NAVIGATING THE REGULATORY LANDSCAPE:

OXITEC CASE STUDY

Context

The yellow fever mosquito, known as *Aedes aegypti (A. aegypti)*, has been known to carry and transmit viruses, including its namesake disease, yellow fever, dengue fever, and chikungunya, according to the Centers for Disease Control and Prevention (CDC).[1] *A. aegypti* is native to Africa, but has spread to other tropical and subtropical regions, where it prefers to occupy open waters with organic matter near populated residential areas to produce offspring. Only the female mosquitos bite and must feed on blood, preferably (but not limited to) human blood, to lay eggs. This feeding behavior of the female mosquito is a key element in the transmission of disease to humans. The lifespan of the *A. aegypti* is around three weeks. Its eggs, however, can survive in favorable climates for six months or longer.

Current methods for controlling populations of these mosquitos include eliminating their preferred habitats (standing water in and around homes); wearing protective clothing to prevent bites (*i.e.*, long sleeve shirts, pants, socks); applying insect repellents; and spraying pesticides. Spray application of pesticides is documented as achieving approximately 50 percent reduction in mosquito populations.[2] This low reduction rate is attributed to their preferred habitat being in close proximity to residential homes and the difficulty in eradicating them using spray methods.

Description of the new technology

Oxitec, Ltd. (Oxitec), a privately-held company organized under English law,[3] has developed a genetically engineered mosquito strain by micro-injection of recombinant DNA (rDNA) into *A. aegypti* eggs designed to kill the subsequent offspring.[4]

The Oxitec rDNA construct contains a dominant lethal gene that is repressed in the presence of adequate concentrations of tetracycline. Mosquitos expressing the rDNA transgene are dependent upon the presence of tetracycline for their survival. Viable adults resulting from the micro-injected eggs were mated in the laboratory to wild-type mosquitos, and the resulting hatched larvae were screened for expression of the fluorescent marker that was also coded in the rDNA plasmid vector.

The heterozygous transgenic strain is described as having a single copy of the rDNA construct at a single site in the mosquito genome. Transgenic heterozygotes are sorted by sex at the pupal stage and, for purposes of implementing the insect control approach dubbed Release of Insects carrying a Dominant Lethal (RIDL),[5] males would be released from the controlled environment of the insectary (lab) into the wild to mate with wild *A. aegypti* females before dying due to the de-repression of their dominant lethal gene in the absence of sufficient dietary tetracycline that is available in their supplemented feed within the insectary, but not in the wild. Half of the progeny of these RIDL/wild type crosses are expected to be RIDL heterozygotes, and half are expected to be wild type. In the absence of dietary tetracycline supplementation, however, the RIDL larval offspring will die before reaching the pupal stage, whereas the wild type offspring will be unaffected genetically, but may suffer adverse effects due to competition for nutrients with the doomed RIDL larvae. According to Oxitec, releasing the appropriate number of RIDL males into the wild could achieve an overall 90 percent reduction in the treated *A. aegypti* population.

Sterilization for population reduction has had favorable results in controlling insect populations in other species, but has not been possible for mosquitos due to technical and regulatory issues. The genetic modification of these RIDL mosquitos includes a fluorescent marker for tracking them once they are released into the wild, as well as the tetracycline controlled kill mechanism used to limit the lifespan of the modified transgenic mosquitos and, in the absence of genetic recombination events, preventing the transmission of the rDNA construct to future generations of *A. aegypti* in the wild. The low transformation efficiency described for this rDNA construct in Phuc's 2007 publication suggests that spontaneous genetic recombination between the rDNA construct and wild type DNA is unlikely, but this is one of the points that must be addressed with actual data during the regulatory approval process. Phuc's 2007 publication's description of the strain from which the current Oxitec transgenic mosquitos are derived also notes that 3-4 percent of the progeny resulting from breeding transgenic males with wild type females resulted in transgenic adults that survived in the absence of tetracycline. The precise genetic status and reproductive capabilities of transgenic mosquitos that do not express the dominant lethal trait in the absence of tetracycline is also important in the assessment of this novel technology.

