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Arthropod Genetics Group

Docket No. FDA-2008-D-0394
The Division of Dockets Management
HFA-305
Food and Drug Administration
5630 Fishers Lane, Room 1061
Rockville, MD 20852
USA

SUBJECT: COMMENT ON THE FOOD AND DRUG ADMINISTRATION'S DRAFT GUIDANCE FOR INDUSTRY, REGULATION OF INTENTIONALLY ALTERED GENOMIC DNA IN ANIMALS, 82 FED. REG. 6561 (PROPOSED JAN. 19, 2017).

DATE: 19TH JUNE 2017.

AUTHORS

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EXECUTIVE SUMMARY

The FDA's revised Guidance for Industry #187, entitled *'Regulation of Intentionally Altered Genomic DNA in Animals'* seeks to place all potential uses of gene editing methods in animals (including humans) under a single regulatory framework. We do not think this is the right approach: the regulatory challenges are different for different species and for different gene editing techniques. We focus on the impact these changes will have on insect research and argue that the risks (of undesirable and/or harmful genetic changes) are not principally dependent on the method by which they are generated (the process), rather on the specifics of the application (the product). This is why we recommend the FDA shifts towards a product-based, rather than a purely process-based approach to the regulation of animals with intentionally altered genomic DNA.

WHO WE ARE

Oxford University's Mathematical Ecology Research Group (headed by Professor Michael Bonsall) is a cross-disciplinary research group based in the Department of Zoology at the University of Oxford. We use mathematical approaches to explore novel problems in ecology, evolution, health and economics. CEM, DK and KK are a group of post-doctoral researchers investigating the ecological and genetic aspects of novel methods to control insect populations. We focus primarily on modelling the genetics and spatial ecology of transgenic mosquitoes. Our current research program is funded by DARPA and seeks to model whether and how gene drives could be used effectively and safely to suppress mosquito populations.

Professor Luke Alphey works in the emerging field of genetic pest management, focusing particularly on mosquitoes. Before moving to The Pirbright Institute in Feb 2014, where he leads the Arthropod

Genetics research group, he was the Research Director of Oxitec Ltd, a spin-out company from Oxford University that he co-founded in 2002. Oxitec successfully conducted the world's first outdoor experiments with a GM insect in the USA in 2006, and in 2010 showed that a wild mosquito population could be suppressed by this genetics-based method. Professor Alphey was selected as a Technology Pioneer of the World Economic Forum in 2008 and Biotechnology and Biological Sciences Research Council (BBSRC) Innovator of the Year 2014 for his work with Oxitec.

BACKGROUND

In January 2017, the FDA invited public comments on a revised Guidance for Industry #187, entitled *'Regulation of Intentionally Altered Genomic DNA in Animals'* (hereafter referred to as 'GFI #187'). Also in January 2017, the White House Office of Science and Technology Policy released an update to the 'Coordinated Framework for the Regulation of Biotechnology' (hereafter referred to as the 'Coordinated Framework'), which provides a common set of principles to ensure a coherent cross-agency approach to the regulation of biotechnology products. The FDA is explicit[1], that GFI#187 should be consistent with this framework's principles.

The purpose of the Coordinated Framework is to ensure participating agencies "develop a coherent and sensible regulatory process, one based on the best available scientific facts and intended to minimize uncertainties, delays, overlaps, and inconsistencies."[2] Two principles in the updated Coordinated Framework are relevant to our comments. First, that the regulatory approach should pay attention to the risk posed by "the biotechnology product, the environment into which it will be introduced, and the application of the product"[3]. Second, that the intensity of Federal oversight should be determined by a risk-based approach to regulation, that can "distinguish between those biotechnology products that require a certain level of Federal oversight and those that do not"[4].

COMMENTS

Our discussion primarily considers how the proposed guidance applies to off-target SNP-type genetic changes[5] involving the knock-out of genes and/or the introduction of indel mutations[6]. We do not consider transgenic techniques which insert exogenous DNA into a cell, in detail.

