Registration Decision for the New Active Ingredient *Wolbachia pipientis w*AlbB strain in male *Aedes aegypti* mosquitoes

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1. Summary

This document announces that the U.S. Environmental Protection Agency (EPA) completed its evaluation of the new bacterial active ingredient *Wolbachia pipientis w*AlbB strain as contained in male *Aedes aegypti* mosquitoes and concluded that it meets the standard for registration under section 3(c)(5) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). On February 21, 2024, EPA proposed to register a pesticide end-use product containing the new active ingredient, *Wolbachia pipientis w*AlbB strain, and released that proposed decision for a 15-day public comment period. EPA received 17 comments on the proposed decision, all supporting the issuance of the registration.

The end-use product, WB1 Males, is intended for use as a population suppression tool for the control of the public health pest, *Ae. aegypti*. WB1 Males have been infected with the naturally occuring bacterium, *w*AlbB, derived from *Ae. albopictus* mosquitoes which harbor *Wolbachia* bacteria naturally whereas *Ae. aegypti* mosquitoes do not (Xi et al. 2005). To achieve mosquito population suppression, live *w*AlbB containing male *Ae. aegypti* are released into the environment where they mate with females of the existing, native *Ae. aegypti* population which are not infected with the *w*AlbB strain. If the female does not carry a compatible *Wolbachia* strain, eggs produced from the mating event will not be viable through a process called cytoplasmic incompatibility. Therefore, with continued releases of WB1 Males throughout the mosquito season, the resulting decrease in reproductive success of *Ae. aegypti* in the treated area is expected to suppress the mosquito population over time. The pesticidal effect of WB1 Males is species-specific as it only affects the reproductive success of *Ae. aegypti* through mating between *Wolbachia w*AlbB strain-containing male *Ae. aegypti* and wild *Ae. aegypti* females that are already present in the release area.

Risk to human health from the release of *Wolbachia w*AlbB strain in male *Ae. aegypti* (hereafter *w*AlbB) is anticipated to be negligible based on lack of demonstrated hazard from exposure. Humans have a history of safe exposure to *w*AlbB, which naturally occurs in the mosquito *Ae. albopictus* and *w*AlbB, like other *Wolbachia pipientis* strains, is not known to infect vertebrate cells. No adverse human health effects have been documented from interactions with *Ae. albopictus* mosquitoes infected with *w*AlbB and the *w*AlbB strain in *Ae. aegypti* has not been modified from the naturally occuring strain found in *Ae. albopictus.* Exposure to *w*AlbB through the releases of the male *Ae. aegypti* containing the bacterium is expected to be minimal since the highest exposure scenario occurs during application and mosquitoes are anticipated to disperse quickly. Exposure potential via dermal or ocular contact is considered negligible because *w*AlbB is contained within the exoskeleton of *Ae. aegypti*, thus, contact with the mosquito itself will not result in exposure to the active ingredient. Additionally, dermal exposure through blood feeding is not plausible since male mosquitoes do not bite and do not feed on blood. Exposure via inhalation is unlikely since *Ae. aegypti* mosquitoes are too large to be inhaled and instead would likely be swallowed. Regarding oral exposure, *w*AlbB containing male *Ae. aegypti* are not proposed for food use, and therefore, an oral exposure scenario to the mosquitoes or their eggs is likely only through ingestion of the released mosquitoes or water containing eggs that result from matings between *w*AlbB

containing male *Ae. aegypti* and wild *Ae. aegypti* females. Accidental ingestion of *w*AlbB, even during releases of large quantities of mosquitoes during application, is not considered likely, and since *Wolbachia* is maternally transmitted, any eggs produced from matings with male *Ae. aegypti* containing *w*AlbB will not contain the active ingredient. This information allows EPA to rely on a well-established history of safe human exposure to *w*AlbB and supports the conclusion that oral, ocular, pulmonary, and dermal contact risk from the use of the active ingredient will be negligible. All acute routes of exposure to *w*AlbB have been classified as EPA Toxicity Category IV (i.e., practically non-toxic and not an irritant). The limited human exposure scenario outlined above is dependent on limiting the release of female *Ae. aegypti* infected with *w*AlbB which would contain the active ingredient be capable of successfully reproducing and blood feeding. Thus, the EPA is requiring MosquitoMate to submit biannual reports detailing quality control measures to ensure that released mosquitoes have an error rate of no more than 1:250,000 (female *w*AlbB-infected mosquitoes to male *w*AlbB-infected mosquitoes) based on the approved manufacturing processes.

