UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

March 26, 2018

MEMORANDUM

SUBJECT: Ethics Review of Research Article by Jean Popovici, et al. (2010)

FROM: Michelle Arling, Human Studies Ethics Review Officer
Office of the Director
Office of Pesticide Programs

TO: Mike Mendelsohn, Chief, Emerging Technologies Branch
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs

REF: Popovici, J., et al. Assessing key safety concerns of a *Wolbachia*-based strategy to control dengue transmission by *Aedes* mosquitoes. *Mem Inst Oswaldo Cruz*. Volume 105(8). pp. 957-964. December 2010.

I have reviewed available information concerning the ethical conduct of the study referenced in the research article "Assessing key safety concerns of a *Wolbachia*-based strategy to control dengue transmission by *Aedes* mosquitoes" by Jean Popovici et al. If the research is determined to be scientifically acceptable, I find no barrier in regulation to the U.S. Environmental Protection Agency's reliance on this research in actions under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) or §408 of the Federal Food, Drug and Cosmetic Act (FFDCA). EPA will ask the Human Studies Review Board (HSRB) to comment on this research article.

Summary Characteristics of the Research

This study enrolled human volunteers to provide blood meals to laboratory-reared mosquito colonies, some of which were infected with *Wolbachia* bacteria. Subjects consented to feed 2-4 cages of mosquitoes twice a week by inserting their arm into a screened cage for about 15 minutes at a time. Each cage contained approximately 150 mosquitoes. The expected duration of participation to maintain a single colony (cage) for its lifespan was about four to six weeks. Later, the research protocol was amended to include an evaluation of whether mosquitoes transmitted *Wolbachia* to the humans providing blood meals. According to the article, the research "investigated the antibody response specific to *Wolbachia* in the human volunteers that regularly feed mosquito colonies." p. 959. The article provides the results of the evaluation of

samples from 17 test subjects who had blood fed mosquito colonies infected with *Wolbachia*, as well as 5 control subjects who had not blood fed the mosquito colonies.

The study design was reviewed and approved prior to implementation by the Medical Research Ethics Committee, an independent ethics committee, at the University of Queensland (Australia). All participants received information about the study orally and in writing, and signed a written consent form prior to enrollment in the study.

To confirm that the study underwent an independent ethics review, EPA's Office of Pesticide Programs contacted the corresponding author on the article, Dr. Scott O'Neill and the Medical Research Ethics Committee at the University of Queensland, by e-mail. Dr. O'Neill noted that he did not have records from the study and the details were difficult to recall, but that the ethics committee could provide all materials submitted and approved. The Medical Research Ethics Committee, the independent ethics body that reviewed the protocol and amendments, provided the original study application, consent documents, and amendments submitted for review, as well as the approval letters. The materials provided by the Medical Research Ethics Committee are included as Attachment 1. Correspondence between EPA's Human Research Ethics Officer and Dr. O'Neill is included as Attachment 2.

1. Value of the Research to Society:

Wolbachia is a type of bacteria that is ubiquitous in the environment, "naturally infecting a large range of insect species including pests of stored food products as well as insects that bite humans such as nuisance mosquitoes." p. 959. The published article summarizes research evaluating the use of *Wolbachia*-infected mosquitoes as a biological control mechanism to reduce the populations of mosquitoes that are carriers of dengue viruses. One objective of the study was to evaluate whether *Wolbachia* is transferred from mosquitoes infected with the bacteria to humans who are bitten by these mosquitoes. The results were published in *Memorias Instituto Oswaldo Cruz* in 2010. EPA is proposing to use the results of this study, in combination with other information about *Wolbachia*, in its human health risk assessment to support the conclusion that humans are not exposed to *Wolbachia* through contact with released *Wolbachia*-infected mosquitoes.

2. Subject Selection:

a. ***Demographics.*** A total of 22 subjects participated in the research reported in the published article. Sera from seventeen subjects who had fed mosquito colonies infected with *Wolbachia* was tested. The sera from five control subjects, i.e., those who had not participated in the bloodfeeding of any mosquito colonies, was also tested.

b. ***Inclusion/Exclusion Criteria.*** According to the application to the IRB, the participants were to be:

> *Volunteer male and female researchers (aged 20-50) working in the O'Neill laboratory (lab 211, Goddard Building, School of Integrative Biology). A maximum of about 5 people will be involved at any time. This includes investigators such as Prof. Scott O'Neill and others, who have fed many*

mosquitoes on their arms in the past without adverse reactions. (IRB package, p. 46)

The IRB submission form noted that no subjects from vulnerable populations would be recruited as none were employed in the lab.

c. ***Recruitment.*** Test subjects were recruited from personnel working in the lab of Dr. O'Neill, at the University of Queensland. According to the application submitted to the IRB, "Personnel working at the O'Neill laboratory will be asked for voluntary unpaid participation in blood feeding *Aedes aegypti* mosquitoes. Senior researchers in the laboratory will provide a Participant Consent Document Form that includes background information on the project, description of the bloodfeeding procedure and a comprehensive list of the possible risks associated with it. They will also provide as much scientific information as necessary. If the person agrees to volunteer, the consent form will be signed in presence of a witness." (IRB Package, p. 47) The same recruitment process was followed to enroll the subjects who agreed to have their blood drawn to feed the mosquito colony and to be tested for *Wolbachia* reactivity.