1. What are off-target genetic changes?

A gene knock-out nuclease (e.g. Cas9-sgRNA complex) is applied to an animal cell (e.g. a fertilised embryo, germline cell or potential germline cell progenitor) in order to make a double-stranded DNA break at a predetermined location (the target site). This is then repaired by cellular machinery. However, as the nuclease is of imperfect fidelity, it also binds to other 'off-target' locations. Consequently, sequence changes may arise at locations on the genome other than the target site due to imperfect repairs (normally small deletions, base changes and/or small insertions)[7]. While there are

[1] Notice of Availability of draft GFI#187, 82 Fed. Reg. 6561, 19, Jan. 2017.
[2] Proposal for a Coordinated Framework for Regulation of Biotechnology; Notice for public comment, 49 Fed. Reg. 50,856, 50,857 (Dec. 31, 1984).
[3] 2017 Update to the Coordinated Framework for the Regulation of Biotechnology, page 7.
[4] 2017 Update to the Coordinated Framework for the Regulation of Biotechnology, page 8.
[5] 'Single nucleotide polymorphisms' (SNPs) are where DNA sequences vary by a single nucleotide.
[6] 'Indel' means the insertion or deletion of single nucleotides
[7] To avoid misunderstanding, while the RNA-guided nuclease complex used for illustration above does have a small nucleic acid component, this is not a heritable element, and an equivalent effect could be obtained with entirely nucleic-acid free nucleases

ways to minimise the occurrence of these off-target genetic changes so they occur only at a low frequency, they are unlikely to be completely prevented, and consequently these mutagenic side-effects are a consideration when regulating the product.

2. How does the draft guidance propose off-target genetic changes are regulated?

The draft GFI #187 regulations state:

> 'Altered genomic DNA may result from random or targeted DNA sequence changes including nucleotide insertions, substitutions, or deletions, or other technologies that introduce specific changes to the genome of the animal.'[8]

From this wording, it is clear the FDA intends off target effects (i.e. random indel mutations) to fall within its definition of 'altered genomic DNA'. Furthermore, any altered genomic DNA in an animal:

> 'is a drug within the meaning of section 201(g) of the FD&C Act because such altered DNA is an article intended to affect the structure or function of the body of the animal, and, in some cases, intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in the animal.'...Therefore, in general, each specific genomic alteration is considered to be a separate new animal drug subject to new animal drug approval requirements.'[9]

As each specific genome alteration is considered a drug, it appears off-target mutations would need to be regulated as such. Moreover:

> '[o]ther than for investigational uses, section 512(a)(1) of the FD&C Act (21 U.S.C. 360b(a)(1)) requires that a new animal drug be the subject of an approved NADA [new animal drug application] based on a demonstration that it is safe and effective for its intended use'[10].

Which implies that every off-target mutation will require a 'new animal drug application'. It further states:

> "one INAD [investigational new animal drug] file may be established for multiple genomic alterations and the file may contain information on investigational animals that contain different numbers or types of intentional genomic alterations, including those occurring at different locations of the genome..."[11].

This mitigates some obvious problems characterising off-target effects as drugs, but leaves important issues, we discuss below.

3. Challenges regulating off-target changes under the proposed rules

a. <u>In practice, how do we get an off-target effect approved as a new drug?</u>

The regulations justify the classification of altered genomic DNA as a drug based on their intended phenotypic effect. However, more-or-less by definition, off-target genetic alterations have no

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such as zinc-finger nucleases or TALENs.

[8] Draft GFI #187, page 7, section II. A.

[9] ibid

[10] Draft GFI #187 page 14, section IV.A.

[11] Draft GFI #187, page 8, section II. B.

intended use. This raises a number of practical questions:

i. How does the FDA propose the regulations will apply when the genetic alteration has no obvious phenotypic consequences?
ii. What burden of proof will researchers have to meet to prove there is no obvious phenotypic effect, and under what experimental conditions will this need to be proved?
iii. Given the likelihood that multiple off-target mutations will occur in a single individual, what combinations of off-target mutations will the FDA expect us to test to demonstrate there are still no obvious phenotypic consequence?
iv. If an off-target mutation is a drug, are the combined effects of multiple off-target mutations to meet the regulatory requirements designed for drug interactions?
v. Given that there are hundreds or thousands of sites in the genome where off-target effects could potentially occur from the application of a single nuclease, does every off-target effect need to be registered as a drug, no matter how rare?

b. How do the regulations apply if we are editing to wild type?

