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Food and Drug Administration
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Comments on the Food and Drug Administration's Draft Environmental Assessment and Preliminary Finding of No Significant Impact for the Release of Genetically Engineered Mosquitoes as an Investigational New Animal Drug (Docket No. FDA-2014-N-2235)

To United States Food and Drug Administration (FDA):

Center for Food Safety (CFS), Foundation Earth, and International Center for Technology Assessment (ICTA), submit the following comments on behalf of themselves and their members in response to FDA's draft Environmental Assessment (EA) and preliminary Finding of No Significant Impact (FONSI) for investigational use of Oxitec, Ltd. (Oxitec)'s genetically engineered (GE) *Aedes aegypti* mosquitoes (OX513A).[1]

CFS is a nonprofit, public interest advocacy organization dedicated to protecting human health and the environment by curbing the proliferation of harmful food production technologies and promoting sustainable agriculture. In furtherance of this mission, CFS uses legal actions, groundbreaking scientific and policy reports, books and other educational materials, and grassroots campaigns on behalf of its 750,000 farmer and consumer members across the country. CFS is a recognized national leader on the issue of GE organisms, and has worked on improving their regulation and addressing their impacts continuously since the organization's inception.

Foundation Earth is a national, nonprofit, public interest advocacy organization founded in 2011. Its focus includes: economic ecology models, technology, biospheric education, and earth jurisprudence. It calls for a major rethink of society from the ground up. Foundation Earth envisions more self-reliant communities embedded in a continental network of bioregional economies that function within the carrying capacity of the planetary boundaries. A rapid shift from a polluting industrial society to a more holistic and responsible approach will require examining the dimensions of a deeply resilient low-impact economy and implementing it broadly. Foundation Earth provides advisory services concerning rapid systems change. Our mission is to bring an earth-centered "True Cost Economy" into reality.

[1] 81 Fed. Reg. 13,371 (Mar. 14, 2016), https://www.gpo.gov/fdsys/pkg/FR-2016-03-14/pdf/2016-05622.pdf.

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ICTA is a nonprofit, nonpartisan organization committed to providing the public with full assessments and analyses of technological impacts on society. ICTA is devoted to fully exploring the economic, ethical, social, environmental, and political impacts that can result from the applications of technology or technological systems. It has assessed new developments in human, animal, and plant biotechnology since its founding in 1994.

British biotechnology company Oxitec recently applied for an investigational new animal drug (INAD) with the FDA to allow the field release of GE *Aedes aegypti* mosquito strain OX513A in Key Haven, Monroe County, Florida. This GE mosquito strain has been genetically engineered to contain a conditional lethality trait and a fluorescent marker. Oxitec prepared a draft EA and the Center for Veterinary Medicine (CVM) of the FDA published a preliminary FONSI for public comment, concluding that the GE *Aedes aegypti* mosquito is unlikely to impact the physical, biological, and human environment; that no cumulative impacts are anticipated; and that the release will have no effect on threatened and endangered species or their designated habitat.

FDA's EA and FONSI related to this proposed release are wholly inadequate and based on incomplete and inadequate science and analyses, lack critical data and vital risk assessments, and ignore potential consequences and uncertainties. Their conclusions are erroneous and indicate FDA's failure to properly evaluate the potential effects of this release as it is required to do under the National Environmental Policy Act (NEPA) and Migratory Bird Treaty Act (MBTA). The information included in the EA raises many questions, contains significant data gaps, and indicates the potential for significant impacts, all of which warrant a full Environmental Impact Statement (EIS). In light of this, FDA's failure to conduct an EIS would be arbitrary, capricious, an abuse of discretion, and would violate NEPA and the MBTA.

I. BACKGROUND: OXITEC AND GE INSECT TRIALS

Oxitec is a company developed by researchers from Oxford University, now owned by U.S. biotechnology company, Intrexon.[2] The company aims to establish a new method of pest control through GE insects, including agricultural pests, such as diamondback moths, and mosquitoes, such as *Aedes aegypti*.[3]

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[2] Oxitec, *Our Team*, http://www.oxitec.com/who-we-are/our-team/ (last accessed April 26, 2016); *see also* Oxford University, *Oxford Spinout Oxitec Sold to Intrexon Corporation for $160 Million*, (Aug. 10, 2015), http://isis-innovation.com/news/oxford-spinout-oxitec-sold-to-intrexon-corporation-for-160-million/ (last accessed April 26, 2016).

[3] Oxitec has been granted patent EP1624749 ("Dilution of Genetic Traits"), which lists more than fifty species of insects it wishes to genetically modify. European Patent Register, *About This File: EP1624749*, https://register.epo.org/espacenet/application?number=EP04732350 (last accessed April 25, 2016). However, its main patent EP1690247 ("Expression systems for insect pest control") is still disputed by the European Patent Office. European Patent Register, *All Documents: EP1649027*, https://register.epo.org/espacenet/application?number=EP04743590&lng=en&tab=doclist (last accessed April 25, 2016). An earlier patent on the technology filed by Isis Innovation (the company which spun out Oxitec from Oxford University) appears to have lapsed. European Patent Register, *About This File: EP1246927*, https://register.epo.org/espacenet/application?number=EP00979774 (last accessed April 25, 2014).

a. Diamondback Moths

Oxitec first tried and failed to conduct trials for GE diamondback moths in the United Kingdom (U.K.) in 2011 and 2012. In 2011, Oxitec sought to make open releases of GE diamondback moths in the U.K. under "contained use" regulations by claiming that its RIDL®[4] technology is equivalent to "biological containment."[5] These proposed releases were controversial and the company did not receive U.K. permission to proceed. GeneWatch, a U.K. organization that CFS works closely with, documented problems with the proposed releases. These problems have never been resolved. Since then, Oxitec has not submitted a formal application to make open releases of its GE moth into the environment in the U.K. or any country aside from the United States. In effect, by applying for release of its GE diamondback moth in the U.S., Oxitec was shopping for lax oversight.

As a U.K. company, Oxitec is obligated to file a transboundary notification with the Cartagena Protocol on Biosafety to the Convention on Biological Diversity prior to exporting GE insects to the U.S. for open release.[6] This notification must include a prior, existing environmental risk assessment that meets European Union (EU) standards. GeneWatch has documented Oxitec's poor record of complying with environmental regulations, particularly the trans-boundary notification of exports of living GE organisms from the U.K. to other countries. GeneWatch found that important issues have been omitted from the relevant environmental risk assessments (ERAs) for export of Oxitec's GE insects, including GE mosquitoes; in some cases the ERA has not been supplied at all.[7] The U.S., as an observer to the meetings of the Cartagena Protocol, should not aid Oxitec in evading the requirements of the Protocol.

Oxitec requested a permit to release its GE diamondback moths in New York with the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) in May 2014. After APHIS published Oxitec's EA in August 2014 regarding the environmental impacts of its proposed release, it received 287 public comments raising

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[4] RIDL is the name that Oxitec gave to its genetic engineering technology. *See* Oxitec, *Oxitec Science*, http://www.oxitec.com/ridl-science/ (last accessed April 25, 2016).

[5] Advisory Committee on Releases to the Environment (ACRE), *Minutes of the 134th Meeting of ACRE at Nobel House, London, Thursday, 1st December 2011*, ACRE/11/M4 (Dec. 1, 2011) (Attached as Exhibit A); Letter from Mike Rowe, Head of GM Policy & Regulation, Department for Environment, Food and Rural Affairs, to Camila Beech, Regulatory Manager, Oxitec Ltd. (Jan. 24, 2012) (Attached as Exhibit B); Letter from Helen Wallace, Dir., GeneWatch UK, to Rt Hon Caroline Spelman MP, Secretary of State, Department for Environment, Food and Rural Affairs (Jan. 27, 2012) (attached as Exhibit C); Letter from Rt Hon Caroline Spelman, MP, Secretary of State, Department for Environment, Food and Rural Affairs, to Helen Wallace, Dir., GeneWatch UK (Feb. 23, 2012) (Attached as Exhibit D).

[6] Regulation (EC) 1946/2003, of the European Parliament and the Council of 15 July 2003 on transboundary movements of genetically modified organisms 2003 O.J. (L 287) 2, http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32003R1946.

[7] Helen Wallace, *Genetically Modified Mosquitoes: Ongoing Concerns*, Third World Network (TWN) Biotechnology & Biosafety Series 15, at 2 (2013), http://twn.my/title2/biosafety/pdf/bio15.pdf; *see also* GeneWatch UK PR, *Lack of Risk Assessment for GM Mosquito Experiments is Negligent, Says GeneWatch* (Feb. 12, 2014), http://www.genewatch.org/article.shtml?als[cid]=566989&als[itemid]=574224.

numerous significant impacts that APHIS failed to evaluate. Many of the comments recognized that APHIS failed to look at the impacts of animal and human consumption of GE diamondback moths other than a single Oxitec-provided study; APHIS did not consider the potential for long-distance dispersal of GE diamondback moths, which meant that areas outside the bounds of the trial were not assessed; there was no indication that those conducting the release had a plan to ensure that crops exposed to moths would be kept out of the food chain; and residents of New York in surrounding neighborhoods were not informed of the field test and had no opportunity to voice their concerns or give consent. Nonetheless, APHIS allegedly approved Oxitec's release permit application, but failed to notify the public of this approval.[8] There are still many unanswered questions regarding the GE diamondback moth trial in New York, including whether APHIS's permit approval is valid.

b. Pink Bollworms

Unlike the GE diamondback moths, the field trial of GE pink bollworms in the U.S. only assessed the dispersion of the GE insect, not the efficacy of the GE "kill switches." In that trial, open releases of a strain of Oxitec's GE pink bollworm, a cotton pest, were attempted in the southwestern U.S.; however, the strain used only the fluorescent trait, not the "early lethality" trait, and was made sterile using radiation. These experiments were halted, partly because of concerns raised by organic farmers about contamination of their crops by the GE insects.

The GE pink bollworm trials prompted a critical report by the USDA Office of Inspector General. This report argued that APHIS's controls over GE insect research were inadequate and that regulations needed to be strengthened.[9] The report also criticized APHIS's Center for Plant Health Science Technology (CPHST) for spending about $550,000 on developing GE plant pests such as the pink bollworm, the Mediterranean fruit fly, and the Mexican fruit fly (in collaborations with Oxitec) without any formal process for selecting which projects would receive funding. APHIS accepted the report's recommendations, which included clarifying its role, drafting specific GE insect regulations, and making research funding decisions more transparent. Scientists at the Max Planck Institute also found the EIS that APHIS published for the GE pink bollworm trials in 2008 to be "scientifically deficient."[10] The scientists reported that the EIS reversed an earlier, more cautious view published by APHIS in 2001, yet failed to provide the substantial body of evidence required to back up its assertions. Alarmingly, this

[8] APHIS, National Environmental Policy Act Decision and Finding of No Significant Impact for Permit Application 13-297-102r Field Release of Genetically Engineered Diamondback Moth Strains OX4319L-PXY, OX4319N-Pxy, and OX4767A-Pxy (2014), http://www.aphis.usda.gov/brs/aphisdocs/13_297102r_fonsi.pdf.

[9] USDA Office of Inspector General, Controls over Genetically Engineered Animal and Insect Research (May 31, 2011), http://www.usda.gov/oig/webdocs/50601-16-TE.pdf.

[10] Reeves et al., *Scientific Standards and the Regulation of Genetically Modified Insects*. PLoS Neglected Tropical Diseases, 6(1), at 1502 (Jan. 31, 2012), http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0001502.

"scientifically deficient" 2008 EIS and later APHIS reports made under the framework criticized by the USDA Office of Inspector General were cited by APHIS in its diamondback moth EA.[11]

c. *Aedes aegypti* Mosquitoes

Oxitec now seeks to release genetically engineered *Aedes aegypti* mosquitoes in Key Haven, Monroe County, Florida, without a full environmental impact statement being developed. The FDA published its preliminary FONSI on March 16, 2016 for public comment, after receiving Oxitec's proposed investigational field trial of GE *Aedes aegypti* mosquitoes under an INAD exemption (21 C.F.R. § 511.1(b)). The preliminary FONSI is based entirely on Oxitec's draft EA, other data submitted by Oxitec, and an FDA and Center for Disease Control (CDC) inspection of the Hatching and Rearing Unit (HRU) and field test site.[12]

Similar to Oxitec's GE diamondback moths, Oxitec has genetically engineered *Aedes aegypti* mosquitoes to express conditional lethality and a fluorescent marker. Oxitec creates its GE mosquito (OX513A) by inserting two genes into the egg of an *Aedes aegypti* mosquito. One gene, a fluorescent marker, helps distinguish the GE mosquito from natural ones. The other gene forces the GE mosquito to rely on the antibiotic tetracycline, which Oxitec inserts into its food in the lab. When Oxitec releases GE mosquitoes into the wild, the mosquito is unable to survive without the presence of the antibiotic. Within days, the males and any offspring they produce will allegedly die off, thereby reducing the population of wild *Aedes aegypti* mosquitoes. Oxitec's mosquito control program involves the repeated release of GE male *Aedes aegypti* to mate with wild female *Aedes aegypti*. Oxitec has already released its GE *Aedes aegypti* mosquitoes in countries that do not require strict environmental analysis such as Brazil, Panama, Malaysia, and the Cayman Islands.[13]

However, GE mosquitoes could have unforeseen consequences for environmental, human and animal health, and they demand proper regulatory oversight before any clinical investigation or release into the wild. Potential concerns include: decline in *Aedes aegypti* creating an ecological niche which other, possibly more harmful pests could fill, including other invasive mosquito species which carry dengue and other diseases;[14] greatly reducing *Aedes aegypti*

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[11] APHIS, Proposal to Permit the Field Release of Genetically Engineered Diamond Back Moth in New York Environmental Assessment, at 48 (October 2014), http://www.aphis.usda.gov/brs/aphisdocs/13_297102r_fonsi.pdf.

[12] FDA, FONSI in Support of an Investigational Field Trial of OX513A *Aedes aegypti* Mosquitoes, at 2 (March 2016), http://www.fda.gov/downloads/AnimalVeterinary/DevelopmentApprovalProcess/GeneticEngineering/GeneticallyEngineeredAnimals/UCM487379.pdf [hereinafter, FONSI].

[13] Notably, Oxitec did not comply with the Cartagena Protocol requirements (and the EU requirements) for Environmental Assessment before shipping their GE mosquitoes to Panama. *See* Email from Unknown, Genetic Modification Team, Department for Environment, Food and Rural Affairs, to Helen Wallace, Dir., GeneWatch UK (Sept. 29, 2014) (Attached as Exhibit E); *see also* Reeves, *supra* note 10, at 1; *see also* Friends of the Earth (FOE), *Genetically Engineered Mosquitoes in the U.S.*, at 1 (2012), http://libcloud.s3.amazonaws.com/93/df/1/959/5/Issue_brief_GE_mosquitoes_in_U.S.pdf.

[14] FOE, *supra* note 13, at 3.

populations could affect other animals that feed on larval or adult mosquitoes;[15] release of female GE mosquitoes, which unlike their male counterparts, bite humans; and the possibility of the dengue virus responding to GE mosquitoes by evolving and becoming more virulent, thus putting human health at greater risk, even if GE mosquitoes help to reduce the population of *Aedes aegypti*.[16]

The novel and unique nature of the traits that Oxitec now seeks to test make it particularly important for FDA to conduct a thorough NEPA analysis and expose Oxitec's proposal to detailed independent scrutiny. Despite the unprecedented nature of its proposed action, FDA is attempting to avoid undertaking the legally-required, rigorous, and overarching analysis of the GE *Aedes aegypti*, or the foreseeable consequences of its release.

II. REGULATORY FRAMEWORK

As an initial matter, FDA does not have formal regulations specific to GE insects and animals. In 2002, the National Academy of Sciences published a report on GE animals stating that aquatic organisms and insects present the greatest environmental concerns because their mobility poses serious containment problems, and because they easily can become feral and compete with indigenous populations.[17] The report expressed concerns about gaps in regulation. In 2004, the Pew Initiative on Food and Biotechnology published a report on gaps in the regulatory system for GE insects in the U.S., and a report of a workshop on the issues.[18] A central finding of the report was that there are gaps in the current regulatory framework to review the many issues raised by the potential introduction of GE insects into wild populations. There is no specific regulation on the release of GE insects, no law that clearly covers all the risks and all of the types of GE insects and no single regulatory body: USDA, FDA, and the Environmental Protection Agency (EPA) could all play a role. Thus, the current but outdated U.S. regulatory system lacks clear oversight of the use of biotechnology, particularly when it is used to eliminate insect vectors of animal and human diseases.

In the absence of a coherent regulatory framework on how to assess the risks of open releases of GE insects in the U.S., it is worth noting that the European Food Safety Authority

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[15] Michael Specter, *The Mosquito Solution*, The New Yorker, at 38, 44 (July 2012) ("the biggest question raised by the creation of [GE mosquitoes] is who will regulate it and how.") http://www.newyorker.com/magazine/2012/07/09/the-mosquito-solution.

[16] FOE, *supra* note 13, at 3 (noting that the connection between the virulence and spread of disease with mosquito and population levels involve complex systems difficult to predict in advance, particularly because researchers do not know the correlation between *Aedes aegypti* population levels and dengue infection in humans).

[17] National Academy of Science, *Animal Biotechnology: Science Based Concerns* (2002), http://www.nap.edu/catalog/10418/animal-biotechnology-science-based-concerns.

[18] Pew Initiative on Food and Biotechnology, *Bugs in the System? Issues in the Science and Regulation of Genetically Modified Insects* (Jan. 22 2004), http://www.pewtrusts.org/en/research-and-analysis/reports/2004/01/22/bugs-in-the-system-issues-in-the-science-and-regulation-of-genetically-modified-insects.

(EFSA) has published guidance for environmental risk assessment under the EU's Deliberate Release Directive for genetically modified organisms (GMOs), although this does not yet cover the important area of food safety assessment. The EFSA Guidance outlines the evidence that Oxitec would need to provide for its GE insects to be placed on the EU market.[19] The EFSA Guidance provides details on the following specific areas of risk for GE insects:

- Persistence and invasiveness of GE insects, including vertical gene transfer (VGT);
- Horizontal gene transfer;
- Pathogens, infections and diseases;
- Interactions of GE insects with target organisms;
- Interactions of GE insects with non-target organisms (NTOs);
- Environmental impacts of the specific techniques used for the management of GE insects;
- Impacts of GE insects on human and animal health.[20]

As mentioned above, although the U.S. is not a party to the Cartagena Protocol on Biosafety to the Convention on Biological Diversity, Oxitec—as a U.K. company—is still obliged to make a trans-boundary notification compliant with the Protocol under Regulation 1946/2003/EC prior to exporting GE *Aedes aegypti* mosquito eggs to the U.S. for open release. This notification must include a prior, existing environmental risk assessment that meets EU standards. Thus the EFSA Guidance is of more than academic interest in the context of the current application, and obligates FDA to be sure that its EA meets the EFSA standards.

a. Federal Food, Drug, and Cosmetic Act (FFDCA)

The FDA recently made it apparent that it intends to exert its jurisdiction to regulate GE insects as new animal drugs (NAD); however, FDA's authority is improper and ultra vires to the FFDCA. The FFDCA's definition of "drug" contains two prongs which could potentially be applied to GE insects: (1) "articles intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease in man or other animals;" and (2) "articles (other than food) intended to affect the structure or any function of the body of man or other animals."[21] The term "new animal drug," on the other hand, does not readily apply to GE animals or insects that pose harm to humans. "New animal drug" is defined as "any drug that is intended for use for animals other than man."[22] In looking at the statute and regulations, FDA's NAD authority is ill-equipped to deal with the unique characteristics of genetically engineering insects to prevent diseases in humans.

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[19] European Food Safety Authority (EFSA), *Guidance on the Environmental Risk Assessment of Genetically Modified Animals*, EFSA Journal 2013, 11(5):3200 (May 23, 2013), http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/3200.pdf [hereinafter, EFSA Guidance].

[20] *Id.* at 73-107.

[21] 21 U.S.C. § 321(g)(1).