Discussion of the legal and procedural issues

The U.S. Food and Drug Administration (FDA) defines "drug" to mean an article intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or animals and/or an article intended to affect the structure or any function of the body of man or other animals.[6] The introduction of a new modification to the structure or function of the body of man or animal is, by FDA definitions, creation of a new drug. The management of drugs within FDA is divided between new human drugs, as administered by the Center for Drug Evaluation and Research (CDER) or the Center for Biologics Evaluation and Research (CBER), and new animal drugs, as administered by the Center for Veterinary Medicine (CVM). New animal drugs are drugs intended for use in animals, other than man; animals are further divided into minor and major species. FDA includes cattle, horses, swine, chickens, turkeys, dogs, and cats among major species and designates all other animals as minor species. FDA defines genetically engineered animals as "those animals modified by rDNA techniques, including the entire lineage of animals that contain the modification."[7] The Oxitec genetically engineered *A. aegypti* strain could be regulated by FDA as both a minor species new animal drug that is subject to pre-market notification processes through CVM and as an article for the mitigation of disease in man that is subject to the requirements for new human drugs.

CVM guidance for genetically engineered animals (GFI 187) indicates that all genetically engineered animals are subject to pre-market approval requirements. FDA has indicated that, in certain cases, it may not enforce the requirements for an investigational new animal drug (INAD) or a new animal drug application (NADA) and intends, in such cases, to post this on its website. FDA is always authorized to initiate enforcement action if the agency becomes aware of a safety concern. In 2003, FDA posted a statement for aquarium fish that were modified to contain genes that were fluorescent and not for food use. FDA concluded that this use of a genetically engineered animal posed no more of a threat than their "unmodified counterparts."[8] FDA conducts a review to comply with the National Environmental Policy Act (NEPA) when it reviews and approves an INAD or NADA. NEPA requires federal agencies to describe in detail and assess the anticipated impacts of all "major Federal actions significantly affecting the quality of the human environment."[9] No NEPA review would occur, however, when FDA exercises its enforcement discretion.

The Oxitec example in this case study is unique in many ways. The field trials Oxitec proposed include the release of a genetically engineered minor species into the environment for population control of insects carrying human diseases. Release of genetically modified insects to mitigate human disease is relatively uncharted territory for FDA. The release into the wild means FDA's enforcement discretion will not be exercised, and that CVM will enforce full pre-market approval as a new animal drug. The decision regarding CVM jurisdiction with this case study has been, and continues to be, debated. Genetically engineered insects being developed for plant pest control are considered under the oversight of the U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS). Previously approved FDA anti-malaria drugs manufactured using synthetic biology

techniques were managed by CDER/CBER, not CVM. The reason for this decision is that the drug for mitigation of human disease was produced by a modified organism, rather than the modified organism mitigating the species responsible for causing the disease. The Oxitec mosquito could be considered an article for mitigating disease. Environmental group Friends of the Earth (FOE) opines in an Issue Brief on this matter that this intended use of genetically engineered mosquitos "should be considered a medical trial and must follow the strict laws and guidelines in place to protect human subjects in medical trials."[10] FOE believes that this includes free and informed consent by all humans in the release area.[11]

GFI 187 indicates a new animal drug is deemed unsafe unless FDA has approved it through a NADA for that particular use. There are exemption processes for conditional approval and indexing unapproved investigational animal drugs for the purpose of pursuing safety and effectiveness investigations by trained scientific experts. The Minor Use and Minor Species Animal Health Act of 2004 also provides additional options for streamlined pre-market approval for minor species and treatment of uncommon diseases in major animals. None of these exemptions or streamlined approaches applies to genetically modified animals.[12] The NADA process involves a detailed demonstration that the drug in its intended use is safe and effective, not only to the animal itself, but also to any food products derived from the treated animal. The process also includes consideration of potential environmental impacts and safety assessments for those responsible for administration of the drug.

Developing a NADA requires extensive technical data supporting the proposed dosage, intended use, and potential environmental impact information. The process is typically done in cooperation with CVM's Office of New Animal Drug Evaluation through the opening of an INAD file. There is a fee structure associated with these activities as required through the Animal Drug User Fee Act of 2003 (ADUFA).[13] This fee structure also includes detailed timelines for responses the agency must provide for the various aspects and steps of the NADA process.