One potential application is to try to eliminate genetic defects/diseases in specific breeds of livestock or companion animals, in particular pedigree dogs. In this case the intended change is to the wild type (or to a wild type, given that wild type may be hard to define). Consequently, it seems perverse to categorise the change as a new drug when it is already present in many, perhaps most of the individuals in that breed or species. Might this be an example of a type of change where it is reasonable to assume "long history of safe use"?

c. How do the regulations apply to gene drive systems?

Some types of application involve the use of nucleated coding sequences integrated into an animal's genome, for example the gene drive systems of Hammond *et al.*[12] and Gantz *et al.*[13], which target malaria vectors. In such designs, the nuclease cuts at a specific site on the genome, leading to copying across of an engineered sequence by homology-directed repair. It is unclear in such a system how to apply the draft guidance. Is this newly copied allele a new drug? Possibly not, as it should be identical to the template copy (which itself would be considered a drug), albeit now in a new haplotype context. But what if the copying is imperfect in some way? More seriously, such nuclease-containing strains presumably have a non-zero rate of off-target cutting and so will generate new genetic changes at some (presumably very low) frequency. For the intended applications in mosquitoes, this is unlikely to be problematic. Moreover, there are unlikely to be any new risks from such new mutations, which are presumably within the normal mutagenic range of the host organism. Therefore, it is very hard to see how this type of application could cope under the proposed regulations that would define any such a change as a new drug.

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[12] Hammond, A., Galizi, R., Kyrou, K., Simoni, A., Siniscalchi, C., Katsanos, D., ... & Burt, A. (2015). A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector Anopheles gambiae. Nature biotechnology. Note, though the designs of Hammond et al. were directed towards population suppression, this is need not be the case.

[13] Gantz, V. M., Jasinskiene, N., Tatarenkova, O., Fazekas, A., Macias, V. M., Bier, E., & James, A. A. (2015). Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito Anopheles stephensi. Proceedings of the National Academy of Sciences, 112(49), E6736-E6743.

d. We cannot always differentiate altered genomic DNA from naturally occurring genetic variation and/or spontaneous mutation

The draft guidance implies that commercialised animals will need to be genetically homogeneous, at least in terms of any alterations potentially attributable to the intended mutagenic event (including "off-target" effects). If the animals are not genetically homogeneous, then following a gene editing event, the observed genetic variants, other than at the targeted locus, will be an unknown and unknowable mix of changes caused by the off-target activity of the gene-editing nuclease (i.e. that would not have happened in those cells had the nuclease not been applied) and unrecognised existing background variation.

This is especially problematic for insects, where there is no single wild type genome against which variants can be assessed for their degree of difference. Take for example, the mosquito *Anopheles gambiae*. The *Anopheles gambiae* 1000 Genomes Consortium has found over 50 million single nucleotide polymorphisms from sequencing just 765 wild specimens of *Anopheles gambiae* and *Anopheles coluzzii*[14]. As these mosquitoes have a genome size of approximately 250 Mbp, this diversity represents approximately one SNP per 5 nucleotides, on average. Individual mosquitoes carry between 1.7 and 2.7 million variant alleles. Presumably sequencing more individuals or sampling more populations would reveal further variation.

While in theory, one may be able to overcome the problem of genetic diversity and compare each mosquito with their parent or parental stock and so identify SNP-type variations, this may not be universal and will still be ambiguous both in terms of differences and in terms of causality. More generally, and even with genetically homogeneous strains, it will be impossible to know if a potential "off-target" effect was caused by spontaneous mutation or application of a nuclease. This leads us to our next point: that the proposed guidance is disproportionate.

4. The regulations are a disproportionate response to the risks of RNA-directed nucleases.

The challenges applying the draft guidance discussed above stem from a common problem: that the response to the risks posed by RNA-directed nucleases is disproportionate. The types of mutation generated by RNA-directed nucleases are, from the authors' reading of the literature, in all cases equivalent in structure and properties (in terms of DNA sequence) to mutations previously generated by classical mutagens or spontaneous mutations. Therefore, both off-target effects and on-target effects where the target mutations are produced simply by generating a double-stranded break (i.e. with no repair template), are within the normal mutagenic range and essentially similar to products of classical mutagenesis.