Non-target organism exposure and toxicity information was evaluated to assess risk from *w*AlbB, including direct and indirect effects. Regarding direct effects, the primary routes of exposure are considered consumption, contact, and the likelihood of the strain to be horizontally or vertically transmitted. Given *w*AlbB is an intracellular bacterium, it is unavailable to non-target organisms dermally and exposure is largely limited to oral consumption of *Ae. aegypti* transfected with the bacterium. Due to the history of safe environmental exposure to *w*AlbB as contained in *Ae. albopictus*, no hazard from consumption or contact to terrestrial or aquatic organisms is anticipated from the use of *w*AlbB in male *Ae. aegypti*. The EPA has concluded that the horizontal transmission of *Wolbachia* is an infrequent occurrence because if interspecific horizontal transmission were to occur under natural conditions, it would happen on an evolutionary scale and the horizontal transmission between insect genera is presumably rare. Vertical transmission of *w*AlbB from transfected male *Ae. aegypti* to the natural *Ae. aegypti* population is limited since *Wolbachia* is maternally transmitted and MosquitoMate has quality control measures established to ensure minimal contamination of females containing *w*AlbB in release devices (see the human health risk assessment summary above).

The EPA is requiring field monitoring for *Ae. aegypti* eggs or larvae containing *w*AlbB. Representative samples of *Ae. aegypti* populations must be collected within a 10 km radius of product release points on a quarterly basis beginning six weeks after the initial application. If more than 10% of the eggs and larvae sampled contain *w*AlbB, then releases of modified mosquitoes must stop until a conventional mosquitocide is applied to reduce the local population or less than 10% of *Ae. aegypti* eggs or larvae are no longer positive for the active ingredient in subsequent monthly testing. MosquitoMate is also required to submit biannual environmental monitoring reports and report any occurrence of *w*AlbB-infected offspring detections within 30 days of testing.

The possibility of indirect effects to non-target organisms due to area-wide suppression of *Ae. aegypti* was also found to be unlikely. There is no evidence in the literature that any non-target organisms are reliant on *Ae. aegypti* mosquitoes as a food source or a pollinator. The

anthropophilic nature of *Ae. aegypti* limits the interactions with predators and predators that do consume mosquitoes (non-species specific) are generalist in nature. Additionally, *Ae. aegypti* is an invasive species in the United States and is frequently targeted for control, factors which both independently limit the likelihood that non-target organisms in the United States are dependent on the presence of this specific mosquito species. Finally, due to the anthropophilic nature of *Ae. aegypti*, it is not expected that the suppression of this species will lead to discernible beneficial effects to non-target organisms.

Since EPA has determined there is a reasonable expectation of no discernible effects to occur to any non-target species exposed to *w*AlbB, EPA also does not expect discernible direct or indirect effects to federally listed threatened and endangered ('listed') species and their designated critical habitats. Therefore, EPA is making a "No Effect" determination under the Endangered Species Act (ESA) for all listed species and their designated critical habitats resulting from the use of *Wolbachia w*AlbB strain.

The EPA's efficacy assessment concluded that releases of *w*AlbB will reduce wild populations of *Ae. aegypti* mosquitoes over the treatment area (20 or more continuous acres), when applied by trained personnel under the direction of MosquitoMate. However, given the difficulty in evaluating live mosquito release products the specific percentage of population suppression that may be expected has not been established consistently in replicated, standardized product performance trials. Accordingly, the product label for WB1 Males does not provide claims as to a specific level of population reduction. Further, to maintain efficacy, releases of *w*AlbB may only be applied by MosquitoMate persons directly contracted by MosquitoMate, or federal, state, tribal, or local government officials for public health or vector control. Overall, the EPA considers the database of studies supporting the product performance of *w*AlbB complete for the supporting the label claims.