[22] *Id*. § 321(v)(1)-(2).

i. Ultra Vires

FDA's decision to analyze Oxitec's GE mosquito application is improper because FDA lacks statutory authority to regulate GE insects as NADs under the FFDCA. The FFDCA does not explicitly grant FDA authority to regulate GE animals or insects. Indeed, Congress never intended or provided a means for FDA to regulate twenty-first century GE animals using its 1938 authority over veterinary animal drugs. To the contrary, GE animals and insects present enormously different risks and impacts than drugs, requiring different expertise, analyses, and regulation than were contemplated when Congress enacted the FFDCA. Nevertheless, FDA issued Guidance interpreting the definition of "new animal drug" under the FFDCA to include GE animals, asserting authority over GE insects under the new animal drug provisions of the FFDCA, and purportedly outlining the steps that FDA will follow when considering application for GE insects.[23] FDA's potential approval of Oxitec's INAD and the issuance of its GE Animal Guidance represent an unlawful effort to extend FDA's regulatory reach far beyond the statutory mandates of the FFDCA. FDA's assertion of jurisdiction under the GE Animal Guidance and its analysis of the Oxitec INAD are ultra vires and contrary to law.

In its Guidance, FDA defines "[GE] animals" as those modified by recombinant DNA (rDNA) techniques and technology, including both animals with heritable rDNA and animals with non-heritable rDNA constructs.[24] FDA notes that "the rDNA construct in a GE animal that is intended to affect the structure or function of the body of the GE animal, regardless of the intended use of products that may be produced by the GE animal," meets the FFDCA's definition of a "drug" and thus FDA intends to assert its regulatory authority over such GE animals.[25]

However, in its Guidance, FDA noted that it does not intend to regulate GE animals that meet the definition of a veterinary biologic and that are regulated by APHIS.[26] Specifically, FDA stated that it does not intend to enforce INAD and NADA requirements for GE insects being developed for animal health protection, and that are under APHIS oversight.[27] Thus, APHIS regulates Oxitec's GE diamondback moth, which is characterized as a plant pest, but FDA regulates Oxitec's GE mosquito, which is characterized as a new animal drug. This is problematic because the genetically engineered traits in both insects are essentially identical—

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[23] FDA, Guidance for Industry: Regulation of Genetically Engineered Animals Containing Heritable Recombinant DNA Constructs, at 3 (May 17, 2011), http://www.fda.gov/downloads/animalveterinary/guidancecomplianceenforcement/guidanceforindustry/ucm113903.pdf [hereinafter, FDA Guidance or Guidance].

[24] *Id.* (FDA's Guidance pertains only to GE animals containing heritable rDNA constructs, and not non-heritable rDNA constructs (e.g., those constructs intended to be used as gene therapy)).

[25] *Id.*at 6.

[26] *Id*. at 6 n.1.

[27] *Id*. at 7.

both contain a lethality gene that kills the insect and a fluorescent marker gene that identifies the insect—yet the regulatory process for each insect is entirely different. The FDA's attempt to fit insects that are genetically modified to prevent diseases in humans under "new animal drug" provisions is ultra vires and not intended by the FFDCA, and the regulatory framework is not adequate to analyze the unique and novel characteristics of GE mosquitoes.

ii. NADA Approval Process

A NADA is an extensive document which must satisfy a number of requirements for proper application and approval by FDA.[28] FDA's Guidance summarizes how developers should address certain NADA requirements submitted for GE animals.[29] In its Guidance, FDA encourages consultation as early as possible in the GE animal development process, even as an early part of the INAD process.[30] For a drug to receive FDA approval, among other requirements, sponsors must comply with NEPA[31] and must prove the safety and effectiveness of the drug under the substantial evidence standard.[32]

Some basic requirements of a NADA include: providing identifying information, such as name and address of the applicant, date, trade name and chemical name;[33] a table of contents and summary of the data including the chemistry and structural formula of the drug; scientific rationale and purpose of the drug; highlights of lab and clinical studies; and conclusions about the major points of effectiveness and safety.[34] "After completion of a NADA, FDA will post a summary of the information in the NADA file, including information used to assess safety and in support of the claims made by the sponsor."[35]

iii. INAD Process

A NAD is generally deemed unsafe unless FDA has approved a NADA for a particular use, or if it is for investigational use and conforms to the terms of an INAD exemption as described at 21 U.S.C. Section 360b(j), codified at 21 C.F.R. Section 511.1.[36] NADs are exempt

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[28] *See* 21 U.S.C. § 360b; *see also* 21 C.F.R. § 514.1.

[29] FDA Guidance, *supra* note 23, at 13.

[30] *Id.* at 14.

[31] 21 C.F.R. § 514.1(b)(14).

[32] *Id*. § 514.1(b)(8); 21 U.S.C. § 360b(d)(1)(E) (requiring "substantial evidence that the drug will have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in the proposed labeling thereof").

[33] 21 C.F.R. § 514.1(b)(1).

[34] *Id*. § 514.1(b)(2).

[35] FDA Guidance, *supra* note 23, at 13 (citing 21 C.F.R. § 514.11(e)).

[36] 21 U.S.C. § 360b(a)(3).

from NADA requirements for (a) "tests in vitro and in laboratory research animals;" and (b) "clinical investigation in animals."[37] FDA notes in its Guidance that INAD requirements apply to investigational GE insects, and that "the development of GE [insects] constitutes clinical investigation because it involves studying the effectiveness of the drug in the target species and the effects of the rDNA construct, including those of its expression product(s), on the animal containing it."[38] However, the release of GE insects into the environment—in particular the breadth of Oxitec's proposed release of its GE mosquitoes three times a week for over twenty-two months—is beyond the scope of a "clinical investigation" and carries significant risks such as escape or contamination.

Generally, an INAD mandates specific labeling and record-keeping duties, the submission of records regarding animal disposition, and of the conditions under which the animals used for clinical investigations could enter the food supply.[39] Before shipment of a NAD for clinical tests, the NAD sponsor must submit to FDA a "Notice of Claimed Investigational Exemption for a New Animal Drug" (INAD Notice), which specifies other detailed information.[40] Further requirements of an INAD include using qualified investigators, monitoring of investigations, and prompt reporting to FDA of any findings that may suggest significant hazards pertinent to the safety of the drug.[41] Lastly, INAD actions are "federal actions" which require NEPA compliance.[42]

iv. Prior FDA Approvals of GE Animals to Address Human Diseases

The other GE animals that have been approved by FDA to address human disease have required a two phase approval process. First, the FDA approves the GE construct in the animal, i.e., goat or chicken producing the human drug in its milk or eggs.[43] Second, the FDA tests the drug to prove its efficacy and safety. The FDA needs to require a similar two step case for this GE mosquito drug. First, the FDA must demonstrate its safety in the environment, and second, the FDA must demonstrate that it is an effective way to treat diseases like dengue or zika.

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[37] 21 C.F.R. § 511.1.

[38] FDA Guidance, *supra* note 23, at 9.

[39] *See* 21 C.F.R. § 511.1(b); FDA Guidance, *supra* note 23, at 9.

[40] 21 C.F.R. § 511.1(b)(4).

[41] 21 C.F.R. § 511.1(b).

[42] *Id*. § 511.1(b)(10); FDA Guidance, *supra* note 23, at 12.

[43] FDA, News & Events, *FDA Approves Orphan Drug Atryn to Treat Rare Clotting Disorder* (Feb. 6, 2009), http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm109074.htm; *see also* FDA, News & Events, *FDA approves first drug to treat a rare enzyme disorder in adult patients* (Dec. 8, 2015), http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm476013.htm.

In its media reports, Oxitec emphasizes that its technology will reduce disease transmitted by viruses carried by the mosquitoes.[44] Thus far, its research does not demonstrate that. If this current test is really being conducted to demonstrate that GE mosquitoes could reduce the transmission of diseases like dengue or zika, then the FDA should require demonstration of the efficacy of this process. The FDA should require a properly designed trial to test the efficacy of this mosquito to prevent viral diseases actually found in Florida such as West Nile virus[45] which can be carried by *Aedes aegypti* (as zika and dengue are not endemic). The efficacy trials should be able to separate out the effects of the GE mosquito from the effects of existing spraying, which of course, would continue to kill the other species of mosquito. Its effectiveness should also be compared to other strategies such as Wolbaccia[46] and vaccines. Indeed, the progress of vaccines for these diseases undercuts calls for rapid action. A vaccine that addresses most serotypes of dengue is approved for use in Mexico, Brazil, the Philippines, and El Salvador.[47] Oxitec should be required to provide a plausible mechanism through which its proposed releases might actually reduce the risk of such viral diseases in the Florida Keys; otherwise the proposed experiment is at best pointless.

b. National Environmental Policy Act (NEPA)

NEPA is "our basic national charter for protection of the environment."[48] NEPA emphasizes the importance of comprehensive environmental analysis to ensure that federal agencies make informed decisions, and requires federal agencies to assess the environmental consequences of their actions before those actions are undertaken. NEPA "ensures that the agency . . . will have available, *and will carefully consider*, detailed information concerning significant environmental impacts; it also guarantees that the relevant information will be made available to the larger [public] audience."[49]

NEPA also established the Council on Environmental Quality (CEQ).[50] The regulations subsequently promulgated by CEQ[51] implement the directives and purpose of NEPA, and "[t]he

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[44] Press Release, *Oxitec and Dengue Fever*, http://www.oxitec.com/news-and-views/topic-pages-safety-and-sustainability/ridl-sit-and-dengue-fever/ (last accessed April 25, 2016).

[45] Turell et al., *An Update on the Potential of North American Mosquitoes (Diptera: Culicidae) to Transmit West Nile Virus*, J Med Entomol, 42(1): 57-62 (Jan. 2005), http://www.ncbi.nlm.nih.gov/pubmed/15691009.

[46] Robert Preidt, Bacteria Experiment May Point Way to Slow Zika's Spread:
Infecting Mosquitoes Led to Lower, Inactive Levels of Virus in their Bodies, Saliva, Health Day (May 4, 2016), https://www.nlm.nih.gov/medlineplus/news/fullstory_158661.html.

[47] Andrew Ward, *Sanofi to Launch Dengue Mass Vaccination*, Financial Times (Apr. 4, 2016), http://www.ft.com/cms/s/0/89b37b20-f865-11e5-96db-fc683b5e52db.html.

[48] 40 C.F.R. § 1500.1(a).

[49] *Robertson v. Methow Valley Citizens Council,* 490 U.S. 332, 349 (1989) (emphasis added).

[50] *See* 42 U.S.C. §§ 4321, 4344.

[51] 40 C.F.R. §§ 1500-1508.

provisions of [NEPA] and [CEQ] regulations must be read together as a whole in order to comply with the spirit and letter of the law."[52] CEQ's regulations are applicable to and binding on all federal agencies.[53] Among other requirements, CEQ's regulations mandate that federal agencies address all "reasonably foreseeable" environmental impacts of their proposed programs, projects, and regulations.[54] This must include analyses of direct, indirect, and cumulative effects.[55] The assessment must be a "hard look" at the potential environmental impacts of its action.[56]

NEPA requires federal agencies, including FDA, to prepare an EIS for all "major Federal actions significantly affecting the quality of the human environment."[57] In other words, if the action may significantly affect the environment, FDA must prepare an EIS.[58] As a preliminary step, an agency may prepare an EA to determine whether the environmental impact of the proposed action is significant enough to warrant an EIS.[59] "An environmental assessment is a 'concise public document' that '[b]riefly provide[s] sufficient evidence and analysis for determining whether to prepare an [EIS] or a finding of no significant impact.'"[60] If an EA establishes that the agency's action may have a significant effect upon the environment, the agency must prepare an EIS.[61] An EIS serves different purposes from the EA already prepared by FDA.[62] An EA aims simply to identify and assess the significance of potential impacts on the environment. An EIS, on the other hand, balances "different kinds of positive and negative environmental effects, one against the other" and "weighs negative environmental impacts against a project's other objectives."[63] "Preparation of an EIS thus ensures that decision-makers

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[52] *Id*. § 1500.3.

[53] *Id*. §§ 1500.3, 1507.1; *see, e.g., Hodges v. Abraham*, 300 F.3d 432, 438 (4th Cir. 2002).

[54] *See* 40 C.F.R. §§ 1502.4, 1508.8, 1508.18, 1508.25.

[55] *See id*. §§ 1508.8, 1508.9, 1508.13, 1508.18.

[56] *Blue Mountains Biodiversity v. Blackwood*, 161 F.3d 1208, 1211 (9th Cir. 1998); *Nat'l Parks & Conservation Ass'n v. Babbitt*, 241 F.3d 722, 731 (9th Cir. 2001) (quoting 40 C.F.R. § 1508.27).

[57] 42 U.S.C. § 4332(2)(C).

[58] *Steamboaters v. FERC*, 759 F.2d 1382, 1392 (9th Cir. 1985); *Idaho Sporting Cong. v. Thomas*, 137 F. 3d 1146, 1150 (9th Cir. 1998) (citation omitted).

[59] *See* 40 C.F.R. § 1508.9.

[60] *Id*. § 1508.9(a); *Anderson v. Evans,* 371 F. 3d 475, 488 (9th Cir. 2004).

[61] *Sierra Club v. Bosworth*, 510 F. 3d 1016, 1018 (9th Cir. 2007) (internal quotations and citations omitted); *see also* 40 C.F.R. § 1508.3.

[62] *See Anderson v. Evans*, 314 F.3d 1006, 1022 (9th Cir. 2002).

[63] *Sierra Club v. Marsh*, 769 F. 2d 868, 875 (1st Cir. 1985).

know that there is a risk of significant environmental impact and take that impact into consideration."[64] FDA's decisions must be "complete, reasoned, and adequately explained."[65]

The CEQ regulations define "significance" as requiring consideration of both context and intensity.[66] Context means that the significance of an action must be analyzed in several contexts such as society as a whole (human, national); the affected region; the affected interests; and the locality.[67] Intensity refers to the severity of the impact, and FDA should consider the following: (1) impacts that may be both beneficial and adverse; (2) the degree to which the proposed action affects public health or safety; (3) unique characteristics of the geographic area such as proximity to historic or cultural resources, park lands, prime farmlands, wetlands, wild and scenic rivers, or ecologically critical areas; (4) the degree to which the effects on the quality of the human environment are likely to be highly controversial; (5) the degree to which the possible effects on the human environment are highly uncertain or involve unique or unknown risks; (6) the degree to which the action may establish a precedent for future actions with significant effects or represents a decision in principle about future consideration; (7) whether the action is related to other actions with individually insignificant but cumulatively significant impacts; (8) the degree to which the action may affect places listed in the National Register, Historic Places, or may cause loss or destruction of scientific, cultural, or historic resources; (9) the degree to which the action may adversely affect an endangered or threatened species or its habitat; and (10) whether the action threatens a violation of federal, state, or local law.[68]

Additionally, CEQ regulations require the preparation of a programmatic EIS "for broad Federal actions such as the adoption of new agency programs or regulations."[69] Under the CEQ regulations, a programmatic EIS is appropriate for a program that exists in fact, but is not necessarily declared by the agency.[70] A programmatic EIS should be "relevant to policy and [] timed to coincide with meaningful points in agency planning and decisionmaking," and "shall be prepared on such programs and shall be available before the program has reached a stage of investment or commitment to implementation likely to determine subsequent development or restrict later alternatives."[71]

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[64] *Anderson*, 314 F.3d at 1022.

[65] *Northwest Coal. for Alts. to Pesticides v. EPA*, 544 F.3d 1043, 1052 n.7 (9th Cir. 2008).

[66] 40 C.F.R. § 1508.27; *see also Marsh v. Oregon Natural Resources Council*, 490 U.S. 360, 374 n.20 (1989).

[67] *Id*.

[68] *Id*.

[69] 40 C.F.R. § 1502.4(b); *see also* 40 C.F.R. § 1508.18(b)(4) (definition of major federal action includes "[a]doption of programs, such as a group of concerted actions to implement a specific policy or plan").

[70] *See id*. § 1508.23 (defining "proposal" to include that a "proposal may exist in fact as well as by agency declaration that one exists").

[71] 40 C.F.R. § 1502.4.

Here, FDA has concluded that its proposed action will not significantly affect the environment, and has thus prepared only an EA. Moreover, although the FDA Guidance establishes for the first time a regulatory approval framework for all GE animals, FDA did not prepare a programmatic EIS or any other NEPA review for the expansive framework it describes in the GE Animal Guidance and the establishment of a GE animal approval process under the FFDCA.

c. Migratory Bird Treaty Act (MBTA)

The MBTA implements the obligations of the U.S. under several international treaties and conventions for the protection of migratory birds.[72] The MBTA mandates that proposed projects must avoid the take of migratory birds entirely and must minimize the loss, destruction, and degradation of migratory bird habitat.[73] The vast majority of U.S. native birds are protected under the MBTA, even those that do not participate in international migrations.[74] Under the MBTA, "[n]o person may take, possess, import, export, transport, sell, purchase, barter, or offer for sale, purchase, or barter, any migratory bird, or the parts, nests, or eggs of such bird except as may be permitted under the terms of a valid permit."[75]

III. INADEQUACIES IN FDA'S EA THAT REQUIRE FDA TO PREPARE A FORMAL EIS PURSUANT TO NEPA

Oxitec's proposed INAD involves reasonably foreseeable and potentially significant impacts based on factors of context and intensity and, therefore, FDA must analyze those impacts in a comprehensive EIS.[76] The project is significant in terms of context because millions of GE mosquitoes will be released in Key Haven, Monroe County, Florida, which has the potential to disrupt the ecology in the region as well as present unique dangers to local residents.[77] Additionally, as explained below, should the lethality trait fail or GE mosquitoes survive, there is potential for GE mosquitoes to move and survive beyond the test site, which could have significant impacts beyond the "effected" region.[78] Oxitec intends to use GE *Aedes aegypti* mosquitoes to suppress wild populations of *Aedes aegypti* around the world, which poses

[72] 16 U.S.C. § 701.

[73] *Id.* § 701-12.

[74] *See* 50 C.F.R. § 10.13.

[75] *Id.* § 21.11.

[76] 40 C.F.R. § 1508. 27(a)-(b).

[77] FDA, Draft Environmental Assessment (EA) for Investigational Use of *Aedes aegypti* OX513A, at 25 (Feb. 2016), http://www.fda.gov/downloads/AnimalVeterinary/DevelopmentApprovalProcess/GeneticEngineering/GeneticallyEngineeredAnimals/UCM487377.pdf [hereinafter EA] (Oxitec intends to release adult male mosquitoes up to three times per week over a time period of up to twenty-two months).

[78] *Id*. at 97, 99; *see* 40 C.F.R. § 1508.27(a).

significant effects for society as a whole; Oxitec has already released its GE *Aedes aegypti* mosquitoes in Brazil, Panama, Malaysia, and the Cayman Islands.[79]

The INAD is also significant in terms of intensity. The impacts that releasing millions of GE mosquitoes will have on public health, safety, and the environment are unique, uncertain, and unknown, and FDA did not adequately analyze the danger posed by oral ingestion, allergenicity, or disbursement of OX531A beyond the trial site.[80] As the EA mentions, the geography contains unique characteristics in close proximity to ecologically critical areas, such as the National Key Deer Refuge and the Great White Heron Refuge, yet FDA assumes the effects are not significant based on the erroneous belief that GE mosquitoes will not survive beyond the test site.[81] Moreover, FDA has identified but not adequately addressed harms to the forty-three listed endangered or threatened species in Monroe County.[82] It has also become apparent that the Florida Keys Mosquito Control District (FKMCD) will not stop the use of existing vector control methods,[83] such as larvicides and adulticides; however, FDA has not evaluated the cumulative effects of releasing millions of GE mosquitoes while using current methods of vector control. If, on the other hand, FKMCD does cease using current vector control methods, FDA would still need to evaluate the cumulative effects that halting other forms of vector control will have on humans or the environment.[84] Lastly, this is the first time in the United States that a company proposes to release genetically engineered insects for the purpose of preventing diseases and viruses in humans, and the FDA's approval of this proposal as an INAD is highly controversial and certain to establish a precedent for future actions with significant effects.[85] Thus, numerous factors of intensity are met, which make this project significant, and the FDA must analyze the impacts of Oxitec's INAD in a comprehensive EIS.

a. FDA Failed to Prepare a Programmatic EIS for its GE Animal Program

FDA has created a GE animal program that is a major federal action, without preparing or engaging in a programmatic or other analysis of the impacts of that program as required by NEPA. Instead, FDA completed an extremely limited EA and FONSI for the approval of Oxitec's GE mosquito INAD, which together fail to discuss or adequately evaluate myriad scientific questions regarding the risk of significant and irreversible environmental and ecological harms related to the release of millions of GE mosquitoes. An EIS is particularly

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[79] FOE, *supra* note 13, at 1; *see* 40 C.F.R. § 1508.27(a).

[80] EA, *supra* note 77, at 76, 85, 96-98; *see* 40 C.F.R. § 1508.27(2), (5).

[81] *Id*. at 43-44; *see* 40 C.F.R. § 1508.27(3).

[82] *Id*. at 43; *see* 40 C.F.R. § 1508.27(9).