Classical mutagens are physical or chemical agents that increase the frequency of mutations. They have been used by genetics researchers and animal and plant breeders for decades. Mutagens have a long history of safe use: drugs with mutagenic-side effects and radiation therapies which are intentionally mutagenic are widely used in anti-cancer and anti-virus treatments, and radiation has been successfully used since the 1950s to create sterile insects for pest control. The mutagens themselves are

[14] Miles, A. et al. (2016) Natural diversity of the malaria vector *Anopheles gambiae*. bioRxiv 096289; http://biorxiv.org/content/biorxiv/early/2016/12/22/096289.full.pdf. Note, though recently classified as separate species, An. gambiae and An. coluzzi are morphologically indistinguishable, often sympatric, and can form viable and fertile hybrids.

regulated, at least in some jurisdictions, based on their potential hazard to human health.

Off-target effects are likely indistinguishable from spontaneous mutation or natural variation. Selection from standing genetic variation/spontaneous mutation has been used since prehistoric times. Therefore, given the degree of natural genetic variation, and the natural mutagenic processes continually producing new variation, it seems hard to argue that there is any novel or special risk associated with the use of nuclease-based methods for targeted mutagenesis. We suggest that the draft guidance therefore is inconsistent with the updated Coordinated Framework's principal that the regulatory approach should consider the risk posed by "the biotechnology product, the environment into which it will be introduced, and the application of the product"[15].

5. Insects will be regulated by three different agencies.

Certain mosquito products will not be covered by draft GFI #187, and will instead be regulated by the Environmental Protection Agency as pesticides. Specifically:

> 'that the phrase "articles (other than food) intended to affect the structure or any function of the body of man or other animals" does not include articles intended to prevent, destroy, repel, or mitigate mosquitoes for population control purposes. Instead, such products are pesticides regulated by the Environmental Protection Agency (EPA).'[16]

This reassignment of primary responsibility for regulating certain insect products means that the USDA will continue to regulate insects that are plant pests, EPA will regulate products intended for population suppression while FDA will regulate other products.

This would leave regulation for the relatively nascent industry relating to insects with targeted genomic changes subject to regulation by three different lead agencies. Closely related products may be regulated by different agencies, for example a gene drive product that spreads a virus-refractory gene may be regulated by FDA, whereas the same genetic system with a different (or no) "cargo" element may be regulated by EPA (many gene drive systems work in part by inducing reduced fitness, e.g. sterility or death, in certain classes of offspring), and the same system in a plant pest species regulated by USDA.

We appreciate the attention given to the need to provide appropriate regulatory oversight of such products, however this division seems clearly at odds with the Coordinated Framework's principles as it is likely to impose unnecessary and onerous bureaucratic requirements on developers. By raising the barriers to entry, this may force smaller players out of the market (or more likely dissuade them from entering in the first place), in particular public sector or not-for-profit entities, or those considering products for smaller niche markets where the accessible market segment is too small to justify incurring heavy regulatory costs. We recognise that this comment may relate more to Draft Guidance for Industry #236 rather than the present #187, however the issue is raised again in the above quote from #187.

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[15] 2017 Update to the Coordinated Framework for the Regulation of Biotechnology, page 7.
[16] Footnote 9, draft GFI #187, referring to draft GFI #236, "Regulation of Mosquito-Related Products".

RECOMMENDATIONS:

We favour regulations for gene-edited products: they give confidence to consumers and certainty to developers, but the rules need to be consistent and proportionate, which is currently not the case.

Recommendation 1: mutations should not be regulated as drugs. If a mutagenic chemical were applied to an animal, one would consider the chemical to be a drug, but not any resulting mutants. One would certainly not consider all offspring down the ages to be "drugged" because they had inherited a genetic change, and even less so for the many non-target mutations with no obvious phenotype, that are indistinguishable from spontaneous mutations or natural variation, that would inevitably be left floating in the strain after such an experiment. Yet this is what the regulations propose should be done if a scientist applies a nuclease (for example delivered as protein, or protein-RNA conjugate), instead of a classical mutagen, even though the situation in terms of heritability of mutations is exactly the same. We suggest that the nuclease is a drug, perhaps, but not the consequence.