After reviewing the submitted and publicly available data and information for *w*AlbB, EPA concludes that the use pattern for WB1 Males will not cause unreasonable adverse effects to human health or the environment. Under FIFRA section 3(c)(5), EPA registering one end-use product, WB1 Males, with provisions to limit the maximum allowable level of female mosquitoes containing *w*AlbB (1 female per 250,000 males), ensure limited environmental escape of the active ingredient through monitoring, and assure product performance standards by limiting commercial application to trained personnel. The product has no application involving food use, and no tolerance exemption for its active ingredient on food commodities was sought by MosquitoMate.

2. Background

On February 19, 2020, EPA received an application from MosquitoMate, Inc. (MosquitoMate) that proposed registration of a pesticide product containing *Wolbachia w*AlbB strain as contained in male *Aedes aegypti* mosquitoes for use in population suppression of wild *Ae. aegypti* populations. MosquitoMate provided data and other information (e.g., scientific

rationales bolstered by published literature) to support the registration of this pesticide product.

Prior to seeking commercial registration, a FIFRA Section 5 Experimental Use Permit (EUP; 89668-EUP-3) was granted to test the efficacy of *Wolbachia w*AlbB strain on October 15, 2015. This EUP was extended in 2017, 2019, and 2020 before expiring on December 31, 2021. Additional information is available in the following dockets (search for "EPA-HQ-OPP-2015-0374" and "EPA-HQ-OPP-2017-0392" at www.regulations.gov) and on the emerging mosquito control technologies website hosted by the EPA (https://www.epa.gov/regulation-biotechnology-under-tsca-and-fifra/emerging-mosquito-control-technologies).

In the Federal Register of March 27, 2020, EPA published a Notice of Receipt (NOR) for this new active ingredient. The NOR provided an opportunity for public comment and no comments were received. (search for EPA-HQ-OPP-2020-0028 at www.regulations.gov).

3. Evaluation

In evaluating a pesticide registration application, EPA assesses a variety of studies to determine the likelihood of adverse effects (i.e., risk) from exposures associated with the use of the product. Risk assessments are developed to evaluate how the active ingredient might affect a range of non-target organisms, including humans and terrestrial and aquatic wildlife (plants and animals).

Based on these assessments, EPA evaluates and approves uses and terms of registration to mitigate any potential risk. In this way, the pesticide label communicates essential limitations and mitigations that are necessary for public and environmental safety. In fact, FIFRA section 12(a)(2)(G) states that it is unlawful for any person to use a registered pesticide in a way that conflicts with the label.

The conclusions conveyed in the assessments described below were developed in full compliance with EPA Scientific Integrity Policy for Transparent and Objective Science, and EPA Scientific Integrity Program's Approaches for Expressing and Resolving Differing Scientific Opinions. The full text of EPA Scientific Integrity Policy for Transparent and Objective Science, as updated and approved by the Scientific Integrity Committee and EPA Science Advisor can be found here: https://www.epa.gov/sites/default/files/2014-02/documents/scientific_integrity_policy_2012.pdf. The full text of the EPA Scientific Integrity Program's Approaches for Expressing and Resolving Differing Scientific Opinions can be found here: https://www.epa.gov/scientific- integrity/approaches-expressing-and-resolving-differing-scientific-opinions.

3.1 Assessment of Human Health Exposure and Risk

To assess risks to human health from use of pesticides, including *Wolbachia w*AlbB strain as contained in male *Ae. aegypti* mosquitoes (hereafter *w*AlbB), EPA evaluates the potential

toxicity of a product and the likelihood, amount, and types of exposure users and bystanders are likely to experience. In conducting a risk assessment, EPA must consider: (1) the hazards of a substance and (2) the exposure to that substance that a person will receive as a consequence of use either directly or indirectly. EPA uses this combined information to assess and characterize the risk(s) and predict the probability, nature, and magnitude of the adverse health effects that may occur from use of the substance in the manner described.

For the purposes of this FIFRA Section 3 registration, the *w*AlbB active ingredient will be contained within male *Ae. aegypti* mosquitoes which will limit exposure. Male mosquitoes do not bite and do not blood feed, and therefore do not significantly contribute to the exposure of humans to *w*AlbB. A key consideration for the human health risk assessment is the potential for unintended release of female mosquitoes containing *w*AlbB if sex sorting standards are not maintained in the manufacturing facility that rears and packages the WB1 Males product. Female mosquitoes do take blood meals which increases the possible routes of human exposure to the active ingredient. Thus, a large part of the focus of the human health risk assessment is regarding the manufacturing process associated with sex sorting prior to the commercial application of the WB1 Males product.