[83] Email from Michael Doyle, Florida Keys Mosquito Control District, to Barry Way, Executive Director, Florida Keys Environmental Coalition (May 10, 2016) (Attached as Exhibit F).

[84] *See* EA, *supra* note 77, at 17 (stating that FKMCD will only continue using its existing control measures if the project is not approved); *see* 40 C.F.R. § 1508.27(7).

[85] 40 C.F.R. § 1508.27(4), (6).

crucial here, when FDA is acting and purporting to establish and apply a new framework regarding novel GE organisms. FDA's continuing failure to prepare a programmatic EIS (or any other NEPA analysis) for its GE animal approval program, as purportedly established by its Guidance, and as now concretely applied to the potential approval of Oxitec's GE mosquitoes, violates NEPA.

b. The EA Fails to Consider Significant Adverse Effects on the Biological, Physical, and Human Environment as Required by NEPA

There are a number of fundamental flaws with FDA's assessment of the impacts of Oxitec's proposed INAD. These flaws, as discussed below, include: (1) the large numbers of GE adult males required to swamp the wild population pose a risk of swallowing them to farm workers and passersby, as well as wildlife, and may also cause wild-type adult *Aedes aegypti* mosquitoes to disperse to surrounding areas; (2) the use of tetracycline as a chemical switch for the genetic killing mechanism is risky because contamination with tetracycline and related antibiotics is widespread in the environment, meaning the killing mechanism may be inactivated; (3) the use of tetracycline to breed the GE *Aedes aegypti* mosquitoes in the lab is likely to facilitate the spread of antibiotic resistance via gut bacteria, in breach of FDA's Guidance on preventing antibiotic resistance; and (4) resistance to the genetic killing mechanism is likely to evolve over time, facilitating greater off-site dispersal. Thus, these GE mosquitoes may no longer require a source of tetracycline to survive. These impacts are potentially significant and reasonably foreseeable, and therefore must be analyzed in a comprehensive EIS. The disease transmission properties of the mosquito must also be analyzed, along with whether using a different strain of *Aedes aegypti* than that found in the Florida Keys affects the potential of disease transmission. It is possible that the Oxitec strain could transmit some viruses more effectively than the strain already present at the site.

i. Significant and foreseeable adverse effects of tTAV and DsRed2

1. Oral ingestion of GE *Aedes aegypti* mosquitoes

Release ratios of GE to wild-type *Aedes aegypti* males are currently unknown but can be expected to be of the order of ten to one or higher. The aim is to replace wild-type offspring with GE offspring that are genetically engineered so that the (majority of the) females die at the larval stage. The dead larvae will contain the DsRed (fluorescent) and tTAV (early lethality) GE traits. They will be consumed by all species that normally consume *Aedes aegypti* mosquito larvae; yet no safety data is provided in the EA for consumption of GE *Aedes aegypti* mosquitoes. Instead, the EA relies on a statement claiming that the DsRed and tTAV proteins expressed in Oxitec's GE mosquitoes are safe to eat (with no data provided), because nucleic acids are generally recognized as safe.[86] The EA also cites one published study by Oxitec, in which OX513A larvae were fed to larvae of two different species of mosquito, *Toxorhynchites* (*T. splendens and T. amboinensis*).[87] The FDA has not adequately analyzed the impacts of oral

[86] EA, *supra* note 77, at 75.

[87] *Id.* at 81 (citing Nordin et al., *Oral Ingestion of Transgenic RIDL Ae. aegypti Larvae Has No Negative Effect on Two Predator Toxorhynchites Species*, PloS One, 8(3): e58805 (2013)).

ingestion of GE mosquitoes and the data provided falls far short of the data or precautions needed.

Although a reference has been provided for toxicity testing of the red fluorescent marker, DsRed2, no evidence exists regarding the safety of the RIDL genetic mechanism and the high level expression of tTA that kills the insects at the larval stage. The mechanism of action is not fully understood and no safety data appears to be available. There is some evidence that enhanced tTA expression can have adverse effects (loss of neurons affecting cognitive behavior) in transgenic mice.[88] Other mouse studies have detected adverse effects on the lung.[89] Considerably more data, based on specific feeding trials in relevant species, are needed to establish that consumption of GE *Aedes aegypti* mosquito adults or larvae is not harmful to humans or wildlife.

Failure to conduct human safety tests prior to conducting open release experiments could damage human health far more widely than in the local area of the trial, due to frequent difficulties in tracing the source of contamination incidents. Journalists have reported that in Brazil, where GE mosquito trials are taking place, "it's impossible to talk during the liberation sessions without accidentally swallowing a few" due to the very large numbers of GE mosquitoes being released to try to swamp the wild population.[90] Therefore, the risk posed to workers or passers-by of swallowing adult GE mosquitoes is legitimate and needs to be assessed. It is of particular concern that staff will be required to wear masks during contained production, but members of the public may be exposed to large numbers of GE mosquitoes during open releases without any protective measures. For example, during Oxitec's experiments with GE mosquitoes in the Cayman Islands, local residents complained about the nuisance caused by the very large number of GE mosquitoes released, which was far higher than the normal expected population density of the wild species.[91]

In determining whether a project is significant, FDA must analyze the context of the project in the region and locality.[92] It is clear that releasing millions of genetically modified

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[88] Han et al., Strain Background Influences Neurotoxicity and Behavioral Abnormalities in Mice Expressing the TetracyclineTtransactivator, J Neurosci, 32(31):10574-10586 (Aug. 2012), http://www.ncbi.nlm.nih.gov/pubmed/22855807.

[89] Sisson et al., *Expression of the Reverse Tetracycline-Transactivator Gene Causes Emphysema-Like Changes in Mice*, American Journal of Respiratory Cell and Molecular Biology, 34(5), 552 –560 (May 2006), http://www.ncbi.nlm.nih.gov/pubmed/16415250; Whitsett & Perl, *Conditional Control of Gene Expression in the Respiratory Epithelium: A Cautionary Note*., American Journal of Respiratory Cell and Molecular Biology, 34(5):519–520 (May 2006), http://www.atsjournals.org/doi/full/10.1165/rcmb.F310.

[90] Vincent Bevins, *Dengue, Where Is Thy Sting?*, Los Angeles Times (Nov. 1, 2012), http://articles.latimes.com/2012/nov/01/world/la-fg-brazil-mutant-mosquitoes-20121102.

[91] Harris et al., *Successful Suppression of a Field Mosquito Population by Sustained Release of Engineered Male Mosquitoes*, Nat. Biotech., 30(9), 828–830 (Sept. 10, 2012), http://www.nature.com/nbt/journal/v30/n9/full/nbt.2350.html.

[92] 40 C.F.R § 1508.27(a).

mosquitoes in Key Haven, Monroe County, Florida, may have potentially significant impacts on the local community that must be analyzed. Releasing large numbers of mosquitoes three times a week for twenty-two months might cause people who accidentally ingest the mosquitoes to develop allergies to the proteins produced by the new genetic constructs in the mosquitoes. Moreover, the degree to which the proposed action affects public health or safety due to oral ingestion is still uncertain and involves unique and unknown risks that the FDA must thoroughly analyze.[93]

2. Allergenicity of GE *Aedes aegypti* mosquitoes

FDA must prepare a full EIS that evaluates the potential allergenicity that could be caused by a GE *Aedes aegypti* mosquito bite. The EA says "levels of exposure to tTAV (and DeRed2) via mosquito bite will be extremely low, if present at all, and unlikely to initiate an immune response."[94] This is based on the assumption that there will be few GE female mosquitoes and not enough to cause humans to develop allergic reactions from what is likely to be a not very significant allergen. However, the company did not do any human trials to examine whether this is demonstrated in actual bites on humans. The EA has entirely failed to analyze the potential allergenicity caused by a GE mosquito bite by relying on unproven assumptions, and it therefore must be analyzed in a comprehensive EIS.

The probable presence of significant numbers of transgenic females in the environment requires that a more complex series of potential hazards would need to be considered in a credible EIS than would be necessary if the presence of females in the environment was highly improbable. For example, the assumption that the transgenic tTA protein is not expected to be secreted into the salivary fluid (which is injected as part of a normal bite) because it does not have a secretory signal peptide sequence is questionable based on the fact that: (1) not all proteins found in the salivary fluid of *Aedes aegypti* have identifiable secretory signal sequences;[95] and (2) levels of expression of tTA proteins are anticipated to be extremely high in all cells (even in heterozygotes).[96] Therefore, it may not be reasonable to assume that physiologically significant amounts of tTA will not be found in the salivary fluid. While it is well established that almost any substance the human body is exposed to have the potential to cause an undesirable allergic response, the probability that a given compound elicits such a response is extremely low. However, the hazard to sensitive humans is sufficiently great that all GE plants intended for human consumption are assessed for allergenicity.[97] The desirability to

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[93] *Id*. § 1508.27(b)(2), (4).

[94] EA, *supra* note 77, at 230.

[95] Almeras et al., *Sialome Individuality Between Aedes aegypti Colonies*, Vector Borne Zoonotic Dis 9(5):531–541 (Oct. 2009), http://www.ncbi.nlm.nih.gov/pubmed/18847318.

[96] Gong et al., *A Dominant Lethal Genetic System for Autocidal Control of the Mediterranean Fruitfly*, Nat. Biotech., 23: 453–6 (Apr. 2005), http://www.ncbi.nlm.nih.gov/pubmed/15750586; see also Phuc et al., *Late-Acting Dominant Lethal Genetic Systems and Mosquito Control*, BMC Biology, 5:11 (Mar. 20, 2007), http://bmcbiol.biomedcentral.com/articles/10.1186/1741-7007-5-11.

[97]World Health Organization (WHO) Food and Agriculture Organization (FAO), *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants*, CAC/GL 45-2003, Foods Derived from Modern Biotechnology (2nd ed. 2009), http://www.fao.org/docrep/011/a1554e/a1554e00.htm.

assess the allergenicity of transgenes in GE insects is specifically mentioned in a 2010 EU/EFSA document that recommends using the food safety framework established for GE plants to assess GE insects.[98] The hazard associated with transgene expression in the salivary glands is specifically mentioned.[99]

The question of whether or not the concern outlined above demonstrates a clear allergen hazard to some humans is not the point. The point is: this needed to be experimentally tested, not just speculated about in terms of the homology of the proteins in question. These tests are conducted on GE plants; they should be conducted on GE insects, too.

The more generally important question is, how could field testing of OX513A progress to the point of large-scale releases into human populated areas without this fairly obvious hazard receiving rigorous scientific consideration, not just a page of speculation at the end of the EA? The failure of the FDA to transparently communicate what scientific consideration this simple hazard should receive raises the question of how more complex hazards have been dealt with.

ii. Off-site dissemination of GE *Aedes aegypti* mosquitoes

The EA relies heavily on claims that the GE *Aedes aegypti* mosquito cannot be disbursed offsite due to a combination of physical, geophysical, geographic, and biological measures. These are unproven assumptions.

1. Biological measures—unintentional survival of GE *Aedes aegypti* mosquitoes

Oxitec's GE *Aedes aegypti* mosquitoes and their progeny are genetically programmed to die at the late larval stage. However, there are several mechanisms that could allow many more of the GE mosquitoes to survive to adulthood. There is a fundamental flaw in Oxitec's approach in using tetracycline as a chemical switch to allow breeding of the GE mosquito in the laboratory, because tetracycline and related antibiotics are widespread in the environment. This omission is especially problematic in light of the EFSA Guidance, which counsels consideration of the "[r]eduction in efficacy of the G[E] insect mediated trait that may result in adverse effects."[100]

Unintentional survival of GE mosquitoes can occur due to failure of the genetic killing mechanism. This can occur if resistance develops to the trait or if the GE moths encounter sufficient levels of the antibiotic tetracycline, or its derivatives, to inactivate the killing mechanism.[101] According to the EA, it is anticipated that >95% of the GE mosquitoes will die in

[98] Benedict et al., *Defining Environmental Risk Assessment Criteria for Genetically Modified Insects to be Placed on the EU Market*, Scientific/technical report submitted to EFSA (EFSA-Q-2009-01081), at 97-99 and 135 (Sept. 10, 2010), http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/71e.pdf.

[99] *Id*. at 135.

[100] EFSA Guidance, *supra* note 19, at 89.

[101] EA, *supra* note 77, at 97.

the environment.[102] This means that at least some females are expected to survive to adulthood, even in the absence of tetracycline. However, contamination with tetracycline and related antibiotics is widespread in the environment and could lead to significantly increased survival rates. The EA erroneously assumes that survival in the environment is expected to be lower due to the harsher environmental conditions encountered, but the studies provided do not indicate that this assumption is true.[103] In the Malaysian study, the average life expectancy for OX513A was 2.0 days, while the average life expectancy for the non-GE comparator was 2.3 days. Therefore the life expectancy did not differ significantly from the non-GE laboratory strain co-released as part of a comparative evaluation.[104] This means that the OX315A strain may live in the environment nearly as long as wild *Aedes aegypti* mosquitoes.

When Oxitec's GE mosquito larvae were fed cat food containing industrially-farmed chicken, the survival rate increased to 15-18%. Oxitec originally hid this information,[105] but later admitted to an 18% survival rate of larvae fed on cat food—which is assumed to contain industrially-farmed chicken contaminated with tetracycline or related antibiotics—in a published paper.[106] The tetracycline derivatives oxytetracycline (OTC) and doxycycline (DOX, used to prevent malaria) could also allow Oxitec's GE insects to breed. OTC can be found at concentrations above 500 µg/g in animal manure and DOX at up to 78.5 µg/g dry weight in broiler manure.[107] A global review reports lower but still relevant concentrations of tetracycline of up to 0.88 µg/g in pig manure, 11.9 µg/g in poultry manure, and 0.208 µg/g in cattle manure.[108] These concentrations are likely to be more than enough to inactivate the killing mechanism in the female GE *Aedes aegypti* mosquitoes if the larvae come into direct contact with contaminated manure. Moreover, it would not be surprising if behavioral adaption beneficial for survival was selected for in the field, leading to females seeking tetracycline contaminated areas in which to lay their eggs.

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[102] *Id*. at 96.

[103] *Id*.

[104] *Id*.

[105] FOE, Press Release, *Company Conceals Evidence that Genetically Modified Mosquitoes May Have High Survival Rate in Wild* (Jan. 12, 2012), http://www.foe.org/news/archives/2012-01-genetically-modified-mosquitoes-survival-rate (last accessed April 26, 2016).

[106] Massonnet-Bruneel et al., *Fitness of Transgenic Mosquito Aedes aegypti Males Carrying a Dominant Lethal Genetic System*, PLoS ONE, 8(5):e62711 (May 14, 2013), http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0062711.

[107] Kyselkova et al., *Cow Excrements Enhance the Occurrence of Tetracycline Resistance Genes in Soil Regardless of their Oxytetracycline Content*, Chemosphere, 93(10): 2413-8 (Nov. 2013), http://www.ncbi.nlm.nih.gov/pubmed/24053942; Ho et al., *Simultaneous Determination of Veterinary Antibiotics and Hormone in Broiler Manure, Soil and Manure Compost by Liquid Chromatography-Tandem Mass Spectrometry*, J Chromatogr A, 1262: 160-8 (Nov. 2012), http://www.ncbi.nlm.nih.gov/pubmed/23026257.

[108] Kim et al., *Occurrence and Environmental Fate of Veterinary Antibiotics in the Terrestrial Environment*, Water, Air, & Soil Pollution, 214(1): 163-174 (Jan. 2011), http://link.springer.com/article/10.1007%2Fs11270-010-0412-2.

The percentage of surviving GE *Aedes aegypti* mosquitoes could also increase if resistance to the genetic killing mechanism evolves over time. This concern is dismissed as unlikely in the EA,[109] despite prior evidence of behavioral resistance developing in a Sterile Insect Technique (SIT) program, i.e., females unreceptive to mating with irradiated males.[110] FDA dismisses this evidence, but there has been little investigation of this phenomenon, which shows the expected development of an evolutionarily-advantageous behavior in the field. Resistance can also develop through the evolution of resistance alleles.[111] This risk must be considered because radiation-induced sterility using the traditional SIT has built-in redundancy that is not provided by molecular genetic approaches.[112] A number of authors have therefore speculated that any genetic or molecular event that allows the GE mosquito to survive and breed successfully could be rapidly selected for during mass production.[113] No laboratory or caged studies have been published to investigate the potential development of resistance through either of these mechanisms. These studies should have taken place *before* Oxitec even applied for an INAD. At the very least, they must be conducted before FDA can approve such a trial.

Oxitec acknowledges that the lethality trait may fail, and therefore biological containment would not be possible, but claims there is no adverse impact if the lethality fails.[114] As explained above, however, such failure could facilitate the establishment or spread of GE mosquitoes offsite. This would exacerbate any adverse impacts such as toxicity or allergenicity to humans or wildlife, and make it impossible to retrieve GE mosquitoes or reverse any unintended effects. These significant impacts are unique and unknown, as well as reasonably foreseeable, and FDA has entirely failed to analyze these impacts. The FDA must analyze them in a comprehensive EIS. In looking at the context of the INAD, should GE mosquitoes survive the lethality trait or move beyond the field trial location, the effects could be significant and well beyond the effected region and locality.[115]

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[109] EA, *supra* note 77, at 97.

[110] Hibino & Iwahashi, *Appearance of Wild Females Unreceptive to Sterilized Males on Okinawa Is. in the Eradication Program of the Melon Fly, Dacus cucurbitae Coquillett (Diptera: Tephritidae)*, Applied Entomology and Zoology, 26(2): 265-270 (Feb. 7, 2008), https://www.jstage.jst.go.jp/article/aez1966/26/2/26_2_265/_article.

[111] Alphey et al., *Modeling Resistance to Genetic Control of Insects*, Journal of Theoretical Biology, 270(1): 42-55 (Feb. 7, 2011), http://www.ncbi.nlm.nih.gov/pubmed/21075122.

[112] Benedict & Robinson, *The First Releases of Transgenic Mosquitoes: An Argument For the Sterile Insect Technique*, Trends in Parasitology, 19(8): 349-355 (Aug. 2003), http://www.ncbi.nlm.nih.gov/pubmed/12901936.

[113] Robinson et al., *Insect Transgenesis and its Potential Role in Agriculture and Human Health*, Insect Biochemistry and Molecular Biology, 34(2): 113-120 (Feb. 2004), http://www.sciencedirect.com/science/article/pii/S096517480300198X.

[114] EA, *supra* note 77, at 97.

[115] 40 C.F.R. § 1508.27(a).

2. Geographical and geophysical containment

The EA assumes that if biological containment fails, there is sufficient redundancy in geographical and geophysical containment to prevent disbursement of GE mosquitoes.[116] The EA states that geographical and geophysical containment measures include temperature, water storage and rainfall, salinity of the water surrounding the release site, and insufficient tetracycline in the environment and breeding sites.[117] However, this argument is fundamentally flawed, as the EA reveals that wild *Aedes aegypti* can survive in the environment in Florida, where it is regarded as an invasive species.[118]

Moreover, The EA completely omits consideration of dispersal via human migration. The EA notes that *Aedes aegypti* mosquitoes are a non-native species introduced into the United States via human migrations and international trade.[119] *Aedes aegypti* are uniquely domestic and tied closely to human habitations and urban areas; the presence of suitable breeding sites, along with the availability of human blood meal, strongly influences both the habitat and geographic range of the mosquito.[120] This indicates that it is reasonably foreseeable for GE *Aedes aegypti* mosquitoes to migrate beyond the field trial site, which must be analyzed in an EIS. In addition, tourism is the main industry in Monroe County, with over 94.7 million visitors to Florida in 2013, meaning that it is also reasonably foreseeable that a GE mosquito could migrate with a tourist well beyond the field trial location.[121]

It is foreseeable that biological, geographical, and geophysical containment will fail, the effects of which are significant, and FDA has entirely failed to analyze the possibility that this failure will occur. FDA must analyze the possibility that biological, geographical, and geophysical containment will fail and evaluate the impacts of widespread disbursement of GE mosquitoes throughout Florida and the U.S. in a comprehensive EIS.

iii. Impact to target organisms: response of the wild *Aedes aegypti* population to the proposed releases

The EFSA Guidance counsels FDA to consider "[c]hanges in [target organism] populations caused by the GE component of the releases (size, age structure, sex ratio, fertility, mortality) that may result in adverse effects leading to environmental harm."[122] Whilst the unstated intention of the releases is to reduce diseases caused by *Aedes aegypti* mosquitoes by

[116] EA, *supra* note 77, at 97-99.

[117] *Id*. at 97.

[118] *Id*.

[119] *Id.* at 38.