3. Risks and Benefits:

a. ***Risks.*** The risks and possible side effects are discussed in the informational materials provided to potential subjects who consented to participate in the bloodfeeding. As explained in the protocol, "minor discomfort, redness and itching can occur as a result of mosquito biting." (IRB Package, p. 57). In addition, there was a risk of developing mosquito bite allergy, which was adequately explained and minimized by allowing subjects to withdraw at any time. The protocol was amended to allow blood to be drawn, and noted that "The participants may experience slight discomfort and/or bruising, these risks will be mitigated by the use of experienced phlebotomists when taking blood from volunteers." (IRB Package, p. 17)

The protocol noted that *Wolbachia*-infected mosquito colonies would be clearly marked, so subjects were aware of the characteristics of the mosquitoes they were feeding. The protocol and consent materials also made clear that there is no risk of *Wolbachia* being transmitted from infected mosquitoes to humans based on how *Wolbachia* is transferred from the host carrying it to other organisms. In addition, the protocol noted that each subject would feed his or her own cage(s) of mosquitoes, so there was no risk of transmission of any blood-borne illness between subjects feeding mosquitoes. If a subject withdrew consent, the cage(s) of mosquitoes he or she was maintaining by bloodfeeding would be destroyed.

b. ***Benefits.*** There are no benefits to the subjects. According to the research and IRB package, the research may assist in development of technology that uses *Wolbachia*, a naturally-occurring bacteria, to shorten the lifespan of mosquitoes. These mosquitoes can transmit diseases, such as dengue fever. Reducing the lifespan of mosquitoes that can transmit diseases to humans could result in fewer cases of vector-borne illness. EPA plans to consider these data in its evaluation of *Wolbachia*-infected mosquitoes.

c. ***Risk-Benefit Balance.*** The potential societal benefits from development of effective methods to reduce vector-borne illnesses outweigh the small risks associated with the study.

4. **Independent Ethics Review**: The study was reviewed and approved by the University of Queensland Medical Research Ethics Committee. This organization is independent of the investigator, and evaluated the research according to Australia's *National Statement on Ethical Conduct in Human Research* (Attachment 3) as well as the regulations governing experimentation on humans (Attachment 4).

The protocol was initially approved on October 31, 2007. The initial approval covered enrolling subjects to feed mosquito colonies, some of which were infected with *Wolbachia*. (IRB Package, pp. 41-58) The protocol was amended several times – extend the project period and add a funding organization, to allow feeding of other species of mosquitoes, and to use blood products from the Australian Red Cross to maintain colonies. On June 18, 2009, the IRB approved an amendment to allow subjects, including those who had been bloodfeeding mosquito colonies infected with *Wolbachia*, to consent to have their blood drawn in part to be tested to determine whether there was any evidence of immunoreactivity to *Wolbachia*. (IRB Package, pp. 13-21)

5. **Informed Consent**: All subjects received information about the study and were offered opportunities to ask questions. The consent form included information about the study, names of investigators, written declaration of informed consent, freedom to withdraw without penalty, assurance of confidentiality, an explanation that participants would get no direct benefits from the study, and an area for signatures from the prospective subject and a witness. Consent was not permitted by a guardian on behalf of another person, and no vulnerable populations were to be enrolled in the study. (IRB Package, pp. 55-58) All test and control subjects signed the informed consent form before participating. An additional consent form was completed by subjects who agreed to have their blood drawn for use in mosquito feeding and to be tested for immunoreactivity to *Wolbachia* or mosquito proteins. (IRB package, p. 19-21) Both consent forms contain similar language regarding the scope of the consent given:

> *I hereby agree to be involved in the above research project as an unpaid volunteer. I have been informed of the background and objectives of this research project and understand the nature of the research and my role in it. I have also been informed of the potential minor discomforts and risks that might arise from getting blood taken. I understand I am free to withdraw at any time without giving any reason for my decision. This decision will not affect my status in the group and I will not suffer any disadvantage as a result of it. My details will remain confidential.* (IRB Package, p. 21)

The consent forms, in combination with the information provided to test and control subjects, appears to meet the requirements of 40 CFR 26.1116. The information provided to participants explains the research study, the purpose, expected duration of participation, and the procedures to be followed; adequately characterizes the risks and discomforts to subjects; and articulates the right to withdraw from the research at any time.

