PREPARING FOR FUTURE PRODUCTS OF BIOTECHNOLOGY

A BRIEF SUMMARY

National Academies of Sciences, Engineering, and Medicine

Chris A Wozniak, Ph.D.

Biopesticides and Pollution Prevention Division

Impetus for the Study

Jul'15	Apr'16	Jul'16	Oct'16	Jan'17	Mar'17

2015 White House Memorandum calling for modernization of the biotechnology regulatory system:

- Update the Coordinated Framework
  - Clarify the roles and responsibilities of the agencies that regulate to "products of biotechnology"
- Formulate long-term strategy for biotechnology regulatory system
  - Efficiently assess risks associated with future products of biotechnology
  - Support innovation, protect health and environment, promote public confidence in regulatory process, increase transparency and predictability, reduce unnecessary costs and burdens
- Commission an external, independent analysis of the future landscape of biotechnology products

Outline of the Report

1. Introduction and Context
2. Emerging Trends and Products of Biotechnology
   - Setting the Stage: Understanding the Key Drivers for Future Biotechnology Products
   - Future Biotechnology Products
3. The Current Biotechnology Regulatory System
   - Overview of U.S. Regulatory System
   - Consumer and Occupational Safety
   - Environmental Protection
4. Understanding Risk Related to Future Biotechnology Products
   - Risks from Future Biotechnology Products: Similarities to the Past and Gaps Going Forward
   - Existing Federal Capabilities, Expertise, and Capacity
5. Opportunities to Enhance the Capabilities of the Biotechnology Regulatory System
   - Consistent, Efficient, and Effective Decision Making for Future Products of Biotechnology
   - Technical Toolbox and Capabilities for Risk Assessment and Regulatory Science
6. Conclusions and Recommendations

STATEMENT OF TASK

- Describe the major advances and the potential new types of biotechnology products likely to emerge over the next 5–10 years.
- Describe the existing risk-analysis system for biotechnology products including, but perhaps not limited to, risk analyses developed and used by EPA, USDA, and FDA, and describe each agency's authorities as they pertain to the products of biotechnology.
- Determine whether potential future products could pose different types of risks relative to existing products and organisms. Where appropriate, identify areas in which the risks or lack of risks relating to the products of biotechnology are well understood.
- Indicate what scientific capabilities, tools, and expertise may be useful to the regulatory agencies to support oversight of potential future products of biotechnology.

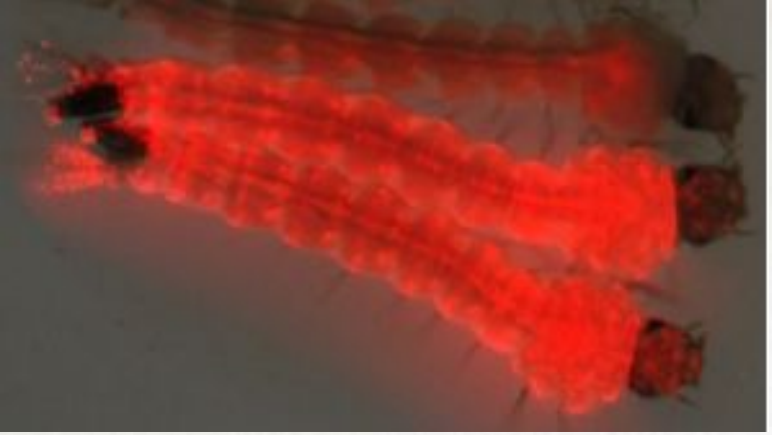
FUTURE PRODUCTS OF BIOTECHNOLOGY

TECHNOLOGY / PRODUCT	TIMEFRAME
Male-Sterile *Aedes aegypti* Mosquitoes for Population Suppression	In review
Wolbachia-based Mosquito Population Suppression	In review
Gene Drives – Plants and Animals (Conservation and Agricultural)	5 – 10 years
American Chestnut with Resistance to Chestnut (*Cryphonectria)* Blight	Soon
Microbial Consortia – Bioremediation, Microbiome Modification, BioMining	Soon
Synthetic dsRNA Sequences for Pest Control	Very soon
Genetically Recoded Organisms – Altered Codons and Amino Acids	3 – 5 years
Gene edited plants, microbes, animals	1-2 years