[120] *Id*.

[121] *Id*. at 39.

[122] EFSA Guidance, *supra* note 19, at 87.

suppressing the target population of *Aedes aegypti* mosquitoes; in practice, the response of the target population is likely to be complex.

The EA completely fails to address whether or not releases of GE mosquitoes could cause an increase in the numbers of mosquitoes in the surrounding areas. This effect is predicted by some models for the release of sterile insects.[123] For releases of GE mosquitoes, Oxitec's Cayman Islands's paper[124] and its graph from Mandacaru, Brazil—the details of which are unpublished, but the graph is in a company brochure[125]—both show increases in *Aedes aegypti* mosquitoes in the control area as population suppression in the target area begins to occur. In the Cayman Islands the control area was next to the target area for the releases, but for Mandacaru there is no public information about the location of the control area. The number of mosquitoes trapped in the untreated area also increased in the final phase of the experiments conducted in Itaberaba, Brazil according to the Projecto Aedes Transgenico (PAT) PowerPoint, which provides some of the only published information on these experiments.[126] Thus, there appears to be a real possibility that wild-type males, when swamped by very high releases of GE males, simply migrate to mate in the surrounding area. More information is needed to either confirm or rule out this possibility. Since Oxitec calculates population suppression based on the difference between the target area and the control area, it is possible that claims of significant drops in population partly reflect significant increases being caused elsewhere. In the context of the EA, it is important to consider the risk that wild-type *Aedes aegypti* mosquitoes will cause increased damage outside the target area. Assessment of this risk requires prior modelling of this potential effect and an altered trial protocol and monitoring to establish whether or not this adverse effect occurs. Further, long-term monitoring of *Aedes aegypti* populations is required in advance of any trials to establish the baseline for assessment of efficacy, and to avoid reliance on a neighboring control that might itself be affected by wild-type *Aedes aegypti* mosquito dispersal from the target site. The EA does not adequately discuss whether in the absence of *Aedes aegypti*, other mosquitoes such as *Aedes albopictus* would become significant transmitters of disease causing viruses. The EA merely notes that where *Aedes aegypti* is not present, *Aedes albopictus* is a carrier of dengue, i.e., parts of China, Seychelles, Japan, and Hawaii.[127]

These significant effects are unique, highly uncertain and unknown, and reasonably foreseeable, and FDA must analyze them in a comprehensive EIS.

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[123] Yakob et al., *Aedes aegypti Control: The Concomitant Role of Competition, Space and Transgenic Technologies*, Journal of Applied Ecology, 45(4):1258–1265 (Aug. 2008), http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2664.2008.01498.x/full.

[124] Harris, *supra* note 91, at 828–830.

[125] Oxitec, *Dengue Fever: The Fastest Growing Mosquito-Borne Disease*, at 6 (October 2013), http://www.oxitec.com/wpcms/wp-content/uploads/OXITEC-Dengue-booklet1.pdf.

[126] Projecto Aedes Transgenico (PAT), PowerPoint, http://ftp.nbiap.vt.edu/documents/animal-biotech/2nd-Intl-Workshop-docs/Day-2/Capurro%20-%20Aedes%20transgenic%20project.pdf (last accessed April 27, 2016).

[127] EA, *supra* note 77, at 100.

iv. Risk of increase in non-target mosquitoes in response to GE *Aedes aegypti* mosquito releases

The EA notes that there is competition among mosquito species, but incorrectly claims that introduction of GE mosquitoes will only affect the target mosquitoes.[128] The EA entirely fails to address how releasing GE *Aedes aegypti* will impact non-target mosquitoes. The FDA must consider whether the proposed releases of GE *Aedes aegypti* mosquitoes will facilitate the dissemination and establishment of other, non-target mosquitoes. To do this correctly, the EA must consider not only exposure of wildlife to direct effects such as potential toxicity, but ecosystem responses to the releases, i.e. indirect effects on the population dynamics of non-target species.

The EFSA Guidance states: "[c]onsidering the aim and type of G[E] insect releases, and also accounting for possible accidental releases, potential impacts on NTO [non-target organisms] that may cause adverse effects include: . . . (b) a change in abundance or species composition of competitors (e.g., insects exploiting the same ecological niches) of G[E] insects and the ecological functions they provide,"[129] and adds "[o]ther pest species (e.g., secondary pests) might exploit the available resource and build up high populations which might have an adverse effect on the environment and on human health."[130]

This situation could be regarded as analogous to problems with GE insect-resistant crops (Bt crops) that have developed in China and Brazil. In China, secondary pests that are not affected by the Bt toxins in its GE cotton crop have become a major problem.[131] In Brazil, the Agricultural Ministry has issued warnings about a massive explosion in corn ear worm (*Helicoverpa armigera*) in areas growing Bt maize.[132] These examples show how reductions in competition or natural enemies can lead to an explosion in another type of insect or pest. These concerns arise as a result of the proposed "single species" approach and do not apply to methods that are effective against multiple species.

Should releases of GE *Aedes aegypti* mosquitoes lead to the expansion or establishment of other mosquitoes, such as *Aedes albopictus* and *Culex spp*, these adverse significant effects

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[128] EA, *supra* note 77, at 78.

[129] EFSA Guidance, *supra* note 19, at 94.

[130] *Id* at 98.

[131] Wang et al., *Bt-cotton and secondary pests*, International Journal of Biotechnology, 10(2/3):113-121 (2008), http://www.inderscience.com/info/inarticle.php?artid=18348; Lu et al., *Mirid Bug Outbreaks in Multiple Crops Correlated with Wide-Scale Adoption of Bt Cotton in China*, Science 328(5982):1151–54 (May 2010), http://www.ncbi.nlm.nih.gov/pubmed/20466880; Zhao et al., *Benefits of Bt Cotton Counterbalanced by Secondary Pests? Perceptions of Ecological Change in China*, Environ Monit Assess, 173(1-3): 985–994 (Feb. 2011), http://www.ncbi.nlm.nih.gov/pubmed/20437270.

[132] Ministerio do Desenvolvimento Agrario (MDA), MDA previne agricultores sobre aparição da lagarta Helicoverpa em plantações (Aug. 9, 2013) [in Portuguese], http://www.mda.gov.br/portalmda/noticias/mda-previne-agricultores-sobre-apari%C3%A7%C3%A3o-da-lagarta-helicoverpa-em-planta%C3%A7%C3%B5es.

may be difficult to mitigate or reverse. *Aedes albopictus* has been shown to transmit the zika virus in Africa.[133] Prior knowledge of the distribution and population dynamics of other mosquitoes, including any competitive effects, at the proposed field site is therefore *essential* before the release can be approved and conducted. Without such data, combined with credible attempts to model likely population responses, open releases of GE *Aedes aegypti* mosquitoes are premature and FDA's approval of such is unlawful.

Finally, the introduction of the GE *Aedes aegypti* mosquitoes might prompt the migration of resident non-GM males into neighboring areas. How do these protocols interact with neighboring area's vector control programs if those communities suddenly have more mosquitoes?

Potential increases in competitor species such as *Aedes albopictus* and *Culex spp*, are a major concern for Oxitec's proposed release of GE *Aedes aegypti* mosquitoes.[134] However, such effects have been omitted from the EA altogether, despite the use of a single-species approach in the likely presence of numerous other mosquito species. In some cases, these competitor species are invasive species and the impact of the proposed releases on their populations are potentially significant and reasonably foreseeable, demanding that it be evaluated in a comprehensive EIS.

v. Potential transfer of antibiotic resistance via *Aedes aegypti* mosquito microbiota

The use of tetracycline to breed the GE *Aedes aegypti* mosquitoes in the lab carries the risk of spreading antibiotic resistance, which could pose a major risk to human and animal health. Insect guts are reservoirs for antibiotic resistance genes with potential for dissemination. Insect production in factories exposed to antibiotics could lead to drug resistance in their microbiota so that the insects disseminate antibiotic resistance when released into the environment. There is growing recognition that antibiotic resistance poses a serious, worldwide threat to public health.[135]

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[133] Grard et al., *Zika Virus in Gabon (Central Africa) - 2007: A New Threat From Aedes Albopictus?*, Plos Neglected Tropical Diseases, 8(2): e2681 (Feb. 6, 2014), http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3916288/.

[134] Beech et al., *Risk analysis of a hypothetical open field release of a self-limiting transgenic Aedes aegypti mosquito strain to combat dengue*, Asia Pacific Journal of Molecular Biology and Biotechnology, 17(3): 99-111 (Jun. 30, 2009), http://www.cdfd.org.in/images/JNRPDF/APJMBB2009b.pdf; National Technical Commission on Biosafety (CTNBio), *Technical Opinion on Examination Request Presented at the 171st Plenary Meeting of the National Technical Commission on Biosafety (CTNBio), held on April 10th, 2014* (April 10, 2014) (Attached as Exhibit G); Bonsall et al., *Transgenic control of vectors: The effects of inter-specific interactions*, Israel Journal of Ecology and Evolution, 56(3-4): 353-370 (Mar. 14, 2013), http://www.tandfonline.com/doi/abs/10.1560/IJEE.56.3-4.353.

[135] M. Wooldridge, *Evidence for the Circulation of Antimicrobial Resistant Strains and Genes in Nature and Especially Between Humans and Animals*, Rev. Sci. Tech., 31(1): 231-247 (Apr. 2012), http://www.ncbi.nlm.nih.gov/pubmed/22849279; Zurek & Ghosh, *Insects Represent a Link Between Food Animal Farms and the Urban Environment for Antibiotic Resistance Traits*, Appl. Environ. Microbiol., 80(12): 3562-7 (Jun. 2014), http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4054130/; Allen et al., *Resident Microbiota of the Gypsy Moth Midgut Harbors Antibiotic Resistance Determinants*, DNA Cell Biol., 28(3): 109-17 (2009), http://handelsmanlab.sites.yale.edu/sites/default/files/ResidentMicrobiota.pdf; Tian et al., *Long-Term Exposure to Antibiotics has Caused Accumulation of Resistance Determinants in the Gut Microbiota of Honeybees*, mBio,

The EA states that there is no causal pathway for exposure because gut bacteria is lost during mosquito metamorphosis from larvae to adults, and therefore completely fails to analyze the impacts of gut bacteria.[136] Feeding the GE *Aedes aegypti* mosquitoes tetracycline might affect which diseases the GE mosquito can carry. Recent research published in Nature Commentary[137] demonstrated that another genus of mosquito—*Anopheles*—was more effectively infected with the parasite that causes malaria when it is fed blood that contains antibiotics. The natural microbial mix of the gut of the mosquito is altered in a way that allows the malaria plasmodium to thrive. This research suggests that research on whether the tetracycline diet affects the microbiome of the *Aedes aegypti* mosquitoes in a way that facilitates viral transmission is needed. The simple assertion in the Oxitec EA[138] that the GE mosquitoes will not have access to tetracycline after transitioning from larvae to adult stages assumes that the adult GE mosquitoes will not escape and find tetracycline. Females could obtain this from both animals and humans when they seek the blood of mammals that use tetracycline. Research should establish that any escaped GE females would not be more effective transmitters of viruses like dengue and zika.

Reliance on antibiotics for breeding GE *Aedes aegypti* mosquito in the lab is a serious downside compared to the use of the traditional SIT based on the use of radiation, or compared to the "No Action" alternative that does not contribute to the spread of antibiotic resistance. In its Guidance for Industry #209, FDA recognizes that "the administration of medically important antimicrobial drugs to entire herds or flocks of food-producing animals would represent a use that poses qualitatively higher risk to public health than the administration of such drug to individual animals or targeted group of animals."[139] Combined with the potential for survival of female *Aedes aegypti* mosquitoes in the presence of tetracycline contamination in the environment, as discussed above, this suggests a fundamental flaw in Oxitec's technology.

3(6):e00377-12 (Oct. 2012), http://mbio.asm.org/content/3/6/e00377-12.abstract; Levy & Marshall, *Honeybees and Tetracycline Resistance*, mBio, 4(1): e00045-13 (Feb. 12, 2013), http://www.ncbi.nlm.nih.gov/pubmed/23404397; WHO, *WHO's First Global Report on Antibiotic Resistance Reveals Serious, Worldwide Threat to Public Health* (Apr. 30, 2014), http://www.who.int/mediacentre/news/releases/2014/amr-report/en/ (last accessed April 28, 2016).

[136] EA, *supra* note 77, at 76.

[137] Gendrin et al., *Antibiotics in Ingested Human Blood Affect the Mosquito Microbiota and Capacity to Transmit Malaria*, Nature Communications, 6: 5921 (Jan. 6, 2015), http://www.ncbi.nlm.nih.gov/pubmed/25562286.

[138] EA, *supra* note 77, at 76.

[139] FDA, Guidance for Industry #209: The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals (2012), http://www.fda.gov/downloads/animalveterinary/guidancecomplianceenforcement/guidanceforindustry/ucm216936.pdf.

vi. Restricted purpose, inadequate monitoring, and lack of prior studies

The stated purpose of the requested field release is to assess the *efficacy* of GE *Aedes aegypti* strain OX5123A in reducing wild populations of non-GE *Aedes aegypti* mosquitoes.[140] However, biosafety issues are still not yet fully understood for this new technology and must also be assessed. This requires greater prior assessment of the release environment, especially background populations and fluctuations in both target and non-target organisms, and of the GE *Aedes aegypti* mosquito strain proposed for release, as detailed above (in particular, thorough safety testing of the impacts of ingestion on humans and animals) prior to any release. The application for open release is therefore premature. Further, were the releases to precede following the provision of this important additional data, additional monitoring would be required to detect potential adverse effects, i.e., the purpose of the experiment would need to be extended to include additional monitoring. This should include for example, monitoring to detect potential adverse effects on beneficial insects, predators, and wildlife; monitoring to detect any migration of *Aedes aegypti* mosquitoes to neighboring islands and persistence or dispersal of GE *Aedes aegypti* mosquitoes; monitoring of non-target mosquitoes to detect any unintended increases in such mosquitoes due to population suppression of a competitor; and monitoring of antibiotic resistance and its spread through gut bacteria. In other words, Oxitec's INAD experiment does not take into account how it proposes to monitor the unique and reasonably foreseeable impacts that make this project significant.

c. The EA Fails to Consider Cumulative Impacts as Required by NEPA

NEPA requires agencies to consider the cumulative impacts of proposed actions.[141] "A cumulative impact is defined as 'the impact on the environment which results from the incremental impact of the action when added to other past, present, and reasonably foreseeable future actions regardless of what agency . . . or person undertakes such other actions. Individually minor, but collectively significant actions, taking place over time, can generate cumulative impacts."[142] Cumulative impacts must be fully considered in an EA." Given that so many more EAs are prepared than EISs, adequate consideration of cumulative effects requires that EAs address them fully."[143] Specifically, an EA must provide a quantified assessment of a project's environmental impacts when combined with other projects.[144] The EA cannot simply discuss the direct effect of the project and conclude that there are no cumulative impacts.[145] Instead, cumulative impacts must be evaluated along with the direct and indirect effects of a

[140] EA, *supra* note 77, at 13.

[141] 40 C.F.R. § 1508.27(b)(7).

[142] *Id*.

[143] *Kern v. U.S. Bureau of Land Mgmt.*, 284 F.3d 1062, 1076 (9th Cir. 2002) ("We have held that an EA may be deficient if it fails to include a cumulative impact analysis….").

[144] *Great Basin Mine Watch v. Hankins*, 456 F.3d 955, 972 (9th Cir. 2006).

[145] *Id.*

project and its alternatives. As the United States Court of Appeals for the District of Columbia Circuit has explained,

> "[A] meaningful cumulative impact analysis must identify (1) the area in which the effects of the proposed project will be felt; (2) the impacts that are expected in that area from the proposed project; (3) other actions—past, present, and proposed, and reasonably foreseeable—that have had or are expected to have impacts in the same area; (4) the impacts or expected impacts from these other actions; and (5) the overall impact that can be expected if the individual impacts are allowed to accumulate."[146]

In stark contrast to what NEPA requires in an EA, FDA's FONSI cursorily concludes that

> "The investigational trial is short in duration and any unanticipated adverse effects are unlikely to be widespread or persistent in the environment. Most importantly, the status of the environment is restored when releases are stopped (i.e. the releases mosquitoes all die, and the environment reverts to the pre-trial status). FDA has therefore made the preliminary finding that the proposed field trial would not individually or cumulatively have a significant effect on the quality of the human environment."[147]

As discussed at length above, FDA has failed to consider many potential impacts on the physical, biological, and human health environments, and has erroneously concluded that such impacts are unlikely to occur. Likewise, FDA has entirely failed to examine the significant cumulative impacts that its action will have on the environment. For example, the FKMCD will not stop the use of its existing control measures for wild *Aedes aegypti* mosquitoes during the trial.[148] FKMCD currently utilizes integrated mosquito managements practices, which involve a variety of methods to reduce *Aedes aegypti* mosquitoes including adulticides, larvicides, source reduction, and biological controls.[149] FDA must analyze the cumulative impact of using other control measures at the same time as releasing millions of GE mosquitoes. The fact that FKMCD is not ceasing to use other vector control measures is problematic because it will be nearly impossible to determine whether wild *Aedes aegypti* populations are suppressed—if at all—from Oxitec's experiment or other control measures.

On the other hand, the EA implies that FKMCD will only continue using current control measures if the "no action" alternative is chosen.[150] If FKMCD does cease using current vector

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[146] *Grand Canyon Trust v. FAA*, 290 F.3d 339, 345-46 (D.C. Cir. 2002) (internal citations omitted).

[147] FONSI, *supra* note 12, at 6.

[148] Email from Michael Doyle, *supra* note 83.

[149] EA, *supra* note 77, at 17.

[150] *See id.* (stating that FKMCD will only continue using its existing control measures if the project is not approved).

control measures, the FDA must still analyze the cumulative effects that ceasing control measures has on humans and the environment, including the potential for an increase in mosquito populations and an increase in the risk of diseases. Proper protocols need to be developed to "integrate" Oxitec's GE mosquito technique into existing vector control, due to the need to suspend spraying during Oxitec releases. Suspending current vector controls has to be proven to be safe, and effective, otherwise it is dangerous (another way to increase *Aedes albopictus* and *Culex ssp*. populations), and an expensive waste of time and money because *Aedes aegypti* is not the only disease carrying vector in Florida or the U.S. These issues need to be dealt with thoroughly. An effective protocol would specify when FKMCD needs to resume spraying. If FKMCD needs to spray anyway, what additional benefits does the Oxitec technique bring, or does it just get in the way of effective mosquito control? Who would monitor all this, in what manner, and at what cost to the community?

Countries or regions with endemic disease also need to consider additional cumulative risks due to potential impacts of partial or temporary population suppression on human immunity. In areas of high mosquito abundance, where dengue is endemic, reducing the frequency of biting can increase the incidence of the more serious and often fatal disease, dengue hemorrhagic fever (DHF), which occurs by reducing cross-immunity to the four different serotypes of the dengue virus, or increasing the incidence of dengue fever (DF) due to age related affects. WHO has stated that full-scale programmatic deployment is not currently recommended for Oxitec's GE mosquitoes and that Randomized Controlled Trials (RCTs) with epidemiological outcomes should be carried out to build evidence for routine programmatic use of OX513A *Aedes* against *Aedes*-borne diseases. Such trials would need to be conducted in dengue-endemic areas and thus would proceed or not proceed independently of any trial in Florida, taking into account the additional risks associated with impacts on human immunity to the relevant diseases and relevant local conditions (such as the role of other vectors in transmitting relevant tropical diseases).

The risks associated with these and other cumulative actions must be considered comprehensively in an EIS *prior* to approval of the release.

d. The EA Fails to Identify Alternatives as Required by NEPA

FDA has failed to take the required "hard look" at possible alternatives to approving the release of GE *Aedes aegypti*. Section 102(2)(E) of NEPA requires all agencies to "[s]tudy, develop, and describe appropriate alternatives to recommended courses of action in any proposal which involves unresolved conflicts concerning alternative uses of available resources."[151] Regardless of whether an EA or EIS is prepared, NEPA "requires that alternatives be given full and meaningful consideration."[152] In fact, the alternatives section is considered the heart of an environmental analysis.[153] "[I]t should present the environmental impacts of the proposal and

[151] 42 U.S.C. § 4331(2)(E).

[152] *Bob Marshall Alliance v. Hodel*, 852 F.2d 1223, 1229 (9th Cir. 1988).