Recommendation 2: the regulatory requirements should vary by application, not just approach. We suggest the "one-size fits all" approach that is proposed is incompatible with the Coordinated Framework's requirement that agencies pay attention to the risk posed by "the biotechnology product, the environment into which it will be introduced, and the application of the product"[17]. We agree with the National Academy of Science that the right approach to the regulation of genome editing is to focus on the 'product' (i.e. the application of a particular genetic technique) and not the 'process' (i.e. the technique itself). To quote from their report "emerging technologies have blurred the distinction between genetic engineering and conventional plant breeding to the point where regulatory systems based on process are technically difficult to defend"[18]. A product-based approach would allow regulators to match the type of regulatory oversight to the risks posed by the application.

Recommendation 3: regulate each application based on its risk profile. We suggest all genetic variants made by any process are regulated by a new system, where the specific regulatory burden is determined on a case-by-case basis based on the risk profile of the intended application. Relevant factors informing the degree of oversight required would include the target species, whether transgenes were being introduced and the frequency of on- and off-target effects. In some cases, the ability of any of these elements to spread within a population into which they are introduced (e.g. fitness and/or selfish DNA characteristics of the element) will also be relevant; perhaps more so for interventions in wild species/populations than managed ones. In respect of artificial selfish DNA elements ("gene drive systems"), it is commonly the case that the location of each element is important as well as what it does, so elements with very similar DNA-cutting abilities may have quite different performance profiles in this regard (e.g. is the nuclease inserted into its target site (a gene drive), or not; for an X-shredder is it inserted on the Y chromosome, or not, etc.), again suggesting that DNA-cutting ability is not an ideal focus for regulation.

Our proposed system would allow the regulatory requirements to be proportionate to the risks. For example, as the oncogenic risks posed by off-target mutations is a major consideration for human gene

[17] 2017 Update to the Coordinated Framework for the Regulation of Biotechnology, page 7.
[18] National Academy of Sciences (2016). Genetically Engineered Crops: Experiences and Prospects. https://nas-sites.org/ge-crops/

therapy, all clinical applications, regardless of any other factors, would require close monitoring by regulators. By contrast, pedigree dog breeders wishing to prevent congenital disease by editing to wild type, i.e. restoring allelic diversity by taking alleles from another dog breed, should be able to do so with only basic oversight. For insect applications, the regulatory burden would be determined by a range of factors e.g. a gene drive causing infertility with a high homing rate that rapidly spreads through the population requires much more stringent controls than a non-homing nuclease that causes a double strand break at a specific target site. Unlike humans, off-target effects are less of an issue for insects; if the aim is to reduce a pest species' numbers, deleterious off-target effects may even be a good thing. As these examples illustrate, moving away from the "one-size fits all" approach would be more in line with the Coordinated Framework's recommendation that agencies "distinguish between those biotechnology products that require a certain level of Federal oversight and those that do not"[19].

CONCLUSION:

The draft regulations GFI#187 are unworkable in their current form and would seriously impede the development and application of genome-editing technology. We urge the FDA to take into account the vastly differing risks posed by different applications of a genetic modification technology. We note that many other comments on the draft GFI #187 and experts share our option[20].

We appreciate this opportunity to provide comments and sincerely thank the FDA in advance for its careful consideration. Please do contact us if you have any questions.

Sincerely,

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[19] 2017 Update to the Coordinated Framework for the Regulation of Biotechnology, page 8.

[20] For example, comment from Jeffrey Taylor, FDA-2008-D-0394-0328, posted 1st May 2017; comment Amanda Plimpton, FDA-2008-D-0394-0330, posted 1st May 2017; comment from David Arnosti, FDA-2008-D-0394-0346, posted 18th May 2017; also see Carroll, Dana, et al. "Regulate genome-edited products, not genome editing itself." Nature biotechnology 34.5 (2016): 477-479.