3.1.1. Product Characterization

Developed in 2005, the WB1 Males product (Waco strain; *w*AlbB infection; Line 1) is a strain of *Ae. aegypti* ("Waco" strain) that is stably infected with the *Wolbachia* "*w*AlbB" strain from *Ae. albopictus* ("Houston" strain) (Xi et al., 2005). Below, pertinent life history of *Wolbachia* and *Ae. aegypti* is provided followed by a description of the generation of the *w*AlbB-infected *Ae. aegypti* strain.

Surveys estimate that a majority of insect species are naturally infected with *Wolbachia* bacteria (Hilgenboecker et al., 2008), and these infections are common in mosquito species (Kittayapong et al., 2000; Ruang-Areerate et al., 2003). The *Wolbachia* bacterial species are maternally transmitted, obligate intracellular parasites that cannot survive outside of host cells and die within a short time span of the death of the host organism. *Wolbachia* infection in mosquitoes can lead to several reproductive alterations in the host, primary of which is cytoplasmic incompatibility which results in embryonic mortality of eggs from matings between infected and uninfected individuals (Yen et al., 1971; Stouthamer et al., 1999).

Ae. aegypti is a mosquito species invasive to the U.S. (Nelson 1986; Powell and Tabachnick, 2013). *Ae. aegypti* mosquito lifecycles are composed of an aquatic larval and pupal stage followed by a terrestrial adult stage. *Ae. aegypti* is highly anthropophilic (Powell and Tabachnick 2013) and is rarely found more than 100 meters from human dwellings (Nelson 1986). As such, *Ae. aegypti* usually uses man-made containers for egg laying and larval development, such as gutters, water containers, cans, and tires as breeding sites, and adults typically rest on walls and in shaded areas within and around human dwellings (Christophers 1960; TunLin et al., 1995; Hribar et al., 2001). Like other mosquitoes, females require vertebrate blood to support egg production while males do not blood feed. In the process of blood feeding, *Ae. aegypti*

females can acquire and subsequently transmit pathogens, some of which are of human health concern. These include yellow fever virus, chikungunya virus, dengue virus, and Zika virus. Notably, *Ae. aegypti* mosquitoes are not known to contain *Wolbachia* bacteria (Ross et al. 2020).

To create the WB1 Males product, cytoplasm containing the *Wolbachia w*AlbB strain from *Ae. albopictus* eggs was injected into *Ae. aegypti* eggs in a process called transfection. Because *Ae. albopictus* can naturally carry two different strains of *Wolbachia* (*w*AlbA and *w*AlbB), polymerase chain reaction (PCR) was used to confirm the presence of only the *w*AlbB strain in the recipient *Ae. aegypti* line. Given wild-type *Ae. aegypti* mosquitoes are not known to contain *w*AlbB or *Wolbachia* generally, any matings with *Ae. aegypti* transfected with *w*AlbB will not be viable; thus, reducing the overall population of *Ae. aegypti* mosquitoes in the treated area. Neither the recipient mosquito (*Ae. aegypti*) nor the transfected bacterium (*Wolbachia w*AlbB strain) were genetically modified in the process of creating the end-use product, WB1 Males.

3.1.2 Manufacturing Process

WB1 Males are produced using two different manufacturing processes which include details pertaining to the production of the mosquitoes in arthropod containment level 1 (ACL-1) insectary facilities. Details of the manufacturing process include the rearing of the mosquitoes, gender separation process, confirmation of the presence of the *w*AlbB strain in the mosquito colony, the degree of cytoplasmic incompatibility, the longevity of the mosquitoes, and testing scheme for ensuring absence of relevant human pathogens. These quality control and assurance measures outlined in the manufacturing process ensure the product identity of the released mosquitoes and ensure the fitness of WB1 mosquitoes as a commercial product.

Much of the human health and ecological risk assessments below are founded on lack of exposure to female *Ae. aegypti* mosquitoes containing *w*AlbB that are capable of blood feeding. Sex sorting in facilities producing WB1 Males is conducted through approved manufacturing processes with an estimated female contamination rate of no more than 1 female per 250,000 males released as the upper, allowable limit.