[153] 40 C.F.R. § 1502.14.

the alternatives in comparative form, thus sharply defining the issues and providing a clear basis for choice among options by the decisionmaker and the public."[154] Agencies must therefore rigorously explore and objectively evaluate all reasonable alternatives, including the no action alternative.[155]

Despite the rigor required by NEPA, FDA's EA presents no serious analysis of potential alternatives. Instead, FDA merely provides a cursory review of just two options it purports to have "evaluated" to satisfy this requirement: the proposed release approval action and the "no action" disproval.[156] It is a classic NEPA violation to limit the consideration of alternatives simply to (1) action or (2) no action.[157]

FDA's alternatives analysis is also fundamentally flawed because it is—like the rest of the EA—far too limited in scope. An agency's alternatives analysis should be a function of the "purpose and need" of the action under review.[158] FDA states that the purpose of the investigational field trial is to "evaluate the mating ability of released OX513A mosquitoes with local wild type *Aedes aegypti* females, to assess the survival of the resultant progeny in order to estimate the mortality related to inheritance of the OX513A rDNA construct, and to determine the efficacy of sustained releases of OX513A mosquitoes for the suppression of a local population of *Aedes aegypti* in the defined release area in Florida Keys."[159] As part of the need for the NAD, FDA states "*Ae. aegypti* is a known vector for human diseases; zika virus, dengue fever, chikungunya."[160] However, the prevalence of locally acquired diseases in Florida—particularly Monroe County—is low; in 2014 there were only six cases of locally acquired dengue, all of which occurred in Miami-Dade County, not Monroe County.[161] In 2015, there was only one case of locally acquired dengue, which occurred in Broward County, and there have been no reports of locally acquired dengue in 2016.[162] In 2014, there were eleven reported

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[154] *Id*.

[155] *Id*.

[156] EA, *supra* note 77, at 16-17.

[157] *See, e.g.*, *Am. Oceans Campaign v. Daley*, 183 F. Supp. 2d 1, 17-21 (D.D.C. 2000); *Muckleshoot Indian Tribe v. U.S. Forest Serv.*, 177 F.3d 800, 813-14 (9th Cir. 1999) (consideration of only unqualified deregulation and the no action alternative is presumptively too limited to comply with NEPA).

[158] *See* 40 C.F.R. § 1502.13 (agency must "specify the underlying purpose and need to which the agency is responding in proposing the alternatives…."); *City of Carmel-By-The-Sea v. U.S. Dep't of Transp*., 123 F.3d 1142, 1155 (9th Cir. 1995) ("The stated goal of a project necessarily dictates the range of 'reasonable' alternatives and an agency cannot define its objectives in unreasonably narrow terms.") (citation omitted).

[159] EA, *supra* note 77, at 13.

[160] *Id*. at 15.

[161] Florida Department of Health, *Mosquito-Borne Disease Surveillance*, http://www.floridahealth.gov/diseases-and-conditions/mosquito-borne-diseases/surveillance.html (last accessed April 28, 2016).

[162] *Id*.

cases of locally acquired chikungunya, though none in Monroe County, and there were no reported cases of locally acquired chikungunya in 2015 or 2016.[163] There have been no known cases of locally acquired zika virus in Florida.[164]

When local transmission of dengue fever was reported in Florida Keys in 2009 and 2010, twenty-two people were diagnosed in 2009 and a further sixty-six people in 2010.[165] The reasons for the outbreak are unknown but action taken in response—including prompt diagnosis, increased disease surveillance, increased control of larval and adult mosquito populations, and door-to-door canvassing to find and eliminate mosquito breeding sites—appears to have been successful. The 2010 cases appeared to be a continuation of the 2009 outbreak, suggesting local transmission for a period of one or two years. However, further local transmission has been essentially non-existent since then, particularly in Monroe County.

Thus, the purpose of determining the efficacy of suppressing wild populations of *Aedes aegypti* mosquitoes is overly-narrow and ignores the larger problem of diseases caused by *Aedes aegypti*, which would require FDA to consider alternatives in addition to the release of GE varieties to address the problem. Moreover, the purpose and need are not complimentary; the need exists because *Aedes aegypti* is a known vector for diseases, and the purpose of the trial is to determine the efficacy of sustained releases of OX153A for the suppression of a local population of *Aedes aegypti*; however, there is a low prevalence of the diseases within the field trial location. Thus, if the FDA is successful in vector control, it will not provide sufficient data to determine whether the vector control successfully reduced the prevalence of diseases caused by *Aedes aegypti*. At a minimum, FDA should evaluate other potential test sites than Monroe County, which should be included in its alternatives analysis. It is not enough for FDA to say "should Oxitec wish to select another location in the United States to conduct a field trial, it would prepare an environmental assessment for that investigational release."[166] The purpose of the EA is to evaluate the potential alternatives, and then choose a test site; not choose a test site, and ignore all other alternatives.

Even with that aside, however, FDA fails to assess any of the numerous other feasible means of testing the efficacy of GE mosquitoes. Some of these alternatives include a closed release in an indoor facility or closed-net greenhouses, or siting the release in a more isolated location with respect to threatened and endangered species. FDA instead inexplicably assumes that an open-air field release is the *only* viable option, and in doing so improperly restricts itself from considering any other options that could feasibly, effectively, and safely fulfill its identified purpose. Not only that, FDA intends to release mosquitoes three times a week for twenty-two months, but did not evaluate field trials that involve less releases or last a shorter duration. If it

[163] *Id*.

[164] *Id*.

[165] EA, *supra* note 77, at 15.

[166] *Id.* at 17.

is possible to achieve the same purpose with less environmental harm, the FDA must identify and analyze those alternatives before rejecting them.[167]

Lastly, if the need is to reduce vector borne illnesses such as zika or dengue, FDA should analyze alternatives to releasing GE mosquitoes. Vaccines are already emerging as important alternatives, in one case with proven impacts on disease. The first dengue vaccine, Dengvaxia (CYD-TDV) by Sanofi Pasteur, was first registered in Mexico in December, 2015. CYD-TDV is a live recombinant tetravalent dengue vaccine that has been evaluated as a 3-dose series on a 0/6/12 month schedule in Phase III clinical studies. It has been registered for use in individuals 9-45 years of age living in endemic areas. The Philippines has just launched the world's first mass dengue vaccination program using this vaccine. There are approximately five additional vaccine candidates under evaluation in clinical trials, including other live-attenuated vaccines, as well as subunit DNA, and purified inactivated vaccine candidates. Additional technological approaches, such as virus-vectored and VLP-based vaccines, are under evaluation in preclinical studies. An NIH-sponsored phase 2 clinical trial of chikungunya vaccine opened in late 2015, after promising results in a phase 1 trial. Research on a zika vaccine is also being accelerated.

In Florida, a more likely consequence of refusing the trial (the "No Action Alternative") is that alternative approaches are developed and implemented instead, including the development and deployment of vaccines for travelers to countries where the relevant diseases are endemic. FDA's failure to consider other options, locations, or scope of the project is thus arbitrary and capricious and in violation of NEPA.

e. The EA Fails to Consider and Prescribe Adequate Mitigation Measures

FDA dismisses the few risks that it does acknowledge in the EA nearly out-of-hand, rather than applying its authority to require mitigation measures to address known risks. CEQ defines "mitigation" to include:

> (a) Avoiding the impact altogether by not taking a certain action or parts of an action;
> (b) Minimizing impacts by limiting the degree or magnitude of the action and its implementation;
> (c) Rectifying the impact by repairing, rehabilitating, or restoring the affected environment;
> (d) Reducing or eliminating the impact over time by preservation and maintenance operations during the life of the action; and
> (e) Compensating for the impact by replacing or providing substitute resources or environments.[168]

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[167] *Lands Council v. Powell*, 395 F.3d 1019, 1027 (9th Cir. 2004) ("The purpose of NEPA is to require disclosure of relevant environmental considerations that were given a 'hard look' by the agency, and thereby to permit informed public comment on proposed action and any choices or *alternatives that might be pursued with less environmental harm*" (emphasis added)).

[168] 40 C.F.R. § 1508.20.

Despite this expansive definition, which gives FDA broad power to impose conditions on its approval of the permit, FDA has failed to prescribe *any* mitigation measures to address the known risks of the release.

Moreover, the EA fails to adequately explain or analyze how FDA will monitor compliance with the conditions of the release. Mitigation must be enforceable, which includes the duty of on-going monitoring to ensure compliance,[169] and is essential where mitigation is part of the justification for the agency's determination not to prepare an EIS.[170] Even if FDA had prescribed mitigation measures, such measures would not substitute for actually analyzing environmental impacts.[171] Yet here FDA relies on Oxitec's claims that the release is low-risk and mitigation measures are unnecessary because the likelihood of escape survival, and establishment of OX153A is highly unlikely due to a combination of physical, geophysical, geographic, and biological measures; however, FDA did not analyze the reasonably foreseeable effects of a field trial with such a massive scope as the one proposed by Oxitec, in which GE mosquitoes will be released three times a week for nearly two years.[172] FDA also failed to analyze the potential impacts should/when any or all of those conditions fail or change, and has not conducted a failure mode analysis to test the reliability of these conditions.

Absent a more complete explanation of how Oxitec intends to release and monitor such a significant amount of mosquitoes for an extended period of time, FDA's attempt to delay adequate review at this time—before the release—is arbitrary, capricious, and unlawful.

f. The EA Fails to Adequately Consider Effects on Endangered and Threatened Species Under NEPA.

Under NEPA, one of the factors to determine the significance of an action is the extent "to which the action may adversely affect an endangered or threatened species or its habitat."[173] The Endangered Species Act (ESA), which is the federal statute that regulates threatened and endangered species, requires FDA to determine whether any threatened or endangered species or critical habitats "may be present" in the action area. To determine whether threatened or endangered species are present, FDA must inquire with either the National Marine Fisheries Service (NMFS) or the Fish and Wildlife Service (FWS), or both, under the ESA. Here, The EA acknowledges that there are a total of 43 threatened, endangered, or candidate species identified

[169] CEQ, Appropriate Use of Mitigation and Monitoring and Clarifying the Appropriate Use of Mitigated Findings of No Significant Impact, at 7 n.18 (Jan. 14, 2011), https://ceq.doe.gov/current_developments/docs/Mitigation_and_Monitoring_Guidance_14Jan2011.pdf; *id.* at 2 (explaining that when agencies do not "monitor mitigation commitments to determine if mitigation was implemented or effective, the use of mitigation may fail to advance NEPA's purpose of ensuring informed and transparent environmental decisionmaking").

[170] *Id.* at 10.

[171] *See, e.g., Northern Plains Res. Council, Inc. v. Surface Transp. Bd.*, 668 F.3d 1067, 1085-86 (9th Cir. 2011).

[172] EA, *supra* note 77, at 14, 25,102.

[173] 40 C.F.R. § 1508.27.

in Monroe County, yet the FDA has not done its duty to informally consult FWS or NMFS to determine whether the species "may be present" in the action area.[174] Even without informal consultation under the ESA, FDA has not evaluated reasonably foreseeable impacts to the forty-three threatened or endangered species that it has identified within the County of the action area, which is required under NEPA.

Despite acknowledging the existence of forty-three at risk species, the FDA completely ignores the majority of those species. The EA states that the only species found in the physical vicinity of the proposed trial site is the Stock Island Tree Snail, but concludes that the snail will not be affected because none of its critical habitat overlaps with the domestic habitat of *Aedes aegypti*. The EA, however, erroneously assumes that no GE mosquitoes will escape the test trial site, and thus it need not evaluate the potential harm to federally listed species outside the field trial location. As explained above, this is a serious flaw, for any exposure to tetracycline may allow OX153A to survive the lethality trait and migrate beyond the field trial location and as "house" mosquitoes, the OX153A may reside in cars and trucks and be easily transmitted beyond Key Haven. Thus, threatened and endangered species "may be present" in the action area, despite not being present at the field trial location. Since it is reasonably foreseeable that OX153A could survive the lethality trait and migrate beyond the field trial location, FDA must analyze the potential and significant impacts to threatened and endangered species, particularly in Monroe County.

In addition, adverse ecosystem effects cannot be ruled out without assessing the impacts of consuming GE *Aedes aegypti* mosquitoes on all of the potential main predator species for adult and larval *Aedes aegypti* mosquitoes. These include species that are endangered, threatened, or of special concern, such as the Cape Sable seaside sparrow (*Ammodramus maritimus mirabilis*), piping plover (*Charadrius melodus*), and Bachman's warbler (*Vermivora bachmanii*). The EA makes the assumption that birds that eat mosquitoes will not be impacted because nucleic acids, including DNA, are presumed to be generally recognized as safe (GRAS) for food consumption.[175] However, that GRAS presumption for GE food applies only to food additives that are intended for human consumption, and only when the genetic modification does not present different structural, functional, or compositional characteristics than its traditional counterpart.[176] Mosquitoes are not considered food for humans, a genetic modification to mosquitoes are not regulated as a food additive, and the FDA has not determined that GE mosquitoes present no different structural, functional, or characteristics than wild *Aedes aegypti* mosquitoes. Therefore, the GRAS presumption would not apply to threatened or endangered species of birds that eat mosquitoes, and the FDA must still analyze the impacts that consuming GE *Aedes aegypti* mosquitoes may have on threatened and endangered species of birds.[177]

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[174] EA, *supra* note 77, at 43.

[175] EA, *supra* note 77, at 75 (citing 57 Fed. Reg. 22,984, 22,990 (May 29, 1992)).

[176] *See Alliance for Bio-Integrity v. Shalala*, 116 F. Supp. 2d 166, 176 (D.D.C. 2000) (citing 57 Fed. Reg. at 22,990).

[177] FDA further stated that birds and other mammals that prey on mosquitoes will not be impacted because studies on chicken and cows fed with glyphosate tolerant soybean, and pork, dairy cows, beef steers, and broiler chickens fed with recombinant *Bacillus thuringiensis* corn, indicated that scientists were unable to detect the recombinant DNA and concluded there were no safety concerns. However, the inability to detect the DNA does not mean there

FDA's failure to carry out its duties to consider the effects of its action on threatened and endangered species and their habitat constitutes a violation of NEPA.

IV. THE EA FAILS TO PROPERLY CONSIDER MIGRATORY BIRDS UNDER THE MBTA

In the EA, FDA fails to properly consider and disclose its obligations to migratory birds, never even mentioning its responsibilities under the MBTA. The MBTA prohibits the take of migratory birds entirely and mandates that the loss, destruction, and degradation of migratory bird habitat must be minimized. The release of GE mosquitoes has the potential to affect species of birds protected under the MBTA. Rather than determining whether the release would have adverse effects on species protected under the MBTA, FDA simply ignores this significant issue.

Further, FDA's consideration of impacts to migratory birds pursuant to its obligations under Executive Order 13186 is cursory at best. The FDA acknowledges that National Wildlife Refuges (NWRs) nearby contain migratory birds and other wildlife; however, the FDA has made the assumption that GE mosquitoes will not have an effect on those species because the prevalence of *Aedes aegypti* in both NWR's is rare, meaning there were less than 20 species in the total refuge.[178] A baseline determination of current *Aedes aegypti* populations in the NWRs does not relieve FDA of its duty to determine whether the impacts of releasing millions of GE mosquitoes will affect migratory birds. FDA makes no attempt to consider the actual impacts of the proposed action on these species, instead relying on assumptions to deny the potential for impacts.[179] FDA failed to provide any data or actually consider the risks to migratory birds. This constitutes a failure to take the requisite "hard look" at impacts to migratory birds under NEPA and could potentially lead to take under the MBTA.

V. RECOMMENDATIONS

CFS has identified numerous, significant gaps in FDA's EA. The proposed release therefore carries unnecessary risks and is premature. Prior to considering any application for open release of GE *Aedes aegypti* mosquitoes, FDA should require and consider the following additional information:

- Safety testing for consumption of GE *Aedes aegypti* mosquito adults or larvae by humans and wildlife, including children, pets and threatened and endangered species;
- Prior baseline assessment of wild *Aedes aegypti* mosquitoes;
- Modelling of population responses of wild *Aedes aegypti* mosquitoes to the proposed

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is no risk to the animals, and different genetic modifications have different impacts, meaning that glyphosate tolerant soybean or *Bacillus thuringiensis* corn could have different effects than OX315A. Thus, those studies do not relieve FDA of its duty to analyze the effects that consuming OX135A has on threatened and endangered species.

[178] EA, *supra* note 77, at 43-44.

[179] *Id*.

releases;

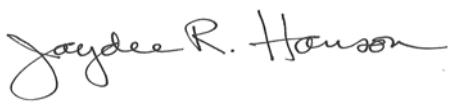
- Studies of dispersal of *Aedes aegypti* mosquitoes from the test site to other sites;
- Studies of dose responses of OX153A proposed for release to tetracycline and its analogues;
- Studies of insecticide resistance and disease transmission properties in the GE *Aedes aegypti* strain;
- Studies on human allergenicity to the proteins in the GE *Aedes aegypti* mosquitoes;
- Studies on effects on the GE *Aedes aegypti* mosquitoes on threatened and endangered species;
- Physically well contained caged trials of all GE *Aedes aegypti* mosquitoes;
- Laboratory studies of resistance mechanisms;
- Laboratory studies of antibiotic resistance;
- Physically well contained caged studies of the competitive effects on wild *Aedes aegypti* mosquitoes;
- Studies of the effects of releasing large numbers of the GE *Aedes aegypti* on populations of other mosquitoes such as *Aedes albopictus* and *Culex* species which carry human and animal diseases; and
- Independent replication of Oxitec laboratory results, including studies of proteins in saliva and larval survival rates in the presence of tetracycline contamination.

VI. CONCLUSION

FDA's EA is wholly inadequate and based on incomplete and inadequate science and analyses, lacks critical data and vital risk assessments, and ignores reasonably foreseeable significant impacts and uncertainties. The EA's conclusions are erroneous and indicate FDA's failure to properly evaluate the potential effects of this release under NEPA and the MBTA. FDA must conduct an EIS to fully evaluate the impacts of its proposed action, and failure to do so would be arbitrary, capricious, an abuse of discretion, and a violation of the statutes discussed herein.

We thank you for the opportunity to comment on this EA, but urge FDA to delay further consideration of this INAD until the deficiencies detailed herein have been corrected and until FDA has developed formal regulations for the oversight of GE animals and insects.

Sincerely,



Jaydee R. Hanson
Senior Policy Analyst

Ryan Berghoff
Legal Fellow

Exhibit A

to comments on Docket No. FDA-2014-N-2235

ACRE/11/M4

ADVISORY COMMITTEE ON RELEASES TO THE ENVIRONMENT
MINUTES OF THE 134TH MEETING OF ACRE AT NOBEL HOUSE, LONDON, THURSDAY, 1ST DECEMBER 2011

Present:
Prof Chris Pollock (chairman)
Prof Jim Dunwell
Mr Jim Orson
Prof Keith Lindsey
Prof Jeff Bale
Prof David Hopkins
Dr Ieuan Joyce
Prof Les Firbank
Dr Mike Bonsall
Prof Kathy Bamford

Invited expert:
Dr Mike Skinner

Assessors:

Dr Jonathan Davey	SASA
Dr Simon Warne	HSE
Mr Dave Jefferies	FSA

Defra:
Dr L Ball (secretary)
Ms S Brown
Dr S Popple
Mr M Rowe
Mr D Sherlock

Apologies were received from Prof Hails, Prof Peters and Prof Bullock. The chairman welcomed Dr Mike Skinner who was assisting ACRE in its assessment of a GM vaccine application. Dr Skinner is a senior lecturer in the Department of Medicine at Imperial College London and member of SACGM(CU).

The Committee was notified that Dr Kath Bainbridge has been on a career break since October but would be back in time for the next meeting.

1. Minutes of the 133rd meeting, 4th August 2011 ACRE/11/M3

The minutes were agreed with one amendment.

2. Policy update

2.1 Update on the national decision making proposals
Members were informed there had been two working groups under the Polish presidency but there had been little progress, with the debate remaining very polarised. This will go as a progress report to the December Environment Council and then pass to the incoming Danish presidency.

2.2 ECJ ruling on honey
This ruling had concluded that pollen was an ingredient of honey, so that there would be a requirement for GM labelling if GM content was greater than 0.9%. The Food Standards Agency leads on this but Defra is working closely with them because of the broader implications, including for field trials. There was also an impact on third country imports where there could be non-authorised GM content. The solution may be a change in legislation to rectify the situation, but the UK will continue to push for pragmatic and proportionate policies. This will be discussed in Standing Committee on 12th December.

3. Matters arising

Since the last ACRE meeting Member States have voted on 2 applications, for A5547-127 soyabean (ref. EFSA-GMO-NL-2008-52) and a renewal of 40-3-2 soyabean (EFSA-GMO-RX-40-3-2) to import and use GMOs as food and feed. As there was there was no qualified majority the applications have been referred to the Appeals Committee which is expected to consider them in January. This is the first time the new comitology rules have been applied to a GM food and feed dossier.