The NADA general application provisions are detailed in 21 C.F.R. Part 514. The requirements generally include the following:

- Basic identification details on the nature of the application, and the trade name and location of the applicant. For genetically engineered animals, the details on the rDNA construct, including the number and characterization of the insertion sites is also necessary.
- A summary of the chemistry, clinical purposes, and laboratory and clinical studies is included.
- Proposed labeling for adequate instructions for use must accompany the application. The labeling for genetically engineered animals should include a description of the common name, genus, and species with instructions for handling throughout the animal's lifecycle.
- Details on the composition and

components utilized in the production of the drug. The GFI 187 recommends providing the molecular characterization of the article in sufficient detail to facilitate evaluation of potential risks due to genetically engineered animal rDNA that might encode pathogens, toxicants, allergens, mobile DNA sequences, or sequences that deregulate growth control.

- Extensive details on the manufacturing methods, production facilities, and controls to allow for sufficient evaluation that the methods described will "preserve the identity, strength, quality and purity of the new animal drug" are to be provided.[14] Evaluation of any "interruption of coding or regulatory region (insertional mutagenesis)" is also recommended in the GFI 187.[15]

- CVM could, upon request, also require four identical sets of representative samples for each strength of the finished dosage with all the articles used as components along with reference standards and detailed analytical assaying procedures used to determine quality specifications. This can include detailed experimental protocols for establishing dosage, and when used in animals that are also a food source, substantial information on tissue residuals and elimination rates. Samples of the genetically engineered animal could also be required, upon request. CVM encourages specific dialogue as part of the INAD file, as to how to address this aspect of the application process.

- The application is to include evidence of the establishment of safety and effectiveness, including proposed labeling. This evidence must include reports of all the tests, scientific literature, and clinical investigations utilized to support the claims, including favorable and unfavorable results.

- Commitments to manufacture in accordance with current Good Manufacturing Practices (cGMP) and conform to advertising requirements are included in the application. FDA has indicated it will provide guidance for how genetically engineered animals are to "commit to cGMP" aspects of this process at a later date. Non-clinical studies are expected to be conducted in compliance with the Good Laboratory Practice regulations in 21 C.F.R. Part 58, and the reason for any non-compliance must be provided.

- Each application is to include a claim for categorical exclusion or an environmental assessment that demonstrates that the new animal drug or the genetically engineered animal will not significantly impact the quality of the human environment.

These details are assembled in accordance with 21 C.F.R. § 514.1(b)(15), and submitted to CVM for review.

Conferences with CVM prior to submission of a NADA are described in 21 C.F.R. § 514.5 and include conducting field studies, if necessary. Oxitec is currently seeking approval to conduct field studies within the United States. Oxitec reports field trials have been and are ongoing in other locations, and discussions are currently ongoing with FDA

as part of their INAD. The NADA approvals in general are carried out in stages, and the reviews involve experts in many areas of science, including veterinarians, animal scientists, biostatisticians, chemists, microbiologists, pharmacologists, and toxicologists.[16] All aspects are reviewed, including the product's final labeling, packaging, and possible directions for use, prior to CVM approval. Review of genetically engineered animals may involve inclusion of addition technical experts and possible interaction with other agencies (*i.e.*, U.S. Environmental Protection Agency (EPA), CDC, and USDA). Interactions with EPA and CDC have been part of the ongoing Oxitec field trial discussions within CVM. After the NADA is complete, the approval process requires notification through the *Federal Register* (FR). Once approved and listed in the FR, any significant changes as detailed in 21 C.F.R. § 514.8 must be re-substantiated through a supplemental approval process.

All approved animal drugs are expected to maintain all aspects of the processes detailed in their application in accordance with FDA regulations at all times, and are subject to inspection. All adverse events are to be investigated and reported. Drug listing, recordkeeping, and periodic reporting are all required post-approval. Any significant deviation in quality controls, equipment, facilities, labeling, etc., must be reviewed and approved prior to sale or distribution.