MOSQUITO POPULATION REDUCTION

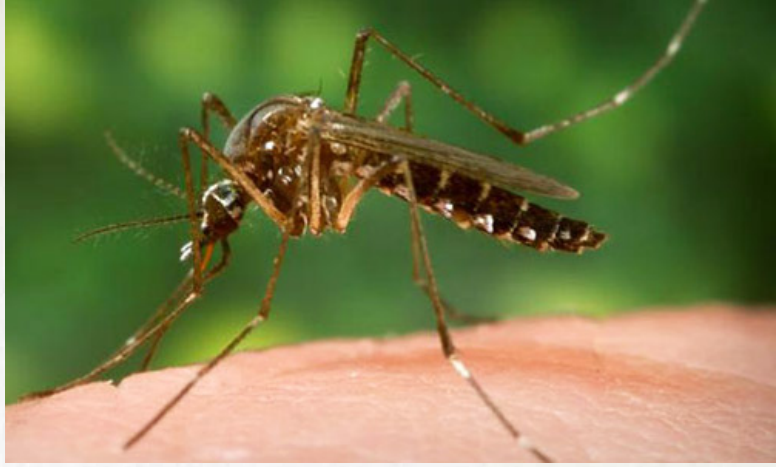
- Two different approaches which accomplish the same thing:
- 1) *Wolbachia pipientis*, a bacterium living within the mosquito host
  - *Wolbachia* males give rise to non-viable eggs when mating with native mosquitoes
- 2) Mosquito genetically engineered to express conditional lethality (sterility)
  - GE males give rise to viable eggs when mating with native mosquitoes, but larvae do not molt to become adults

- Both technologies work through release of male mosquitoes which are incapable of reproducing and do not remain in the environment



Oxitec OX513A *Aedes aegypti*

Genetic Sterility



- Tetracycline antibiotic is necessary for the GE mosquitoes to grow and reproduce
- In absence of tetracycline, leads to gene expression being disrupted– lethal!
- A Red Fluorescent protein marker is used to track the larvae which result from mating with the released GE mosquitoes
- Population suppression is stated goal of environmental release of sterile males

OXITEC OX513A MALE STERILE MOSQUITOES

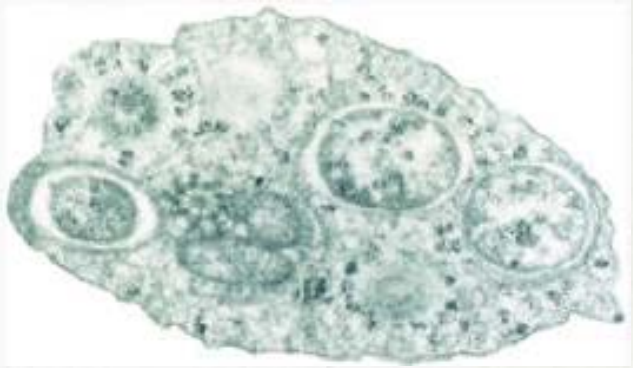
- Oxitec mosquitoes have credible efficacy data from Brazil, Cayman Islands, Panama and Malaysia
- Released 2-3x per week
- Requires ongoing release and monitoring
- No environmental persistence of genetic construct / mosquito; GE males last 2-3 days

FEDERAL OVERSIGHT OF GE MOSQUITOES

- FDA Guidance document #236 – public comments
  - Awaiting signature from FDA authorities
- Will provide for Federal Government oversight at some point where primary goal is mosquito population suppression
- EPA provided technical support to the EA / FONSI effort for FDA-CVM along with CDC

WOLBACHIA-BASED MOSQUITO MANAGEMENT

- *Wolbachia* – intracellular bacterium
- Cannot be cultured
- Present in ~60% of all insect species
- Naturally present in some mosquitoes
- Maternally inherited
- Not naturally present in *Aedes aegypti,* the yellow fever mosquito



TREATMENT OF FIELD SITES WITH *WOLBACHIA*

- *Wolbachia* -infected male mosquitoes released
- Treatment of entire neighborhoods with few application sites.
- One release site covers ~200 yards (diameter)
- Application 1-2x per week throughout mosquito season
- Eggs resulting from mating of *Wolbachia* males and wild type females don't hatch
- Population suppression is primary goal
- No establishment of *Wolbachia*-infected population

REGULATORY STATUS OF *WOLBACHIA* IN US

- *Wolbachia*-infected mosquitoes are treated as microbial pest control agents under FIFRA
- Field trials have been underway for a couple years in California, Kentucky and New York with *Aedes albopictus* ZAP
- Field trials (Section 5 EUP) approved for *Ae. aegypti wAlbB* – FL and CA
- *Ae. albopictus* ZAP – pending Section 3 registration in 2017

GENE DRIVES - REGULATION

- The skewed inheritance of a particular **gene** to increase its prevalence in a population; non-Mendelian inheritance patterns
- May function in sexually reproducing organisms (not viruses or bacteria)
- No gene drive carrying organisms are currently pending at EPA for approval
- Which agency regulates a particular gene drive will depend on the specific characteristics of that gene drive : host combination
- There is a self-imposed moratorium presently on any field releases
- Gene Drives: National Academy of Sciences, Engineering and Medicine report: 2016
  - https://www.nap.edu/catalog/23405/gene-drives-on-the-horizon-advancing-science-navigating-uncertainty-and

GENE DRIVES FOR RODENT CONTROL

- IslandConservation.Org has performed considerable research on gene drives for management of mice and rats on islands infested with rodents
- Rodents with an engineered gene drive may lead to establishment of a population which produces only male offspring.
- A naturally occurring gene drive in the mouse genome could cause almost exclusive bias male-only inheritance.
- The devastation of invasive rodent populations is extensive!
- Island release of a gene drive mechanism provides for a meaningful isolation of the organism(s) and their effects, as well as isolation of the test organism