4. Matters agreed by circulation

Since the last ACRE meeting and prior to the vote at standing committee, ACRE's advice has been published on the application for A5547-127 soyabean. Advice has been agreed by circulation and published since the last meeting on 356043 (ref. EFSA-GMO-UK-2007-43) and MON87701 soyabeans (ref. EFSA-GMO-BE-2010-79). These applications are for food and feed uses, import and processing, excluding cultivation. ACRE agreed with EFSA's opinion, which was that these GMOs do not pose a greater risk to human health or the environment than their conventional counterparts, in the context of their proposed uses.

5. Update on notifications for authorisation under the GM Food and Feed Regulation (EC) No. 1829/2003 ACRE/11/P16

The secretariat informed ACRE that four new applications had been submitted under the GM Food and Feed Regulation since ACRE's meeting in August 2011. All four applications are for import and processing, food and feed use (excluding cultivation). These are FG72 soybean (ref. EFSA/GMO/BE/2011/98), Bt11 x 59122 x MIR604 x 1507 x GA21 maize (ref. EFSA/GMO/DE/2011/99), MON87705 x MON89788 soybean (EFSA/GMO/NL/2011/100) and MON88302 oilseed rape (ref. EFSA/GMO/BE/2011/101).

This is the first time that a GM soybean containing event FG72 has been notified under the GM Food and Feed Regulation. As such, ACRE was provided with a summary of the application. Given the extremely limited potential for environmental exposure of this GMO in the UK, ACRE advised that it would discuss the application after EFSA had published its opinion.

ACRE was informed that an application to cultivate Bt11 x MIR604 x GA21 maize (ref. EFSA/GMO/UK/2010/84) had been validated. The Committee will be asked to consider Bt11 x MIR604 x GA21 maize when the risk assessment for all single events has been finalised.

ACRE was also informed that there have been two new EFSA opinions on applications to cultivate GMOs: MON88017 maize and 1507 maize. EFSA's opinion on 1507 maize updates elements of its existing opinion. ACRE will be consulted on this by circulation. ACRE will be asked to produce final advice on MON88017 maize at its February meeting.

6. Application from BN ImmunoTherapeutics, Inc. under Part B of Directive 2001/18/EC to carry out a trial involving a therapeutic vaccine consisting of attenuated GM viruses – ref. 11/R44/01 ACRE/11/P17

ACRE invited Dr Mike Skinner from the Science Advisory Committee on Genetically Modification (for Contained Use) to join it in assessing this application from BNIT to release a GM vaccine (PROSTVAC V/F) at study sites in England and Wales. Dr Skinner is a virologist with particular expertise in poxviruses.

PROSTVAC V/F is designed to eradicate prostate serum antigen-expressing tumour cells in men with prostate cancer.

The vaccine comprises two live attenuated GM viral vectors. PROSTVAC- V is a modified, attenuated vaccinia virus whereas PROSTVAC- F is a modified, attenuated fowl pox virus. Both GMOs contain the same transgenes - a PSA gene and genes encoding three immunological co-stimulatory molecules (referred to as TRICOM).

ACRE was asked to advise on the risks posed to the environment and to humans that are not patients involved in the trial. Patient safety will be assessed by the MHRA who are responsible for clinical trial authorisations.

ACRE noted that the parental, non-recombinant strain of the vaccinia virus was derived from the same seed stock virus as the Dryfax vaccine, which was used to vaccinate humans against smallpox for over 200 yrs. The applicants have demonstrated through a neurovirulence test in mice that it is more attenuated than the mix of vaccinia viruses comprising the Dryfax vaccine. The parental strain of the fowlpox virus is a USDA licensed poultry vaccine widely used for vaccinating chickens against fowlpox. Therefore, ACRE considered that there was a history of safe use.

ACRE first considers the molecular characterisation of the GMOs, taking into account the stability of the genotypes and methods for their identification. It discussed hazards associated with the insertion of the transgenes into a gene (that has homology to the ankyrin repeat gene family) in PROSTVAC-F. The committee discussed evidence on the role of genes in the ankyrin repeat family in pox viruses. It concluded that the parental strain of PROSTVAC-F is highly attenuated and that knocking out this single gene will not restore it to the full virulence of the wild type virus. ACRE concluded that the molecular characterisation of these GMOs had been carried out to a high standard.

ACRE also considered evidence on the characteristics of these viruses that demonstrates that they are very unlikely to recombine with each other or with other viruses or to insert into the genome of the host cell.

ACRE then assessed the environmental risks associated with the release of these two GMOs by considering routes of potential environmental exposure and by considering the consequences for the environment and humans (who are not patients in this trial).

ACRE noted that PROSTVAC-F is replication defective in humans and that fowlpox is a virus that infects chickens and turkeys – it would not be expected to infect pet species or pigeons etc.

ACRE considered potential routes of shedding and likely duration. For both GMOs, it concluded that this will be restricted to the site of vaccination and that shedding will be minimised through intramuscular injection and, in the case of PROSTVAC-V, through bandaging the wound. It concluded that shedding from other sites is unlikely. The applicant noted that this could be associated with complications. However, ACRE noted the exclusion criteria proposed for patients by the applicant, which will significantly reduce the likelihood of complications.

In the case of PROSTVAC-V injection, ACRE noted that patients would have been vaccinated against smallpox previously. Consequently, patients are unlikely to develop sequel and replication is unlikely because the patients' immune systems will react against the vaccine.

The applicant describes how patients will dispose of contaminated material and dressings associated with the wound-site. ACRE was sceptical about patient compliance in returning this material to the clinic. Whilst the committee considered that the risk to the environment and to human health would be negligible if this material were disposed of in the sewer system or via municipal waste, it considered that procedures likely to result in higher compliance should be adopted. For example, requiring patients to sterilise/ disinfect material prior to disposal.

ACRE discussed the potential for transmission to healthcare staff involved in the trial and in particular, through needle stick injury. ACRE considered that the risk of harm was negligible; it noted safety data from previous clinical trials. However, the committee considered that the applicant should consider local best practice rather than referring to WHO protocol. It advised that procedures should be proportionate but clearly defined. For example, with respect to disposing of material from the trial.

In conclusion, ACRE considered that the applicant had provided a comprehensive and clear environmental risk assessment. However, it advised that if the release of these GMOs is approved that conditions for handling material involved in the trial should be described clearly.

ACRE will not finalise its advice until the public consultations on these applications (submitted to the English and Welsh authorities) have concluded on December 19th. ACRE will consider any representations that have a scientific content and reflect this in its written advice.

Action: ACRE to agree written advice to Defra and Welsh Ministers after the public consultations on the applications have concluded.

7. Research report: Environmental risks from research trials and marketing of genetically modified (GM) veterinary and human medicines ACRE/11/P20

ACRE was asked to comment on the draft report for the research project CB0303: Environmental risks form research trials and marketing of genetically modified (GM) veterinary and human medicines and particular to advise the secretariat of quality and robustness of the study.

ACRE highlighted some short-comings in the research and noted that the scientific language used in places should be improved. The committee provided advice on how to take the work forward.

Action: Secretariat to provide feedback from ACRE to project officer.

8. Authorisation of glufosinate ammonium-tolerant genetically modified MS8, RF3 and MS8 x RF3 oilseed rape – ref. EFSA-GMO-BE-2010-81 ACRE/11/P18

MS8/RF3 oilseed rape has received a number of authorisations for placing on the EU market. These cover import, processing and industrial food/feed uses but not cultivation. ACRE was asked to reconsider the advice it published in 2004 on this GMO in the light of new information produced by the applicant, EFSA opinions and information on oilseed rape imported into the UK. Committee members were asked to consider and comment on the likelihood and consequences of MS8/RF3 plants growing from spilled grain during import; and what if any, management/ monitoring measures would be appropriate.

ACRE considered that the risk to the environment posed by spilled grain was no greater than for non-GM oilseed rape. This was on the basis of three layers of evidence that in combination indicate a negligible risk:

- limited environmental exposure. This is because of the proximity of the crushing and processing plants to the receiving ports (i.e. only non-living material will be transported inland).

- if MS8/RF3 grain was spilled it would not persist or invade new habitats to a greater extent than non-GM OSR. In addition, ACRE noted that the use of glufosinate ammonium herbicides is not significant in semi-natural environments.
- feral oilseed rape populations in the UK are not self-perpetuating and therefore will decrease over time in semi-natural environments unless the grain is replenished through further spillage[1]

ACRE noted that the management guidelines for dealing with spillage supplied by the applicant were thorough.

In considering its previous advice, ACRE noted that coexistence measures were not within its remit because these concern choice rather than risk to the environment and to human health. It requested that the secretariat update its advice on MS8/Rf3 oilseed rape to better reflect its responsibilities.

Action: ACRE secretariat to amend existing ACRE advice in the light of ACRE's discussion and to circulate to the committee for comment and agreement.

9. Framework document governing the working relationship between Defra and ACRE and updated Code of Practice for Scientific Advisory Committees

ACRE/11/P19

In line with Cabinet Office and Treasury guidance, sponsoring departments are required to draw up a written agreement with their arms length bodies that sets out the relationship between them. Members considered a draft framework document which incorporated the existing terms of reference for ACRE and gathered together in one place existing advice on good practice. ACRE was broadly content with the draft framework document and asked for the Devolved Administrations to be consulted to ensure the relationship with them was accurately reflected.

ACRE members were given copies of the updated Code of Practice for Scientific Advisory Committees, published at the end of November. ACRE had contributed to the consultation on the draft of this document. The update has expanded and clarified advice from the previous Code but will not impact on ACRE significantly. The Code outlines good practice for committees, which ACRE is already following, but there are some new responsibilities imposed on the secretariat and additional advice on its role.

ACTION- Secretariat to circulate framework document to members for any comments and check with Devolved Administrations

10. Items for information

[1] Devos et al 2011 Feral genetically modified herbicide tolerant oilseed rape from seed import spills: are concerns scientifically justified? Transgenic Res 10.1007/s11248-011-9515-9

10.1 Oral update on post-market environmental monitoring

The draft report would be circulated shortly and discussed at the next meeting, in February. The methodology and preliminary findings have been presented at an EU working group. The focus of the report is on the use of existing surveillance networks.

10.2 EFSA scientific opinion – statistical significance and biological relevance ACRE/11/INF14

ACRE noted this document and commented that it was of a high quality.

10.3 Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA ACRE/11/INF15

This paper, published in Cell Research, had been identified by ACRE members as presenting interesting but as yet, uncorroborated results and conclusions. The committee noted that its relevance would be primarily for diet and health. The paper reports the first evidence that small regulatory RNAs, called microRNAs, produced by plants can regulate gene expression in mammals. The researchers detected plant-derived microRNAs produced in the blood and tissues of humans and other plant-eating mammals. One particular microRNA, MIR168a, which is present naturally in high concentrations in rice and cruciferous vegetables was found to inhibit a protein that helps to remove low-density lipoprotein ('bad cholesterol'). The researchers acknowledge in their paper that these findings are surprising.

ACRE considered that animal and plant material containing these molecules has been part of the human diet for hundreds of thousands of years and that humans have therefore evolved in the presence of such molecules. The committee noted that the current regulatory pipeline does not include any GMOs that have been modified to produce microRNAs. There are GM plants that have been modified so that they produce small silencing RNAS. ACRE considered that current risk assessment procedures were appropriate for addressing possible risks to the environment on a case by case basis.

A member of the secretariat for the Advisory Committee on Novel Foods (ACNFP) attended the meeting and informed ACRE of the discussion that had taken place during the ACNFP meeting on November 24th. Both committees agreed that further work would be needed to validate the findings and that they would track the issue with interest

Action: ACRE to keep appraised of research in this area and to coordinate with the ACNFP as necessary.

10.4 Potential trial of a 'genetically sterile' insect under the Contained Use Regulations ACRE/11/INF16

ACRE was informed of a request sent to HSE by a small biotechnology company, Oxitec who develop GM insects for use as agents of biological control. The company had queried whether trials involving insects modified to express a repressible dominant lethal trait could be carried out under the contained use regulations and if so, what physical barriers would be required. HSE consulted its Advisory Committee

on Genetic Modification (Contained Use). The Secretary of SACGM(CU) attended the ACRE meeting and summarised the SACGM's discussion. Defra is part of the competent authority for the contained use of GMOs and as such it had asked ACRE members as well as an external expert to comment ahead of SACGM's meeting, which was held on November 7th. ACRE agreed with SACGM in concluding that, in theory, the technology would confer a high degree of genetic containment. However, it considered that more empirical evidence was needed to confirm that this would be the case in practice; in particular, with regard to the level of penetration of the lethal trait into wild type populations and the rate of loss of the associated transgenic construct. The secretariat asked ACRE to consider what information it would expect to see if an application to release this GMO was submitted in the future. It was asked to consider whether there would be a conundrum in proving the requisite information i.e. whether data from open field trials would be needed to support applications to carry out such trials. ACRE did not consider this would be the case.

Dr Bonsall declared a conflict of interest as he had been working with the company, Oxitec Ltd, on this insect. He left the room while this item was discussed.

10.5 Statement complementing the EFSA GMO Panel scientific opinion on maize MON89034 x 1507 x MON88017 X 59122, to cover all sub-combinations - ref. EFSA-GMO-CZ-2008-62 ACRE/11/INF17

ACRE noted this paper to update EFSA's risk assessment on a stacked event, which now takes into account the sub-combinations of this event. The overall conclusion on the risk posed by this GMO has not altered.

10.6 Executive summary of an evaluation of the EU legislative framework in the field of cultivation of GMOs under Directive 2001/18/EC and Regulation (EC)No 1829/2003, and the placing on the market of GMOs as or in products under Directive 2001/18/EC ACRE/11/INF18

ACRE welcomed this as a useful contribution to the debate on the legislative framework.

10.7 Field-evolved resistance to Bt maize by western corn rootworm ACRE/11/INF19

ACRE noted this document, describing the first example of field evolved resistance in western corn rootworm.

11. Any other business

None

12. Date and time of the next meeting

Thursday 9th February at 10.30am in Nobel House.

ACRE Secretariat
December 2011.

Exhibit B

to comments on Docket No. FDA-2014-N-2235

Head of GM Policy and Regulation,
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defra
Department for Environment
Food and Rural Affairs

By Email only

Camilla Beech
Regulatory Manager
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71, Milton Park
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Oxfordshire
OX14 4RX

24 January 2012

Dear Camilla,

Further to the letter dated 5th December you received from Simon Warne at the Health and Safety Executive I am writing on behalf of Defra and HSE to further explain some of the legislative considerations for the trials you are considering. I hope that you find this additional information useful as you consider how to proceed.

Defra and the HSE, acting jointly as the UK competent authority, have considered your request for clarification as to whether your RIDL technology confers sufficient containment to allow for any or all of the three designs of experimental trial described in your background document dated 8 November 2011 to be carried out under the Genetically Modified Organisms (Contained Use) Regulations 2000 ("the Contained Use Regulations").

The contained use legislation applies to activities in which organisms are genetically modified or in which GMOs are used, and for which barriers are used to limit their contact with, and provide a high level of protection for, humans and the environment. If however the organisms are to be "released" within the meaning of Part VI of the Environmental Protection Act 1990 ("the EPA") then the proposed trial or trials may only be conducted in accordance with a consent granted by the Secretary of State under section 111 of that Act and the procedures set out in the Genetically Modified Organisms (Deliberate Release) Regulations 2002 ("The Deliberate Release Regulations"). In such circumstances the Contained Use regulations explicitly provide that they do not apply.

Under Part IV of the EPA, an organism is deemed to have been released by a person if that person deliberately causes or permits it to cease to be under his or her control and it enters the environment. An organism is under a person's control within the meaning of the EPA if they are kept contained by measures designed to limit contact with humans and the environment and prevent or minimise the risk of harm.

Following consideration of the information you have provided, we have concluded that in this particular case biological barriers alone are not sufficient to bring the proposed 'open' field trial within the scope of the Contained Use Regulations. This is because you would be deliberately allowing GMOs to cease to be under your control and they would have entered the environment within the meaning of the definition of a release in the EPA. As stated above such an open trial could only proceed pursuant to a consent granted by the Secretary of State. However, in the context of your proposals, we consider that the use of a closed polytunnel in addition to the biological barriers you have described could provide the necessary level of control for the trial to fall outside the definition of a "release". Alternatively, you may wish to consider undertaking further work in a closed off area within a large glasshouse as this approach might enable you to follow the decline of the frequency of the RIDL allele over more generations than would be possible in a polytunnel. In either case, depending on the adequacy of the containment measures selected (e.g. the closeness of the fit of any insect proof screening) we envisage that a trial could proceed as a contained use. It would thus not be necessary to apply for a consent to release in accordance with the meaning of the EPA and the procedures set out in the Deliberate Release Regulations (regulations 6 – 13). This is subject to a) your obtaining approval from the Food and Environment Research Agency (FERA) in relation to Plant Health legislation and b) your complying with the requirements of the contained use regulations, including as they relate to notification of premises.

Considerations of this nature need to reflect the specific circumstances on a case-by-case basis. Therefore, in reaching a view on the appropriate regulatory system for controlling trials that involve levels of physical containment that are intermediate between a closed polytunnel and an open field trial, further considerations are required. These would take into account the specific factors relating to, for example, the design of the trial and the number of moths involved. Such factors will determine the extent to which there will be environmental contact (before the genotype dies off in the population) and the consequent environmental impact.

It should be noted that if following trials there is a desire to market the GM moths for wider release into the environment, the procedure for obtaining a consent set out in Part III of the GMOs Deliberate Release Regulations must be adhered to before doing so. Such consents (known as Part C consents under Directive 2001/18/EC on deliberate release) may only be granted once the procedures concerning notification of other Member States and EU authorisation in the case of objection have been followed.

It would be helpful to be kept informed of how you intend to proceed in light of this guidance and the parallel advice from SACGM.

Yours sincerely,

Mike Rowe
Head of GM Policy & Regulation
Defra

Exhibit C

to comments on Docket No. FDA-2014-N-2235

GeneWatch UK

Rt Hon Caroline Spelman MP
Defra
Nobel House
17 Smith Square
London
SW1P 3JR

27 January 2012

Dear Secretary of State

Plans for experiments with genetically modified diamondback moths and other GM insects

We write regarding plans by Oxitec Ltd to conduct trials in Britain of genetically modified (GM) diamondback moths, as discussed by the Health and Safety Executive's Scientific Advisory Committee on Genetic Modification (SACGM) (Contained Use) on 8th November 2011, and the Advisory Committee on Releases to the Environment (ACRE) on 1st December 2011. We aware that Oxitec and Certis Europe have also entered a collaboration to develop GM tomato leaf miners, using the same technology, and that plans to release other GM insects may follow.

We are concerned that Oxitec has implied, wrongly, that releases of GM diamondback moths or other GM insects into open fields or polytunnels could be regarded as a "contained use" under the Genetically Modified Organisms (Contained Use) Regulations 2000. We wish to draw your attention to the definition in the Regulations:

"contained use" means an activity in which organisms are genetically modified or in which genetically modified organisms are cultured, stored, transported, destroyed, disposed of or used in any other way and for which physical, chemical or biological barriers, or any combination of such barriers, are used to limit their contact with, and to provide a high level of protection for, humans and the environment;

and to the following points:

(1) Oxitec's patented RIDL technology does not provide biological containment in the sense of the definition in the Regulations, because its GM male insects are intended to come into contact with and mate with wild females of the same species, which cannot be regarded as limiting their contact with the environment. This is also the case for insects released inside polytunnels or greenhouses;

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Phone: 01298 24300 ◆ E-mail: mail@genewatch.org
Website: www.genewatch.org

(2) This process is intended to substantially alter ecosystems in the sense of reducing the population of the target insect species: any such effects may impact negatively on beneficial species and on biodiversity and/or lead to increases in other types of pest. In the longer term, adaptive and evolutionary responses could also lead to adverse impacts. Proposed releases of such insects therefore constitute a deliberate release of a GMO and must be subject to a thorough environmental impact assessment and full public consultation;
(3) Most of the offspring of Oxitec's GM insects, which will be transgenic, are intended to die before reaching adulthood at the larval (caterpillar) stage. Large numbers of GM insect eggs and dead GM caterpillars will therefore remain on any crops (such as cabbages) at the experimental site (whether it is fully open or a polytunnel) and could potentially enter the food chain. Potential impacts on human health, such as allergies, therefore need to be addressed, as do issues of traceability and labelling or disposal of such crops;
(4) Problems with Oxitec's technology mean that some transgenic insects will survive to adulthood, when they will be able to fly and mate: evidence suggests that the numbers surviving could increase significantly in the presence of low levels of contamination with the antibiotic tetracycline, which is commonly used in agriculture.[1] This increases the risk of dispersal of the GM insects beyond the trial site and the likelihood that they survive to breed for multiple generations. Insects are easily dispersed hidden in crops or attached to any object, soil or clothing, as well as by flying.