6. **Respect for Subjects**: Subjects were free to withdraw from participation at any time. In addition, the application to the IRB noted that "people showing any minor discomfort or reason to stop blood feeding the mosquitoes can inform their supervisors at any time in the laboratory, and they can withdraw immediately if they wish." (IRB package, p. 49) Further, although the subjects were recruited from staff at the lab where the research occurred, the application to the IRB noted that coercion would be avoided by stating specifically that a decision not to participate would not be detrimental to a person's career:

> Written consent will be obtained from volunteer participants. Before any participation is required, they will be explained the reasons for their help, the objectives of the research project, the expected benefits, associated risks and they will be given a bibliography to read if necessary. *Right to refuse will be not considered detrimental to their research or work status in the group. Any volunteer will be able to refuse at any time once the experiment is underway and no explanation will be required for their decision*." (IRB package, p. 48; emphasis added)

The information provided in the consent form explained that subjects' information would be kept confidential. The subjects' identities were not revealed in the published article.

Applicable Standards

Standards Applicable to the Conduct of the Research

The portions of EPA's regulations regarding the conduct of research with human subjects, 40 CFR part 26 subpart A - L, do not apply since the research was neither conducted nor supported by EPA, nor was it conducted by a person with the intention to submit the results to EPA.

The IRB approval notes that "This project complies with the provisions contained in the *National Statement on Ethical Conduct in Research Involving Humans* and complies with the regulations governing experimentation on humans." (IRB Package, p. 13) The applicable regulation is the National Health and Medical Research Council Act 1992 (updated 1 July 2006). This Act establishes the Australian Health Ethics Committee and charges it with developing guidelines on human research and providing them to the government for review and issuance. This Committee's work resulted in the *National Statement on Ethical Conduct in Research Involving Humans (*The National Statement). The National Statement outlines the responsibilities of both researchers and bodies reviewing the ethical aspects of proposed research. The key ethical principles of The National Statement are respect, research merit and integrity, justice, and beneficence. Under The National Statement, research protocols must be reviewed by an independent ethics body prior to implementation, participants must be provided with information about the research before giving consent to enroll, participation must be voluntary, participants must be free to withdraw at anytime without negative effects, and participants must be respected (e.g., confidentiality of data, adequate compensation, insurance coverage for study-related adverse effects). In addition, research must have merit – a study that is not is not scientifically valid does not meet the standards for ethical conduct of the research.

Standards Applicable to EPA's Reliance on the Research

The Agency's rule (40 CFR part 26 subpart Q) defines standards for EPA to apply in deciding whether to rely on research—like this study—involving intentional exposure of human subjects. The applicable acceptance standards from 40 CFR part 26 subpart Q are these:

> **§26.1703.** Except as provided in **§**26.1706, EPA must not rely on data from any research subject to this subpart involving intentional exposure of any human subject who is a pregnant woman (and therefore her fetus), a nursing woman, or a child.
>
> **§26.1704(b).** EPA must not rely on data from any research subject to this section if there is clear and convincing evidence that: (1) The conduct of the research was fundamentally unethical (e.g., the research was intended to seriously harm participants or failed to obtain informed consent); or (2) The conduct of the research was deficient relative to the ethical standards prevailing at the time the research was conducted in a way that placed participants at increased risk of harm (based on knowledge available at the time the study was conducted) or impaired their informed consent.

FIFRA §12(a)(2)(P) also applied to this research. This provision reads:

> In general, [i]t shall be unlawful for any person . . . to use any pesticide in tests on human beings unless such human beings (i) are fully informed of the nature and purposes of the test and of any physical and mental health consequences which are reasonably foreseeable therefrom, and (ii) freely volunteer to participate in the test.

EPA will submit this study for review by the Human Studies Review Board (HSRB) in conformance with 40 CFR §26.1604.

Standards Applicable to the Documentation of the Research

This article was submitted by a registrant in support of EPA's consideration of an action under FIFRA. Consequently, the requirements for the submission of information concerning the ethical conduct of completed human research contained in EPA regulations at 40 CFR part 26, subpart M apply. Under 40 CFR §26.1303, the entity submitting data to EPA is required to provide, at the time of data submission, information concerning the ethical conduct of human subject research. If the data submitter cannot access such information, they are required to describe, at a minimum, their efforts to obtain the information.