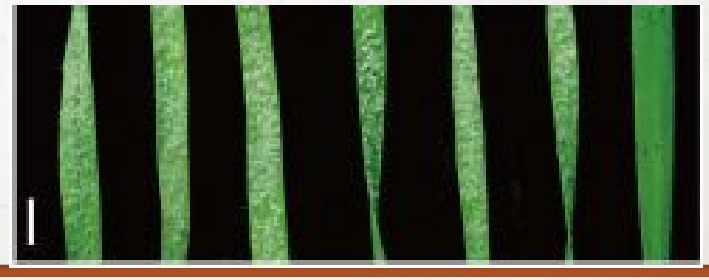
RNA INTERFERENCE FOR PEST CONTROL

- dsRNA may be used as a pesticidal substance to counteract Gypsy Moths and the Brown Marmorated Stinkbugs for example
- Delivery of a dsRNA solution to plant leaves as a spray leads to consumption by larvae and adults
- The dsRNA targets a specific gene(s) in the insect which is important for normal function; inhibition of that gene leads to demise of the pest
- dsRNA can be targeted to virtually any gene in an organism
- dsRNA does not persist in the environment and is target specific
  - http://entomology.umd.edu/news/-rnai-the-insecticide-of-the-future

GENE EDITING FOR PLANT DISEASE RESISTANCE

- Bread Wheat has 3 sets of chromosomes (hexaploid) making breeding for disease resistance and traditional gene additions problematic, if not impossible
- Researchers used TALEN and CRISPR/Cas9 technologies to simultaneously edit DNA sequences in 3 pairs of genes in wheat
- Resulted in 530 DNA base pair changes in a total of 6 genes (gene knockouts)
- Resistance to Powdery Mildew fungus (*Blumeria graminis*) has been long sought in wheat as it is very costly to yield and requires lots of chemical fungicides to combat

- Mildew Resistance in Bread Wheat - Wang et al., Nature Biotechnology 32:947-951 July 2014; doi:10.1038/nbt.2969
- Editing Out Pesticides - http://www.pbs.org/wgbh/nova/next/nature/crispr-grapes/



American Chestnut Research and Restoration Project

American Chestnut once covered an extensive range across the US and was a valuable source of wood, nuts for human and wildlife consumption, and impacted local economies in a significant manner

AMERICAN CHESTNUT RESTORATION PROJECT

- Chestnut blight decimated American Chestnut, *Castanea dentata*, from 1910 – present
- Fungal pathogen, *Cryphonectria parasitica*, relies on oxalate production for canker formation and invasion of tissues
- Bill Powell, SUNY, introduced oxalate oxidase (OxO) from wheat into American Chestnut
- Reduced canker size in transgenic trees is promising and in field trials (APHIS permit)
- Ultimate goal is to establish trees into eastern forests without management

AMERICAN CHESTNUT RESTORATION PROJECT

- USDA-APHIS regulates transgenic chestnut due to use of plant pest sequences
- EPA-OPP regulates transgenic chestnut due to pesticidal action of transgene
- The American Chestnut Foundation is working through genetic backcrosses to improve the habit and phenotype / diversity of the final tree type for planting
- Establishing a once native species into forests w/o management will require consideration of impacts on any native species present in that community and a shift in regulatory paradigm for EPA
- Risk analysis is simplified by the history of safe use / exposure from many OxO containing plants / foods, however, potential impacts to native communities may require modeling-based analysis to ascertain effects on various species

GENERAL PREDICTIONS IN NASEM REPORT

- The scope, scale, complexity and tempo of future products of biotechnology will increase in the next 5 – 10 years
- Future products will include a wider array of organisms and be increasingly diverse
- To serve a large number of markets such as health, energy, environment, food, and personal care
- The overall number of products entering the regulatory system will increase

CONCLUSIONS AT THIS POINT

- The NASEM report on Future Products of Biotechnology is lengthy and covers many different aspects of regulation, analyses, research and socioeconomic drivers
- EPA will continue to consider the recommendations from the report as novel technologies arise, but will also invest in ongoing horizon scanning
- With the technologies involved in gene editing, genome engineering and synthetic biology, we are clearly entering a new arena in regulation of biotech
- Computer modeling of environmental risks will likely be increasingly important as comparators from the scientific literature may not exist in cases of organisms created through synthetic biology (i.e., de novo sequences, nucleotides)
- An increase in the diversity of organisms utilized for agricultural, medical, environmental remediation, mining and other purposes is evident

FEEDBACK TO EPA-OPP

- How do these novel technologies address concerns you may have with regard to pesticide use or solving environmental issues?
- Do these novel technologies generate new concerns in and of themselves?
- What other stakeholders might the Agency be missing input from?
- How best would the Agency reach out to these people / groups?
- Are there mechanisms by which the Agency could help those unfamiliar with our regulatory system to navigate more effectively to see their technologies reach the marketplace?
- Anything else you would like to provide feedback on?