3.1.3 Human Health Risk

Hazard

In support of the application, MosquitoMate submitted waiver rationales intended to fulfill data needs for the human health risk assessment. Information supplied by MosquitoMate and Agency literature reviews supported that: 1) *Wolbachia* has not been reported to infect mammalian cells, 2) infection of mammalian cells has only been observed once under controlled laboratory conditions *in vitro* and the temperatures at which infection was maintained were below human body temperature, and 3) no reports have been made demonstrating that the *w*AlbB strain is toxic, infectious, or pathogenic to humans. Thus, no

hazard has been identified for human health from the *w*AlbB strain or from *Wolbachia* in general.

Exposure

Potential exposure to *w*AlbB via the oral, dermal, inhalation, and ocular routes was discussed in detail in the human health risk assessment. In general, any interaction with male *Ae. aegypti* containing *w*AlbB is expected to be brief because males do not seek out humans and are likely to quickly disperse once the holding container is opened (e.g., to find shaded areas and look for food (nectar) and mating opportunities). Additionally, since *w*AlbB is localized within cells of *Ae. aegypti* and males do not feed on human blood, the likelihood of exposure to the active ingredient is reduced.

The most likely population to encounter *w*AlbB in significant quantity over a short window of time are commercial applicators. The likelihood of dermal, ocular, and inhalation exposure is discussed below in context of applicator exposure as a conservative scenario. Applicators are exposed to *w*AlbB when releasing mosquitoes via hand containers or through vehicular methods. Exposure from vehicle releases is considered negligible as the wind is expected to facilitate dispersal of the mosquitoes. Applicators releasing mosquitoes on foot may be exposed to *w*AlbB when mosquitoes leave the holding container. Together with the label instructions to point the opening of the mosquito container away from the face and the general compulsion of *Ae. aegypti* to disperse, the likelihood of extended ocular or dermal contact is reduced to negligible levels. Further, applicators handling the *w*AlbB are not expected to be exposed through the inhalation route because mosquitoes are too large to be inhaled (Gorguner and Akgun, 2010). Given the size of *Ae. aegypti* mosquitoes, accidental inhalation is more likely to result in swallowing rather than inhalation.

Regarding oral exposure, *w*AlbB is not proposed for food use, and therefore, an oral exposure scenario to the adult mosquitoes or their eggs is likely only through ingestion of the released mosquitoes or water containing eggs that result from matings between *w*AlbB containing male *Ae. aegypti* and wild *Ae. aegypti* females. Accidental ingestion of *w*AlbB in large quantities during release is not considered likely. Further, eggs resulting from matings between male *Ae. aegypti* that contain *w*AlbB and wild *Ae. aegypti* females will not contain *w*AlbB since *Wolbachia* is transmitted maternally and thus ingestion of these eggs would not result in exposure to wAlbB.

The likelihood of exposure to *w*AlbB through all routes is increased if female mosquitoes are incidentally released alongside *w*AlbB males (e.g., blood feeding, accidental consumption of eggs containing *w*AlbB). The low female contamination rate (1:250,000) for the end-use product, WB1 Males, specified in the manufacturing process section allows EPA to conclude this exposure scenario is negligible. In the case individuals are exposed to females containing *w*AlbB, EPA notes one study that found *Wolbachia* is absent from the salvia of infected *Ae. aegypti*, providing additional confidence humans will not be exposed to the active ingredient through mosquito bites (Moreira et al., 2009).

In addition to concluding that exposure to *w*AlbB from the release of male *Ae. aegypti* transfected with the bacterium is negligible, the Agency conducted a literature review to address the possible hazard posed by *w*AlbB and *Wolbachia* generally. Publicly available information conveyed that *Wolbachia* is present in approximately 1 million species and the *w*AlbB strain is present in the mosquito, *Ae. albopictus*, which is found in many parts of the world, including the U.S. Given the ubiquitous presence of *Wolbachia* in different insect species, including biting mosquitoes, and the natural presence of the *w*AlbB strain in *Ae. albopictus*, EPA concludes that there has been an extensive history of safe human exposure to *Wolbachia* and the *w*AlbB strain.