The legal and regulatory takeaway

The intricate details and ongoing jurisdictional debate are interesting parts of this complex case study. An argument could be made that a technology designed to control a pest should be regulated by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). As discussed, past genetically engineered pest control technologies have fallen under the jurisdiction of APHIS. Technologies that control animal populations by sterilization, however, have been regulated by CVM. Some argue that if the Oxitec mosquitos are primarily intended to prevent or mitigate a human disease, the product should be regulated as a human drug rather than as an animal drug.

After review and consultation, however, the various regulatory authorities determined that the Oxitec mosquitos are most appropriately regulated by CVM as an animal drug. CVM's precedent of regulating other animal sterilants used for animal population control as animal drugs is guiding FDA and the other regulatory stakeholders in determining a regulatory pathway for the Oxitec mosquito. As with almost all FDA-related regulatory inquiries, of equal importance is the initial determination of the product's "intended use." Here, Oxitec and other stakeholders have been careful to describe the use of the product as limiting or controlling the population of certain mosquitos. Notably, the product makes no claim to prevent or mitigate disease in humans; the product only claims to control or reduce the population of certain mosquitos.

The Oxitec mosquito control technology is a novel case for CVM because it employs rDNA technology in an organism that is intended to be released into the wild, not simply used to produce an animal drug that would then be used under controlled conditions. Nonetheless, limiting the Oxitec product's claim to one already within the ambit of CVM's prior regulatory experience supports the rationale for regulating the

Oxitec mosquito as an animal drug. Any future claims that this technology prevents or mitigates human disease -- such as dengue fever or chikungunya -- rather than simply controlling a mosquito population would likely raise questions of whether the technology is a human drug, and thus subject to CDER jurisdiction.

Given the complexity of the jurisdictional gauntlet, it is completely unclear how a new product developer would begin the regulatory approval process, as none of these issues is intuitively self-evident. Little guidance exists to direct private entities to the appropriate government office to begin the review process, let alone outline what that process is, how long it might take, and how much it might cost before the product can be commercialized. These are business realities that must be known to bring a product to market. This case study crystalizes just how unclear the jurisdictional divide is and how even the government can be at a loss to specify which agency has the lead, let alone outline coherently what the review process might include.

Endnotes

1 CDC, Dengue Homepage: Entomology & Ecology, available at http://www.cdc.gov/dengue/entomologyecology/.

2 Florida Keys Mosquito Control District, Questions and Answers on GM Mosquitoes, available at http://keysmosquito.org/question-answers-on-gm-mosquitoes/.

3 Oxitec, available at http://www.oxitec.com/.

4 H.K. Phuc, et al. (2007). Late-Acting Dominant Lethal Genetic Systems and Mosquito Control. BMC Biology 5:11.

5 http://www.oxitec.com/ridl-science/understanding-ridl-science/molecular-biology/.

6 FFDCA § 201(g)(1), 21 U.S.C. § 321(g)(1).

7 CVM (2009), Guidance for Industry Regulation of Genetically Engineered Animals Containing Heritable Recombinant DNA Constructs (GFI 187), available at http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM113903.pdf.

8 FDA (2003), Statement Regarding Glofish, available at http://www.fda.gov/animalveterinary/developmentapprovalprocess/geneticengineering/geneticallyengineeredanimals/ucm413959.htm.

9 42 U.S.C. § 4332(2)(C).

10 Friends of the Earth, Issue Brief: Genetically Engineered Mosquitoes in the U.S., at 3, available at http://libcloud.s3.amazonaws.com/93/df/1/959/5/Issue_brief_GE_mosquitoes_in_U.S.pdf.

11 Id.

12 21 U.S.C. §§ 360ccc(a)(3)(A) and 360ccc-1(a)(2).

13 FDA, ADUFA, available at http://www.fda.gov/ForIndustry/UserFees/AnimalDrugUserFeeActADUFA/default.htm.

14 21 C.F.R. § 514.1(b)(5).

15 GFI 187 at 16.

16 FDA, From an Idea to the Marketplace: The Journey of an Animal Drug through the Approval Process, available at http://www.fda.gov/AnimalVeterinary/ResourcesforYou/AnimalHealthLiteracy/ucm219207.htm.

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