Experiments involving DNA vaccines that are expected to be short-lived in the environment are treated as deliberate releases by ACRE. It is therefore difficult to understand on what basis deliberate releases of GM insects with comparable or longer lifespans and higher potential for reproduction and dispersal could be regarded as "contained use".

Attempts by Oxitec to conduct commercial releases of GM bollworms in the United States were prevented partly because US organic standards do not allow the presence of GMOs in organic crops. In Europe, the presence of GM insect eggs or larvae may breach both organic and conventional standards and therefore require labelling. In any event, consumers may wish to avoid such products, out of health concerns or because they have environmental or other grounds for opposing such production methods. If GM insects were to be used in agriculture, traceability and labelling of crops likely to contain GM insect eggs or larvae would also be important to address potential liability for unforeseen effects.

We therefore seek your assurance that:

(1) DEFRA does not support proposals to conduct trials of GM insects under "contained use" regulations;
(2) Impacts on consumer choice, trade and liability issues will be fully considered before a decision is taken on whether or not to allow any trials of GM insects to take place;
(3) A full environmental impact assessment and public consultation will be minimum requirements prior to any such trials.

[1] Nimmo, D, Labbe, G, Gray P. Oxitec confidential information: Eliminating tetracycline contamination. On: http://libcloud.s3.amazonaws.com/93/de/e/986/MosquitoDocOriginal.pdf

We would be very happy to meet with you should you require further information on this important matter.

Yours sincerely,

Dr Helen Wallace
Director
GeneWatch UK
Email: helen.wallace@genewatch.org

Pete Riley
Director
GM Freeze
Email: pete@gmfreeze.org

CC: Geoffrey Podger, Chief Executive HSE;

CC: Professor Christopher Pollack CBE, Chair ACRE.

Exhibit D

to comments on Docket No. FDA-2014-N-2235

Nobel House
17 Smith Square
London SW1P 3JR

Telephone 08459 335577
Email helpline@defra.gsi.gov.uk
Website www.defra.gov.uk

defra
Department for Environment Food and Rural Affairs

Dr Helen Wallace
GeneWatch UK
60 Lightwood Road
Buxton
SK17 7BB

Our ref: PO259907/RGW

23. February 2012

From the Secretary of State
The Rt Hon Caroline Spelman MP

Dear Helen,

Thank you for your letter of 27 January about genetically modified diamondback moths.

Since the 1950s there have been various examples around the world of harmful insect populations being reduced or eradicated by the release of males that have been sterilized by radiation. This is known as the Sterile Insect Technique (SIT), and it may be used to control insects that are agricultural pests or those which transmit human diseases. The British company Oxitec Ltd has developed a new method of altering the genetic make-up of insects so that they do not produce viable offspring, and this has potential advantages over the normal SIT approach using irradiation. To date, Oxitec has focused its efforts on producing GM mosquitoes to control the spread of dengue fever, and there have been trial releases of these insects in Malaysia and the Cayman Islands, with other trials currently under consideration in Brazil and the USA.

Oxitec now wants to explore the potential of its technology to control the diamondback moth, which is a serious crop pest in the UK and other countries. This could provide an alternative means of protecting crops than the use of chemical insecticides. The company is therefore looking at the possibility of undertaking trials of GM diamondback moths in England, and has sought initial advice from the Health and Safety Executive (HSE) and Defra about the terms on which these might be taken forward. This has been considered by the independent scientific expert groups that advise the HSE and Defra respectively on GM safety issues, and both have asked for the company to provide further information about its plans. When this has been received a view will be taken on what type of regulatory controls will need to apply for the sort of trial that Oxitec has in mind.

As with GM issues in general, our approach will be to ensure that an appropriate level of control is exercised so that human health and the environment are not compromised, whilst at the same time allowing for innovation and the development of safe new products.

Yours ever,

Caroline

CAROLINE SPELMAN MP

Exhibit E

to comments on Docket No. FDA-2014-N-2235

Subject: RE: FW: GMO transboundary notification: information request - Panama. ref. RFI4379
From: [redacted]
Date: 29/09/2014 12:14
To: "Helen Wallace" <helen.wallace@genewatch.org>

Dear Helen

We haven't had any new notifications since your last request.

Best wishes

[redacted]
GM Team
Defra
Area 3B Nobel House
17 Smith Square
London SW1P 3JR
020 7238 2051

From: Helen Wallace [mailto:helen.wallace@genewatch.org]
Sent: 29 September 2014 12:03
To: [redacted] (Defra)
Subject: Re: FW: GMO transboundary notification: information request - Panama. ref. RFI4379

Dear [redacted],

Could you please let me know if you have had any new transboundary notifications since my last request? And, if so, please supply me with copies of the documents.
In particular, have you received the transboundary notification for GM Mediterranean Fruit Flies to Brazil?

Thanks,
Helen

Dr Helen Wallace
Director
GeneWatch UK
60 Lightwood Rd
Buxton
SK17 7BB
Tel: +44-(0)1298-24300
Website: www.genewatch.org

On 22/07/2014 13:43, [redacted] (Defra) wrote:

> Dear Helen
>
> My apologies for not getting back to you sooner We don't have any new transboundary notifications but will send you documentation on the fruit flies once we have it.
>
> We have nothing further to report on the Panama export. I assume you received Lord de Mauley's letter of 21 June on this but please let me know if this did not reach you.
>
> Best wishes
>
> [redacted]
> GM Team
> Defra
> Area 3B Nobel House
> 17 Smith Square

[redacted]
GM Team
Defra
Area 3B Nobel House
17 Smith Square
London SW1P 3JR
020 7238 2051

-----Original Message-----
From: Helen Wallace [mailto:helen.wallace@genewatch.org]
Sent: 14 January 2014 14:05
To: [redacted] (Defra)
Subject: Re: GMO transboundary notification: information request - Panama. ref. RFI4379

Dear [redacted],
Could you please let me know if you have had any new transboundary notifications since my last request? And, if so, please supply me with copies of the documents.
We are aware that new open release experiments using Oxitec's GM mosquitoes have been approved in Panama (which should require a transboundary notification):
http://horacero.com.pa/nacionales/102215-minsa-aprueba-uso-de-mosquito-transgenico-en-el-combate-contra-el-dengue.html
Thanks,
Helen

Dr Helen Wallace
Director
GeneWatch UK
60 Lightwood Rd
Buxton
SK17 7BB
Tel: +44-(0)1298-24300
Website: www.genewatch.org

On 12/11/2013 11:06, [redacted] (Defra) wrote:

> Dear Helen
>
> Nothing new to report
>
> Best wishes
>
> [redacted]
>
> -----Original Message-----
> From: Helen Wallace [mailto:helen.wallace@genewatch.org]
> Sent: 12 November 2013 11:06
> To: [redacted] (Defra)
> Subject: Re: GMO transboundary notification: information request - Panama. ref. RFI4379
>
> Dear [redacted],
> Could you please let me know if you have had any new transboundary notifications since my last request? And, if so, please supply me with copies of the
> documents.
> Thanks,
> Helen
>
> Dr Helen Wallace
> Director
> GeneWatch UK
> 60 Lightwood Rd
> Buxton
> SK17 7BB
> Tel: +44-(0)1298-24300
> Website: www.genewatch.org

London SW1P 3JR
020 7238 2051

From: Helen Wallace [mailto:helen.wallace@genewatch.org]
Sent: 21 July 2014 16:16
To: [redacted] (Defra)
Subject: Re: GMO transboundary notification: information request - Panama. ref. RFI4379

Dear [redacted],
I don't think I have an acknowledgement for this request.
Please can you let me know the response?
Thanks,
Helen

Dr Helen Wallace
Director
GeneWatch UK
60 Lightwood Rd
Buxton
SK17 7BB
Tel: +44-(0)1298-24300
Website: www.genewatch.org

On 24/06/2014 15:04, Helen Wallace wrote:

> Dear [redacted],
>
> Could you please let me know if you have had any new transboundary notifications since my last request? And, if so, please supply me with copies of the documents.
>
> Open release experiments using (GM) Mediterranean fruit flies (*Ceratitis capitata*) were approved by Brazil's regulator CTNBio on 10th April: http://www.ctnbio.gov.br/upd_blob/0001/1880.pdf . We would like a copy of the transboundary notification documents, if a notification has been made.
>
> Open release experiments using Oxitec's GM mosquitoes began in Panama in late April/May: http://tvn-2.com/Noticias/Paginas/Liberados-300-mil-mosquitos-transgenicos.aspx
> We have still not been provided with a risk assessment for these experiments which meets the requirements of the regulations.
>
> Best wishes,
> Helen
>
> Dr Helen Wallace
> Director
> GeneWatch UK
> 60 Lightwood Rd
> Buxton
> SK17 7BB
> Tel: +44-(0)1298-24300
> Website: www.genewatch.org
>
> On 14/01/2014 15:45, [redacted] (Defra) wrote:
>
> > Dear Helen
> >
> > We haven't received any new notifications. I have checked with Oxitec and they have told me all shipments to Panama to date have been for contained use so the transboundary regulations have not applied. Oxitec will submit a transboundary notification as soon as it is required.
> >
> > Best wishes

On 17/09/2013 10:45, [redacted] (Defra) wrote:

Dear Helen

Nothing new to report

Best wishes

[redacted]

Department for Environment, Food and Rural Affairs (Defra)

Exhibit F

to comments on Docket No. FDA-2014-N-2235

From: Michael Doyle <mdoyle@keysmosquito.org>
Date: Tuesday, May 10, 2016 at 3:51 PM
To: Barry Wray <support@fkec.org>
Cc: Andrea Leal <aleal@keysmosquito.org>, Larry Hribar <lhribar@keysmosquito.org>, Beth Ranson <branson@keysmosquito.org>
Subject: Re: GMO Experiment Pesticide Usage Plan

Barry,
All control measures on Key Haven , with the exception of adding Oxitec males, would remain the same as normal FKMCD measures before, during and after the effectiveness trial.
As long as the treated area (streets d,e,f) and the untreated area (a) are treated the same other than Oxitec releases, then they are comparable for FDAs purposes.
- Mike

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On Mon, May 9, 2016 at 3:59 PM -0700, "Barry Wray" <support@fkec.org> wrote:

Hi, Michael,

I just wanted to get a quick clarification on the status of pesticide, or larvicide usage in Key haven during the proposed experiment. Would any treatments at all continue even if to address other species? If so, please explain at a high level what and why this wouldn't affect the experiment.

Thanks,
--

Barry Wray
Executive Director
Florida Keys Environmental Coalition
PO Box 205
Key West, FL 33041
www.fkec.org
barry@fkec.org
305-304-9898

Exhibit G

to comments on Docket No. FDA-2014-N-2235

Technical Opinion on Examination Request presented at the 171st Plenary Meeting of the National Technical Commission on Biosafety (CTNBio), held on April 10th, 2014

Procedure: 01200.002919/2013-77
Applicant: Oxitec do Brasil Participações Ltd.

1. Presentation

The Oxitec do Brasil Participações Ltda. (CQB 357/13) requests authorization for the commercial release of the OX513A lineage of *Aedes aegypti*, genetically modified for control – by population reduction – of the wild mosquito, carrier of the dengue virus (DENV).

Filed on 03/07/2013; Protocol 28300/2013; Previous Statement 3676/2013 published on 15/07/2013. The process received favorable opinions of the drafters Mário Hiroyuki Hirata, João Santana da Silva and Odir Antônio Dellagostin (in the Permanent Sector Subcomissions of Human and Animal Health) and Francisco José Lima Aragão and Fernando Hercos Valicente (in the Permanent Sector Subcomissions of the Plant and Environmental Areas).

The present report corresponds to an examination request of the commercial release process, solicited at the 170th Ordinary Meeting of CTNBio on March 13th, 2014, under the responsibility of Leonardo Melgarejo and Antônio Inácio Andrioli. Allan Edver (Permanent Sector Subcomissions of Human and Animal Health) and Orlando Cardoso (Permanent Sector Subcomissions of the Plant and Environmental Areas) who serve as advisors for CTNBio;

2. Initial Comments

The importance of the theme is unmistakable. The dengue fever advancing in the country, the emerging resistance – among vectors – to insecticides used, the harm to the health of the population , social and environmental economic costs and the need for innovative methods to combat the disease, which are more than well known, provide pressure for quick acceptance of alternative proposals.

The project is well informed and the three studies referred in Cayman, Malaysia and Brazil (Juazeiro, State of Bahia, during 2012 and 2013) present interesting preliminary results, showing it to be a promising alternative in the fight against dengue.

However, data is insufficient to assert a steady position, as is demonstrated below. In this perspective, the present report recommends the process should be put into DILIGENCE until the gaps referred to here are solved in a consistent manner.

Among the highlighted points, consider that:

2.1. The treatment provided by CTNBio deserves revision, for it differentiates itself from others in ways that are exceptional

The process regarding the Planned Release into the Environment (LPMA) that precede the request for commercial release are not yet concluded. It is possible to affirm this situation is unprecedented and the precedents already revealed threaten CTNBio's credibility. The LPMAs are instruments that provide inputs to commercial release processes and should be conducted in all ecosystems relevant to risk assessment and in all Brazilian biomes, in order to meet the demands of the current legislation.

What motives would justify the premature acceptance of preliminary data by CTNBio that, in this case, configures an anticipated assessment of the final reports, opposing the practices used so far, that are recommended by this commission? Furthermore, what circumstances would justify the fact representatives for the applicant of the technology have been invited to attend a meeting where the technology would be evaluated and, perform an exposure of merit that could be confused with institutional marketing and creating possibility of inducing CTNBio members to the approval of its demand?

If these conditions weren't enough to suspend the present assessment on their own, the impact of these concessions should be considered, regarding equality of treatment, considering all processes being currently evaluated and the ones to be evaluated in the future, forwarded by applicants of innovative technogies in the field of genetic engineering. From now on, are the requests for commercial release exempt from including completion reports of LPMA requests that sustain them?

What arguments justify the contempt for the Biosafety Law that demands LPMA studies in all Brazilian biomes? Would it be acceptable that allegedly "preliminary" information collected in Bahia, should attend to peculiarities from Pampa, the Amazon or Pantanal, where the environmental conditions that affect the dynamics of mosquito populations are clearly distinct? In addition, in this case, would it be prudent that

CTNBio continued breaching this requirement when a Brazilian court decision recently suspended the release of transgenic T-25 corn, based on the argument that no studies had been conducted in the North and Northeastern biomes, prohibiting its cultivation in those regions?

2.2. There is a glaring inadequacy of CTNBio protocols to assess winged insects risks

The implications of this matter are evident: when adequate guidelines to assess winged insects are not available, CTNBio is likely to decide on the unprecedented possibility to authorize the release of a living transgenic being that do not have effective restrictions in regards to spread, based on guidelines created for the purpose of assessing risks associated to cultivated plants. The fact that the vector to be controlled by transgenic mosquitoes that were to be eradicated from Brazil in the 1970s, is present throughout the country, does not make it a less severe issue, despite the mosquitoes' autonomous flight capability not exceeding 200 meters. Additionally, the fact that the basic control systems (release of males and sterility) possess recognized failures is anything but irrelevant. Even the mortality rate of larvae in the absence of tetracycline presents failure levels of 5%, in ideal lab conditions for research.

Therefore, the consideration that the valid guidelines have been met, does not seem sufficiently safe. They just do not apply to the problems in question. The applicant itself recognizes the serious fact that Normative Resolution No. 5 of CTNBio does not contemplate the peculiarities in the case, and does not offer an annex to specifically assess topics on health and environmental risks related to transgenic insects. It is worth noting that only cases related to "organisms consumed as food" and "microorganisms used as vaccines" are planned, concerning risk assessment efforts for human and animal health.

In this sense, since there are no normative instructions to assess the transgenic organism submitted by Oxitec, it is surprising that one of the opinions approved by the Permanent Sector Subcomissions of Human and Animal Health related to risks to animals that would eventually consume that mosquito affirmed that "the evaluation of these parameters was a result of complying with requirements on human and animal health, as present in CTNBio's Normative Resolution No. 5". In respect to the Precautionary Principle , the establishment of robust guidelines in advance would be wise, capable of guiding the evaluation process of transgenic insects, with effective conditions to decide their own implications for human health and the environment.

It has to be stressed that all opinions that support the request for commercial release (including the consolidated one) consider the OX513A mosquito **Risk Class I**, when the applicant company understands the issue as distinct and deserving of greater caution. On page 67 of the dossier presented by the applicant it can be read that "the risk classification of the *Aedes aegypti* OX513A was evaluated and in accordance with Normative Resolution No. 2 of November 27th, 2006, it was established as **Risk Class II**: moderate individual risk and low collectivity risk".

This topic should be clarified before any decision. In its statement, the company affirmed that it works with Risk Class II events, and it benefited from a Quality Certificate in Biosecurity Class NB-1 (in accordance with the evaluator Mário Hiroyuki Hirata) and developed the planned release into the environment based on CTNBio Normative Resolution No. 7, which is restricted to genetically modified, Risk Class I organisms. If the transgenic mosquito is classified as Risk Class II, the LPMA then followed, at least, the guidance of an "inadequate" Normative Resolution.

3. Risk assessment associated with the introduction of massive quantities of OX513A into the environment

The dossier presented by the applicant company presents a vast set of scientific data, complemented by a rich bibliographic review, covering aspects pertaining to the biology of the *A. aegypti*, associated risks on environementincluding the OX513A in trophic chains and potential consequences of releasing genetically modified females undesirably. However, the process lacks certain biosafety aspects:

3.1. The occupation of the ecological niche of A. aegypti by A. albopictus has not received sufficient attention from the dossier and the other evaluators

The large-scale release of OX513A, altering the reproductive performance of the *Aedes aegypti*, can trigger a population explosion of other vectors, with implications for adaptive dengue virus mechanisms in epidemiological terms and consequences for public health. Therefore, it is important to check the possibility of alterations in hosts, vectors, or even infectious profiles.

The data pointed to as preliminary were collected in three locations evaluated on a planetary scale, and suggested high effectiveness of the technology. The reduction of 95% of the local population of *A. aegypti* in

Brazil is impressive, after treating the area for six months (adult population estimated by marking-release-recapture statistics, according to page 36 of the dossier submitted by the company). These field results, in spite of the adversities of studies of this type would have surpassed even those obtained under controlled laboratory conditions. This successful endeavor should also be perceived as an additional reason for repeating tests.

The alterations made by releasing hundreds of thousand transgenic mosquitoes with the characteristic of letality passed down to *Aedes aegypti* descendants will benefit other insects. As local populations of *A. aegypti* compete with local populations of *A. albopictus* (species that have invasive ecologic characteristics) wouldn't the suppresion of the first favor a population explosion of the second?

Available references suggest *A. albopictus* is adapted to the peridomestic environment just as *A. aegypti*, where it feeds from human and animal blood, laying eggs in many natural and artificial water-accumulating containers (Hawley, 1988, quoted in Lambrechts et al., 2010). Scientific reports support the fact that up to the XVIII and XIX centuries, *A. albopictus* was the most frequent daytime biting species in the majority of the cities in Asia (Gilotra et al., 1967 quoted in Lambrechts et al., 2010), having since lost space due to conditions that benefited its main competitor. As the naval industry expanded (commerce, then tourism), *A. aegypti* started to dominate ecological niches occupied by *A. albopictus*, becoming progressively the main daytime biting species in some Asian cities. Urbanization conditions and *Aedes aegypti*'s greater adaptation to the urban environment (Macdonald, 1956 quoted in Lambrechts et al., 2010) were decisive for such changes, and tend to be eroded following massive releases of OX513A.

The inclusion of *A. albopictus* in the list of the world's 100 most invasive species leaves no doubts as to its agressiveness and potential to occupy that ecological niche. In other words: the almost complete suppresion of local populations of *A. aegypti* by the OX513A will possibly cause migration flows in local populations of *A. albopictus*, compromising the disease-reduction goal, for the simple fact that a new vector of the disease will occupy the ecological niche that was abruptly abandoned by the main competitor.