3.1.4 Human Health Conclusions

The EPA has reviewed pertinent information characterizing the WB1 Males product and possible human health risks from the *w*AlbB strain of *Wolbachia*. The EPA concluded the level of exposure to *w*AlbB anticipated as a result of the release of transfected male *Ae. aegypti* is negligible based on the following factors: 1) the low likelihood that biting females are present in the release batches, 2) the application methods, 3) the intracellular location of the bacterium within the mosquito cells, and 4) the expectation that *Wolbachia* is not present in the saliva of female mosquitoes. Regarding the possibility of human exposure to *w*AlbB, the Agency determined that hazard is unlikely due to the following: 1) humans have a history of safe exposure to the *w*AlbB strain, 2) *w*AlbB is not considered toxic or pathogenic to humans, 3) released male *Ae. aegypti* mosquitoes do not bite, and 4) the manufacturing process limits the accidental release of female *Ae. aegypti* containing *w*AlbB in commercial release.

While human health risk from *w*AlbB is unlikely, both the hazard and exposure conclusions are based on the absence of female *Ae. aegypti* containing *w*AlbB being released in commercial applications of WB1 Males. The manufacturing process provides data that female contamination in commercial batches is minimal, however, to provide a further safety factor, the EPA will impose terms of registration for WB1 Males requiring ongoing quality control assessment to maintain the 1:250,000 gender ratio including submitting biannual reports to EPA, reporting incidences of batches that exceed the gender ratio, and halting production of any batches of WB1 Males that do not meet quality standards. The combination of negligible risk and quality assurance measures allows EPA to conclude that no unreasonable adverse effects for humans will result from the FIFRA Section 3 approval of the *w*AlbB *Wolbachia* strain in the end-use product, WB1 Males.

The database of studies required to support the assessment of risk to human health is complete. For more information on the human health risk assessment of *w*AlbB containing male *Ae. aegypti*, please see the supporting documentation provided in the associated regulatory docket (search for “EPA-HQ-OPP-2020-0028” at www.regulations.gov).

3.2 Assessment of Ecological Exposure and Risk

To assess ecological risks from the use of microbial pesticides, EPA evaluates the likely environmental impacts as a result of exposure of the bacteria to plants and animals and whether that exposure will cause harm or ecological effects. EPA uses this combined information and considers the overall toxicity to characterize the risk(s) in order to identify what levels may cause harmful effects on the plants and animals of concern that may occur from use of the substance in the manner described.

Typically, to evaluate toxicity EPA requires that a wide range of studies are conducted on the following non-target organisms: mammals (acute, subchronic, prenatal developmental, and mutagenicity), birds (acute oral and dietary), aquatic animals (acute freshwater fish and aquatic invertebrates), plants, and insects. Testing is organized in a tiered structure, where Tier I studies address worst-case exposure scenarios and higher tiers (Tiers II and III) generally encompass definitive risk determinations and longer-term greenhouse or field testing.

The majority of information submitted to support the ecological risk assessment for *w*AlbB was comprised of waiver rationales and literature searches in lieu of data generation. The risk assessment found that non-target organisms are already exposed to the *w*AlbB strain of *Wolbachia* in the environment and that there are no current concerns with respect to hazards, exposure, horizontal or vertical transmission, or indirect effects from reduction of *Ae. aegypti* as a food source.

3.2.1 Ecological Exposure

Male *Ae. aegypti* mosquitoes that contain the *w*AlbB strain of *Wolbachia* are intended to be used as a population suppression tool. The ecological risk assessment focuses on the obligate, intracellular nature of *w*AlbB active ingredient which limits exposure scenarios for both aquatic and terrestrial organisms. Thus, dermal contact was considered to be an unlikely exposure pathway and the primary route of exposure was identified as oral consumption.

Regarding dermal exposure, while contact between non-target organisms and *w*AlbB is plausible, the *Wolbachia* bacteria is contained within the exoskeleton of the mosquito and WB1 males do not blood feed. Thus, dermal exposure to the *w*AlbB strain is considered unlikely.