Compliance with Applicable Standards

All of the subjects in this study were adults. There is no evidence to indicate that any female subjects were pregnant or nursing. EPA made several attempts to obtain information about the pregnancy and lactating status of female study participants directly from the corresponding author. (Attachment 2) EPA also corresponded with staff from the Medical Research Ethics Committee, who noted that under the ethics guidelines in Australia, pregnant and nursing women constitute vulnerable populations and therefore an additional level of ethical review would be required for research involving these groups. In the submission to the ethics committee, the researchers noted that no subjects from vulnerable populations would be enrolled. (IRB Package, pp. 46-47) The Office of Pesticide Programs has a long-standing position that,

although there may be gaps in the documentation of the ethical conduct of human research as presented in published research articles, deficient documentation does not itself constitute evidence that the ethical conduct of the study was deficient relative to the standards prevailing when and where the research was conducted. It is reasonable to conclude that the research did not involve intentional exposure of any pregnant or nursing female subjects or any children. EPA's reliance on the research is not prohibited by 40 CFR §26.1703.

The subjects provided written informed consent after receiving information in writing and orally about the study, the risks and benefits of their participation, and their ability to withdraw at any time. The protocol underwent independent ethics review and approval by the University of Queensland Medical Research Ethics Committee. The study involved exposure to lab-reared, pathogen-free mosquitoes, some of which were infected with a naturally-occurring bacterium that is ubiquitous in the environment. Based on these facts, and the absence of any information suggesting that the research was fundamentally unethical or intended to harm participants, I conclude that reliance on the research is not prohibited by 40 CFR §26.1704(b)(1).

Based on my evaluation of the research article and the "IRB Package", along with the Australian standards in effect at the time the study was conducted, I concluded that the conduct of the research was not deficient relative to the ethical standards prevailing at the time the research was conducted in a way that placed participants at increased risk of harm (based on knowledge available at the time the study was conducted) or impaired their informed consent. The protocol took adequate precautions to ensure participants' safety by explaining the potential discomforts of participating, allowing only one subject to feed each cage of mosquitoes, using disease-free and lab-reared mosquitoes, clearly indicating the cages of mosquitoes infected with *Wolbachia*, and allowing withdrawal from the study at any point without negative impact on the subject. The informed consent forms satisfy the requirements for informed consent in Australia in place at the time the study was conducted. Therefore, reliance on this study is not prohibited by 40 CFR §26.1704(b)(2).

Consistent with the principle of respect for persons, the study purpose and potential risks and discomforts were explained to subjects, only subjects with the capacity to understand the potential risks were allowed to participate, and all subjects provided written informed consent. Consistent with the principle of beneficence, subjects' participation was unlikely to pose more than a minimal risk to subjects, and the research was conducted in a laboratory under trained entomologists.

Finally, there is no clear and convincing evidence to suggest undue influence or lack of fully informed, fully voluntary consent. The subjects received information about the study in writing and orally. Although test subjects were recruited from the primary investigator's laboratory, the protocol and consent materials made clear that a decision not to participate or to withdraw would not have any effect on the subject's position or career. There is no clear and convincing evidence to suggest that subjects were vulnerable to undue influence by the principal investigator or other staff regarding their decision about whether to participate in the research. The study design was reviewed and approved prior to implementation by an independent ethics committee, the Medical Research Ethics Committee at the University of Queensland.

MosquitoMate, the registrant of mosquitoes infected with Wolbachia, satisfied its obligations to provide ethics-related documentation required under 40 CFR 26, subpart M. EPA obtained the

IRB records associated with the study independently. MosquitoMate was not able to obtain any additional information about the ethical conduct of the study.

Based on these facts, I conclude that the study was not deficient relative to the prevailing ethical standards in a way that placed participants at increased risk of harm or impaired their informed consent.

Conclusion

I find no barrier in law or regulation to reliance on the research summarized in the article "Assessing key safety concerns of a *Wolbachia*-based strategy to control dengue transmission by *Aedes* mosquitoes" in EPA actions taken under FIFRA or §408 of FFDCA. I defer to others for a full review of the scientific validity of this research. If it were determined not to have scientific validity, it would also not be ethically acceptable.

cc: Bob McNally
Milutin Djurickovic
Eric W. Bohnenblust
John Kough

Attachments

Attachment 1: IRB Package; O'Neill, S.L, I. Iturbe-Ormaetxe. 2011-2015. Institutional Approval Form for Experiments on Humans Including Behavioral Research. Rearing of Mosquitoes Using Blood from Human Volunteers 21/03/2011 – AMENDMENT. The University of Queensland.
Attachment 2: Emails between Michelle Arling (EPA Human Research Ethics Officer) and Scott O'Neill (corresponding author)
Attachment 3: *National Statement on Ethical Conduct in Human Research* (Australia)
Attachment 4: *National Health and Medical Research Council Act 1992* (Australia)