3.2. The ecological imbalance caused by mass introduction of the OX513A into the environment can cause implications for the epidemiological profile of the dengue virus, aside from transmitting other viral human and zoonotic diseases

In the dossier and opinions favorable to Oxitec's demand, a thesis on a smaller capacity/efficiency of the *A. albopitus* to transmit the dengue virus in an epidemic manner (compared to the *A. aegypti*) was found. Thus, this conclusion omits scientific literature which describes viruses' adaptation/mutation cases to other hosts and vectors. A more careful interpretation considers that evolutionary forces are at stake, highlighting mutation-selection pressures, which tend to stimulate responses to the dengue virus in the absence of its main vector (*A. aegypti*).

Some cases studied demonstrate that arboviruses could rapidly alter associations with hosts/vectors. For example, epidemics caused by the Venezuelan Equine Encephalitis virus (VEE) in several countries in Central and South Americas in the mid-1990s. According to Brault and collaborators, the Mexican epidemic in the 1993-1996 period was unleashed due to the virus adaptating to an alternative vector (with increased epizootic capacity), based on the substitution of a single aminoacid from a glycoprotein envelope (Brault et al., 2002 and 2004). According to Anishchenko et al. (2006), however, the epidemic/epizootic characteristic of the VEE would have been acquired/unleashed by a single mutation in viral strains only present (so far) in its enzootic form. It is possible to perceive in any of the hypotheses above that those studies point to high probability of alterations in the infectious profile of said viruses (starting from a single mutation), reaching high disease transmission capabilities in an epizootic/epidemic form.

Additionally, in the chikungunya epidemic in the island of La Réunion in the 2005-2006 period *A. albopictus* was the main vector, while that role is normally played by *A. aegypti*. Tsetsarkin et al. (2007 and 2009) concluded that a mutation in the CHIK virus was directly responsible for a significant increase of the patogen's inefectiveness, through a vector that was much involved in the transmission of the disease, *A. albopictus*. This mutation would have allowed the virus a greater dissemination efficency of the viral load in the mosquito's secondary organs and, consequently, greater efficacy in transmitting the disease to hosts.

Therefore, considering the hypothesis that mass releasing of the OX513 mosquito will cause mass occupation of the *A. aegypti*'s ecological niche by *A. albopictus*, this could cause changes in the dengue virus' epidemiologic profile, as well as in other viral diseases (human, animal and zoonotic). These are some of issues that were not examined in the dossier.

A reduction in the detected dengue cases can be expected at first. They would then occur sporadically and non-epidemically, due to the slow occupation efforts of ecological niches and the *A. albopictus*' lesser competence (compared to the *A. aegypti*) when transmitting the disease. Next, the suppresion of the virus' main epidemic vector will exert selective pressure potentially favorable to genetic mutations of local strains of the dengue virus, causing implications in the epidemiologic profile of the disease. In these conditions, considering the available scientific literature, we can elaborate at least two hypotheses:

a) Hypothesis based on the experience adquired with the Venezuelan Equine Encephalitis virus

Mutations in the dengue virus strains - which are present today in association with *A. albopictus* but without the capacity to unleash epidemics - could occur. These mutations could infect other vectors which are more prone to causing epidemics. Theoretically, any of the several species of mosquitoes that are vectors for arboviral pathologies present in Brazil (whether from the *Aedes* gender or a genetically close configuration) could take on this role. That species would then become a new epidemic vector for the dengue virus, cohexisting with the *A. albopictus* despite its competitiveness in urban zones.

b) Hypothesis based on the experience acquired with the recent epidemic caused by the chikungunya virus

Mutations in dengue strains that would allow *A. albopictus* to become a highly efficient transmission vector could occur, getting around the immunological properties provided by the symbiote bacteria Wolbachia (as it was with the CHIKV). In that case, *A. albopictus* would become the dengue virus' main epidemic vector.

In both cases, a new epidemic vector for the dengue virus would replace *A. aegypti*, followed by new risks. In said conditions, the change in vector would mean alterations in the infectivity mechanisms of the dengue virus itself, making its control by health agencies more complex.

Additionally, mass releases of the OX513A into urban zones could favor the entry of other human, animal and zoonotic viral diseases, which do not occur today thanks to the occupation of the ecological niche by *A. aegypti*, that is not a vector for these diseases. Considering that *A. albopictus* on its own, facing the current conditions, it is possible to speculate on risks

involving the whole set of viral diseases, whether human, animal or zoonotic which that species hosts.

Considering the predictable hypothesis that some CTNBio members shall take the occurrence of mutation-selection processes as highly speculative, we draw attention to the fact that the greatest part of RNA-based viruses have a mutation frequency so elevated that it could reach 10E-4 (0.0001) mutants per nucleotide, according to Weaver et al. (1993). In the case of the EEV epidemic, Anishchenko et al. (2006) estimated that the mutant capable of creating an epidemic amplification (having suffered only one mutation – as in the chikungunya epidemic case already referred to) could be produced from the moment the total population of VEEV reached 10E4 (10,000) individuals (which represents a relatively small population for arboviruses).

These risks have been approached superficially in the dossier, and the favorable opinions on the commercial release of OX513A mosquitoes do not comment on them very much. The applicant and CTNBio's evaluators who are favorable to the applicant focused on the *A. aegypti*'s biology (adaptation capacity to the DENV and other viral diseases, especially), and did not assess the risks associated with the colonization of urban areas treated with the OX513A by the *A. albopictus* and other vector species.

It is a known fact that the *A. albopictus* is susceptible to infection and is capable of transmitting most viruses that have been tested on it. The list includes 8 alphaviruses, 8 flaviviruses and 4 bunyaviruses, representing the three main types of arbovirus that include human pathogens (revised in Paupy et al., 2009). In this sense, besides transmitting dengue, *A. albopictus* also transmits yellow fever and the chikungunya virus (Hochedez et al., 2006), as well as other viral diseases. It is worth noting the recent chikungunya epidemics in the Indian Ocean islands (especially La Réunion), in Central Africa (Gabon, among other countries) and in Italy, derived from the *A. albopictus* vector (Lambrechts et al., 2010).

Furthermore, *A. albopictus* feeds on a vast variety of animal species, and is recognized as a vector with high potential for transmitting zoonotic pathogens (from animals to humans). This is exactly why the La Cross and Eastern Equine Encephalitis (EEE) viruses are major causes for concern for public health care in the USA. The quoted authors also warn that *A. albopictus* deserves special attention in the South and Central Americas, for it is a vector of yellow fever and Venezuelan Equine Encephalitis viruses. At this point, it is worth noticing that the EEE, VEE and WEE (Western Equine Encephalitis) viruses are present in Brazil (Kotait et al., 2006;

Figueiredo, 2007). It is also worth noticing the West Nile virus (already detected in Brazil, as it is informed on page 350 of the dossier), although it has never caused an epidemic in Brazil. The virus is responsible for a zoonosis that's also transmitted to humans by *A. albopictus*.

Therefore, given the evidence presented in scientific studies, it is necessary to examine the possibility that the abrupt emptying of the ecological niche occupied by *A. aegypti* will tend to strengthen the invasive capacity of local populations of *A. albopictus*. Its implications aren't restricted to the dengue fever, for they extend to other arboviral diseases and several zoonoses that could be brought from peri-urban zones into urban zones. In this sense, considering the Precautionary Principle , this issue needs to be addressed more carefully.

3.3. The dossier presented by the applicant and the favorable opinions tend to minimize the consequences of ecologic disturbances for public health care

The applicant requests that the "target species of biological control" is the *A. aegypti* and in this perspective, elaborates answers for item E 1 in Annex IV of RN5 (p.560). However, the revelance of the matter is in the fact that the dengue fever is a viral disease of dramatic connotations. Thus, the target species only acquires practical sense regarding controlling the dengue virus, so the *Flavivirus sp. (DENV)* would be the target species for Biological Control.

Therefore, the company provided answers that approach the real problem indirectly, and that were wrong for a great deal of the subjects presented in item E. In these conditions the process is weak, omitting health risks associated with the occupation of the *A. aegypti* ecological niche by the *A. albopictus*, as well as possible consequences stemming from this fact, in terms of eventual viral adaptations (of the DENV and other human and animal viruses) and its implicatios, like new epidemics/epizootics and the increased complexity of treatment systems.

On the other hand, the applicant approached this question in a partial manner in item 2.5 of the dossier, where it refers to the "evaluation of the substitution potential for other pathogenic vectors" (p. 338). At that time, the applicant distorts the issue, minimizing its probability tof occurence as well as potential consquences. It literally affirms that: "however, there's still a slight risk that the *A. albopictus* takes over the ecological niche abandoned by the *A. aegypti*.", p.340. But, as we have explained earlier, the probability for this to occur in context, seems to range from "high" to

"moderate". It is worth noting that the group of specialists created within the scope of the *Capacity Building for Implementation of Malaysia's Biosafety Act 2007* project, has pointed out that the risk associated with the *A. albopictus* occupying the ecological niche is moderate (Beech et al., 2009).

The company further states that "the *Aedes aegypti* is an invasive species in Brazil; it was eradicated and returned in the 1970s. As consequence, since the insect does not have a vast history in the country, its suppression or local elimination might be considered a reversion to the pre-introductory stage of the species" (p. 338). This assertion is obviously a mistake. It does not only disregard the set of socio-environmental changes that took place over the last 40 years, with its implications relating to changes in the species' habitat, but it distances itself from the geographic expansion of *A. albopictus*. In addition, it ignores the revolution in urbanization, in means of transportation, in animal breeding systems, in the agroindustries around urban centers, in the standardization of rations and in tetracycline usage, among other factors related to this case of viral epidemiology. It would be naive to assume the specific and abrupt exclusion of *A. aegypti* locals populations today would simply reconstruct the same conditions observed in the 1970s, in terms of epidemiologic risk of viral diseases, including dengue fever.

The company also states that "the possible adverse effects for removing *A. aegypti* aren't specific to the use of OX513A mosquitos, and would apply to any effective methods of mosquito control. Therefore, it is not a new issue". Once again we are facing a piece of information that is clearly mistaken.

We have in our hands an unprecedented situation where, in terms of history of epidemiology, a technology seems capable of eliminating 95% of the local individuals of a specific species (*A. aegypti*) in the short period of 6 months. The control methods were, so far, unspecific, and systemically hit all mosquito populations of the majority of species (if not all) present in the treated area.

Concerning the possible consequences of ecological niche occupation by *A. albopictus* at the sites where the OX513A is to be mass released, the company affirms that "an important recent revision concluded that *A. albopictus* is a lot less effective as a vector for the dengue virus than *A. Aegypti"* and that "Lambrechts et al. (2010) clarified several aspects by observing lineages of *A. albopictus* becoming more susceptible to the dengue virus after various generations created in a laboratory and that,

furthermore, lab studies have the tendency to overestimate the role of this species as a vector for the dengue virus". In this aspect the available scientific bibliography suggests the transmission capacity of the DENV to humans (from *A. albopictus*) might derive from the presence of a symbiotic bacteria – of the Wolbachia genus – that hosts itself in *A. albopictus* individuals. That condition, representing a barrier for the infection of these mosquitoes by the DENV and other arboviruses, reduces its potential to transmit diseases to humans. The recent chikungunya epidemics have shown the arboviruses to be capable of avoiding immunological barriers of *A. albopictus*, - which has become the main disease vector in these specific cases, replacing *A. aegypti*.

On the same topic, the applicant hurries to conclude that "both *A. albopictus* and *A. aegypti* are capable of transmitting viruses and pathogens, but there is no reason to think the replacement of *A. aegypti* by *A. albopictus* might have any negative effect upon human health or the environment (Gratz, 2004; Lambrechts et al., 2010; Moore and Mitchell, 1997)". At this stage, one can notice contempt in regards to the knowledge provided by the chikungunya epidemics – and the alterations in epidemiologic transmission profiles – contradicting references quoted in the dossier to support this conclusion. Lambrechts et al. (2010) indeed conclude – on the natural increase of the *A. albopictus* distribution zone – that this species could present lesser risks in relation to DENV transmission in its epidemic forms, in comparison to *A. aegypti*. But they also concluded that "however, we can not dismiss the fact that at some future date, the occupation of territories by *A. albopictus* will be followed by the virus adapting to this species of vector mosquitoes [*A. albopictus*], invasive and in constant effective increase, followed by a global reemergence of chikungunya among other arboviral diseases". It is worth noticing that the expression "at some future date" should be interpreted in the context hereby described, where the occupation of territories by the *A. albopictus* in "natural" conditions is analyzed, where there is an intense competition between the two species, and not in a context where 95% of the *A. aegypti* pertaining to local populations would be supressed in 6 months.

Therefore, once again: the mass release of OX513A mosquitos shall prevail, unprecedented in the establishment of large and perennial populations of *A. albopictus* in the urban zones, which are normally competition areas against the *A. aegypti*. Alterations on the main competitive species' fitness that are not very deep shall, doubtless, modify the dynamics of the populations of *A. albopictus*. In parallel, altering the fitness of the main vector for specific diseases will also change the dynamics of viral populations which will be unable to complete their

reproductive cycles, favoring any mutation capable of rebalancing their infestation levels in those areas. The VEEV and CHIKV examples picture the high capacity (or in evolutionary terms, "probability") of the arboviruses to change hosts and/or alter the vectoral competence of specific species, including *A. albopictus*.

Finally, it is worth mentioning that, at no time does the dossier evaluate the potential for transmitting zoonoses and epizootics for local human and animal populations, respectively, through *A. albopictus*. This species forms an efficient bridge to conect viral diseases from peri-urban zones to the urban zones to be occupied by it.

The risks for public health in mass releasing OX513A into urban areas due to the occupation of *A. aegypti*'s ecological niche also seem not to be appropriately considered in the favorable opinions submitted to analysis by CTNBio. Doctor Fernando Hercos Valicente, for example, dismisses these risks, affirming that "occupying empty niches left by a different species, in the case of the *Aedes albopictus* which can also be a vector species, is difficult to occur". That is because " *A. albopictus* is essentially wild and only appears at cities close to woods or large gardens with a great number of trees. It never invades the extense areas of the city, far from important plant coverage". These affirmatives could be easily rejected based on the current knowledge on the ecology of *A. albopictus*. According to Lambrechts et al., 2010 and references quoted, *A. albopictus* can occupy large urban areas, especially in the absence of *A. aegypti*. The statements also neglect the ecologic consequences, in terms of population dynamics, of quick and abrupt supression of the *A. albopictus*' main competitor.

On the other hand, the applicant company emphasizes the risks associated with the occupation of the ecological niche by *A. albopictus*, and recommends the monitoring of these populations. However, it is suggested that this surveillance effort takes place only after the commercial release of OX513A has been approved. What is the justification for analyses in of such great importance to take place only after the commercial release has been approved?

In these evaluators' perspectives it is unnacceptable to delay the data collection to after the approval, for it should result from field studies requested by the Biosafety Law in all relevant biomes. This data should be provided to CTNBio in the dossier that requests the comercial approval of the event. Among the omissions which are necessary for a solid decision, we highlight that the rates and recolonization profiles of areas where the

OX513A was/will be released are not informed/known, both to *A. aegypti* and *A. Albopictus* populations.

It is surprising that in this request for the commercial release of a transgenic insect, the qualitative and quantitative presence of the second species to be impacted the most – *A. albopictus* – is no longer analyzed, and no bibliographic references nor field studies approaching this issue exist. These omissions reveal a structural failure in this commercial release process: the absence of CTNBio guidelines that are coherent with the risks involved in this kind of release.

Lastly, and still relevant, these evaluators consider that the dossier fails by not presenting information relative to the potential of epidemiologic adaptation of the main human, animal and zoonotic viral diseases in the *A. albopictus*, also considering the context at play, when the main vector tends to disappear almost completely from the treated areas, in an extremely reduced time interval.

4. Conclusion

At first, we should reflect on the potential consequences of the administrative mistakes that occurred during this process of commercial release, highlighting:

a) the absence of the Conclusion Report for Planned Release into the Environment (LPMA);

b) contradiction with the RN2 when considering the OX513A as Risk Class I in the LPMA processes and Quality Certificate in Biosecurity;

c) contradiction with the Biosafety Law, having submitted only two LPMAs in Brazil, while the referred law demands the establishment of at least one LPMA in each biome.

Second, it is worth noting the set of unprecedented difficulties CTNBio had to face when assessing this first transgenic insect. The evaluator does not have specific guidelines to assess health-related risks. Besides, the company has made a mistake when considering the target species for biological control was in fact the target insect for the transgenic project (or the commercial release), which also harmed the environmental assessment.
Furthermore, the mass introduction of the OX513A mosquito illustrates the difficulty of socialization between areas of expertise considered to be separate at CTNBio. The position of the evaluators from the Permanent

Sector Subcommissions on Human and Animal Health seems to grant them greater "legitimacy" or "competence" when assessing alterations in the epidemiologic profiles of viral transmissions, after the disturbances in the dynamics of local populations of the main vector and its competitor took place. On the other hand, the Permanent Sector Subcommissions on Plant and Environmental Areas seem to be endowed with greater legitimacy or competence to assess questions pertaining to the population dynamics of insects. Also, the technical decisions will be transformed into conclusions that do not depend on knowledge and arguments involved, for they will be based on the number of votes.

We highlight that this type of decision becomes more fragile as it gets influenced by the procedures, by the non presentation of previous studies, by the admission of the interested party on arguments conducted before some (and not other) members and in the absence of the contradictory views. Evaluators from the Permanent Sector Subcommissions on Human and Animal Health state that they have not addressed environmental factors for there are two other Subcommissions charged with that task. The evaluators from the Permanent Sector Subcommissions on Plant and Environmental Areas, on the other hand, state that they have not addressed human and animal health aspects because there are two other Subcommissions charged with that task. Thus, the existence of a decision facilitator agreement is clear, distorting analytical procedures and running away from the scope of responsibilities attributed to CTNBio.

Finally, contrary to the evaluators who favor the commercial release of the OX513A, we examined a possible route for harm, not treated properly in the process. The damage could be caused through reemergence of human and/or animal viral epidemics of zoonotic origin (or not), pre-existing (or not) to the mass release of the OX513A, with a significant degradation of public health in these areas, as well as potencial negative social and economic consequences for the municipalities affected. The route will be carried out by *A. albopictus* occupying the ecological niche – resulting from mass releasing the OX513A mosquito – with associated changes in the epidemiologic profile of animal, human and zoonotic viruses, providing these with greater infectivity, through exchange of vectors and/or circumvention of immunological barriers of secondary vectors.

In this context, aggravated by the non-fulfillment of the current legislation; the non-existence of evaluation protrocols adequate to the assessment of risks involving flying insects; the insufficiency of studies presented; and the non-inclusion of final results from the field studies approved by CTNBio, we consider that the commercial release of OX513A in these

conditions, presents relevant and irreversible risks for both health and environment, whose probability of occurrence ranges from high to moderate. We recommend the process should be put into DILIGENCE so it can be complemented, and that it should return for analysis in accordance with the guidelines to be established by CTNBio.

5. Forwarding Procedures

Once the diligence is approved, the applicant company shall:

a) Annex the Conclusion Reports on LPMAs carried out in Brazil;

b) Fulfil the Biosafety Law by performing LPMAs in all Brazilian biomes;

c) Provide extensive argumentation based on the published scientific literature and on the information obtained from the LPMAs, on the recolonization rates of the ecological niche left empty by the *A. aegypti*, monitoring the *A. aegypti* and *A. albopictus* species, as well as other vector species for human, animal and zoonotic arboviruses common to the region;

d) Provide extensive argumentation, both quantitative and qualitative on the capacity of epidemiologic adaptation of arboviruses – especially the ones with epidemic and epizootic profiles – to the main secondary vectors present in urban and peri-urban zones in Brazil.

In parallel, we request the Presidency of CTNBio to forward an evaluation request on the social and economic risks related to the OX513A technology to the National Biosafety Council (CNBS), taking into account the fact that information contained in the process suggests a negative/moderate cost-benefit ratio for the municipalities and general public health care services. We point out the human behavior is highlighted among the factors that unleash diseases. Recent studies associate epidemics to cases of assymptomatic infections, involving non-epidemic serotypes, where the role of human dengue reservoirs is not well understood in the dynamic of the disease. In this sense, several authors considerer human populations can disseminate the dengue virus more effectively than mosquitoes (Morrison et al., 1998; Harrington et al., 2005; Morrison et al., 2010; Honório et al., 2009), which raises scientific questions on the real advantages of controlling only the main vector in specific areas. In this sense, it is important to notice that the head of The Neglected, Tropical and Vector Borne Diseases Unit from the Pan American Health Organization (OPS), Luis Gerardo Castellano, said that there is not enough scientific evidence to

clarify the benefits and advantages the genetically modified mosquito could bring to countries (Castellano, 2014).

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