The potential for oral exposure to *w*AlbB is based on the lifecycle and ecology of *Ae. aegypti*. *Ae. aegypti* mosquito lifecycles are composed of an aquatic larval and pupal stage followed by a terrestrial adult stage. Due to *Ae. aegypti*'s high affinity for humans (Powell and Tabachnick 2013), *Ae. aegypti* is rarely found more than 100 meters from human dwellings (Nelson 1986). As such, *Ae. aegypti* usually uses man-made containers for egg laying and larval development such as gutters, water containers, cans, and tires as breeding sites, and adults typically rest on walls and in shaded areas within and around human dwellings (Christophers 1960; TunLin et al., 1995; Hribar et al., 2001). These behavioral and life history traits will be explored further in the section below to account for potential oral exposure of non-target organisms to *w*AlbB.

Aquatic Exposure

As the larval stage of *Ae. aegypti* is aquatic, consumption of *Ae. aegypti* larvae is the most likely route of oral exposure for aquatic organisms. Importantly, matings between *w*AlbB containing male *Ae. aegypti* mosquitoes and wild type female *Ae. aegypti* mosquitoes result in inviable eggs (i.e., do not develop into larvae) and further, *Wolbachia* is transmitted maternally, thus any eggs from such matings will not contain *w*AlbB. Therefore, aquatic exposure to the *w*AlbB strain via eggs is expected to be negligible.

Regarding the possibility of aquatic exposure to adult mosquitoes, female *Ae. aegypti* lay eggs in aquatic environments and will therefore possibly be preyed upon by aquatic non-target organisms. However, given that *w*AlbB containing male *Ae. aegypti* do not lay eggs, aquatic exposure to the *w*AlbB strain via adult mosquitoes is expected to be limited. Moreover, the anthropophilic nature of *Ae. aegypti* and the preferred use of man-made containers as larval habitat and breeding sites further reduces the likelihood of aquatic non-target organisms encountering *Ae. aegypti* male mosquitoes containing *w*AlbB, making the likelihood of such exposure negligible.

Terrestrial Exposure

Regarding possible oral exposure by terrestrial non-target organisms, insectivores may consume male *Ae. aegypti* containing *w*AlbB . Given the anthropophilic nature of *Ae. aegypti* mosquitoes, *w*AlbB containing male *Ae. aegypti* are anticipated to congregate in residential sites. Further, *Ae. aegypti* dispersal is generally limited to around 200 meters based on worldwide release recapture studies (OECD 2018); as such, released *w*AlbB containing male *Ae. aegypti* are not expected to travel far from the release site, thereby limiting exposure to non-target terrestrial organisms. As described previously, the preferential habitat of *Ae. aegypti* is largely limited to areas surrounding human dwellings, which further reduces the potential of exposure of non-target organisms to the bacterium, *w*AlbB.

3.2.2 Non-target Effects

Direct and indirect effects to non-target organisms were evaluated for *w*AlbB. Direct effects from *w*AlbB to non-target organisms was based on possible oral exposure due the active ingredient being contained within the cells of the mosquito. Generally, indirect effects are evaluated as downstream effects due to the use of a pesticide, such as potential impacts to non-target organisms from the reduction of a food source or habitat. Neither direct nor indirect effects to non-targets organisms are expected from *w*AlbB.

Direct Effects

The possibility of direct effects from exposure to *w*AlbB was evaluated based the likelihood of the *w*AlbB strain to pose a hazard to non-target organisms via direct consumption or contact, the likelihood of the strain to be horizontally transmitted to non-target organisms, and the possibility of vertical transmission and subsequent establishment of the *w*AlbB strain in wild *Ae. aegypti* mosquitoes.

Regarding oral consumption, due to the prevalence of *Wolbachia* infection across the Class Insecta, non-target organisms that consume mosquitoes are commonly exposed to *Wolbachia* both through ingestion and contact. The likelihood of interaction with *Wolbachia*-infected arthropods is high and literature searches did not find evidence of adverse effects from *Wolbachia* exposure in birds, fish, or mammals. In addition, the *w*AlbB strain is highly prevalent in *Ae. albopictus*, a mosquito species that is behaviorally similar and geographically overlaps with *Ae. aegypti*. For animals that do consume insects that contain *Wolbachia*, the bacterial species is known to die quickly following the death of the mosquito host (Werren et al., 1995; Pietri et al., 2016). Given the high likelihood of prior exposure to *Wolbachia* and the *w*AlbB strain specifically, coupled with the lack of known adverse effects in predators due to exposure, no discernible effects are anticipated for non-target organisms from the consumption of male *Ae. aegypti* mosquitoes containing *w*AlbB.

