAL4, GAL80 – found in common baker's yeast
- GFP and other fluorescent proteins – found in jellyfish, coral and other marine species
- lacZ – found in common bacteria (*E. coli*)

These genes pose no hazards to humans (i.e. no pathogenicity or toxicity), nor the environment since they do not confer a selective advantage.

In the rare cases in which the proposed genetic modifications inherently pose a higher risk, or confer an advantage on the modified organism, such as gene drive systems, a higher level of containment would be appropriate.

c) Laboratory-kept flies are <u>not found</u> to survive in the wild

Studies have shown that when wild *Drosophila* are brought into laboratory and cultured under standard laboratory conditions they rapidly adapt to 'laboratory life'. A study performed by Hoffmann, Hallas , Sinclair & Partridge (Hoffmann et al. 2001), showed that within three years of laboratory culture (~50 generations), the descendants of wild flies rapidly lost their ability to tolerate environmental stresses of the type normally encountered in the wild (heat, cold, desiccation). This study, as well as those described by Sgro & Partridge (Sgro and Partridge 2001) suggest that *Drosophila* grown under standard laboratory conditions for prolonged periods no longer display the traits required to survive and successfully reproduce in the wild.

From this evidence we would expect that GM *Drosophila* are not likely to survive even if they escape containment. This has now been tested in Europe and America, where for the past 30 years GM *Drosophila* have been kept under PC1 conditions. The 1000 Fly Genomes project has recently fully sequenced over 1,100 separate genomes from wild populations collected from all over the US, Europe and around the world. In this enormous dataset they have not detected transgenes (Lack et al. 2016). Consequently, genetic manipulation of this species under PC1 containment is now known to pose minimal risk to the environment.

d) Other countries use PC1-level containment for standard GM *Drosophila melanogaster* work.

In the United States, almost all transgenic Drosophila research is considered Biosafety Level 1, the least restrictive containment level under the NIH Guidelines (http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html).

However, when the nature of the Genetic Modifications DOES actually constitute a real risk then a higher level of containment is used. Examples would include:

• flies expressing prion sequences

• flies expressing gene-drive constructs

For example the opening paragraph from a recent article about gene drive states:

> *"…**Even though D. melanogaster ordinarily poses no threat to human health or agriculture**, the accidental release of flies carrying gene drive constructs from the laboratory could have unpredictable ecological consequences. This study therefore used institutionally approved stringent barrier methods. Only one experimenter handled the flies, inside an Arthropod Containment Level 2 insectary suitable for work with mosquitoes carrying human pathogens." (Akbari et al. 2015)*

Thus the containment is matched to the real risks. A similar situation exists in the UK and Europe. For GMOs such as insects, a risk assessment in the form of an environmental impact statement is required. If that risk assessment concludes that the risk to the environment is minimal it does not require BioSafety Level 2 containment.

Conclusion:

- The current requirement for PC2-level containment for GM *D. melanogaster*, when the modifications do not confer a selective advantage or produce an infectious agent, is not in line with the environmental risks posed by these flies.
- The environmental risks posed by PC1 containment of *Drosophila* have been studied for decades around the world, and found to be extremely low.
- Further, the current legislation imposes a significantly greater level of containment than that required by other major scientific research countries such as the US and the UK.

It is recommended that research using GM *D. melanogaster*, when the modifications do not confer a selective advantage or produce an infectious agent, be downgraded to PC1-level containment. This will match the current regulations for the containment of other common GM model organisms that are excluded on the basis of "long history of safe use".

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