The potential for *Wolbachia* to be horizontally transmitted from *w*AlbB to other species was evaluated and found unlikely to result in adverse effects to non-target organisms. This conclusion was based on a number of factors including: 1) if interspecific horizontal transmission of *Wolbachia* were to occur, it would happen on an evolutionary scale under natural conditions, 2) horizontal transmission between insect genera is presumably rare in predator-prey species based on a lack of apparent route for naturally occuring transmission, with successful transmission only being accomplished via specific transfers under precise laboratory methods, 3) the Agency is unaware of insect parasitoids of mosquitoes that would have the necessary host-parasite relationship for transinfection by *Wolbachia* to other non-target insects, including predaceous mosquitoes, and 4) no evidence of horizontal transmission from years of testing of WB1 Males under an Experimental Use Permit.

Regarding vertical transmission, the matings between released male *Ae. aegypti* containing *w*AlbB and wild *Ae. aegypti* females do not result in viable offspring limiting the possibility of establishment of *w*AlbB in the natural population of *Ae. aegypti*. It is possible for incidental female release to result in vertical transmission of *Wolbachia*; however, EPA has deemed the maximum allowable level of female contamination (1 female per 250,000 males) to result in negligible levels of exposure. Although this risk is negligible given estimated incidental female release numbers, the potential risk would increase if establishment were to occur. Thus, EPA has imposed terms of the registration for the WB1 Male product that require environmental monitoring to detect and mitigate any *w*AlbB containing *Ae. aegypti* population establishment. The very low maximum allowable female contamination rate for the end-use product, WB1 Males, coupled with the terms of registration requiring monitoring and subsequent remedial

action if females are identified in the environment allows EPA to conclude low likelihood of *w*AlbB establishment in natural *Ae. aegypti* populations.

Indirect Effects

EPA concluded that no adverse indirect effects to non-target organisms are expected should *w*AlbB containing male *Ae. aegypti* successfully reduce the *Ae. aegypti* population based on: 1) literature reviews that indicate that no species are reliant on *Ae. aegypti* mosquitoes as a food source, 2) species-specific behavioral traits of *Ae. aegypti* that limit the potential for interaction with non-target organisms, 3) the generalist nature of predators that consume mosquitoes, 4) the invasive species status of *Ae. aegypti* which reduces the likelihood that any significant co-evolutionary relationships exist with non-target organisms in the U.S., and 5) *Ae. aegypti* is commonly targeted for pest reduction through mosquito control measures which further limits the likelihood that a non-target organism would be reliant upon this species for food.

3.2.3 Environmental Risk Conclusions

The risk assessment concluded that non-target organisms are already exposed to the *w*AlbB strain of *Wolbachia* in the environment and that there are no current concerns with respect to hazards, exposure, horizontal or vertical transmission, or reductions in the pest population. The anthropophilic nature of *Ae. aegypti* limits the degree of exposure of non-target organisms to *w*AlbB since the radius of dispersal of *Ae. aegypti* beyond human homes is considered low. As *Wolbachia* is ubiquitous in nature and the *w*AlbB strain is naturally found in mosquitoes occupying a similar ecological niche as *Ae. aegypti*, no novel hazards or exposures are expected for non-target organisms. Finally, due to the generalist nature of predators that consume mosquitoes, and because there are no predators known to rely on *Ae. aegypti* specifically, no indirect effects to non-target organisms due to the reduction of the pest species is expected from the release of *w*AlbB.

3.2.4. Risk to Federally Listed Threatened and Endangered Species

Since EPA has determined there is a reasonable expectation of no discernible effects to occur to any non-target species exposed to the *Wolbachia w*AlbB strain in male *Ae. aegypti* as a result of the labeled applications, EPA also does not expect discernible direct or indirect effects to federally listed threatened and endangered ('listed') species and their designated critical habitats. Therefore, EPA is making a "No Effect" determination under the Endangered Species Act (ESA) for all listed species and their designated critical habitats resulting from the use of the *Wolbachia w*AlbB strain in male *Ae. Aegypti* mosquitoes and has concluded that consultation with the U.S. Fish and Wildlife Service and the National Marine Fisheries Service under ESA § 7(a)(2) is not required.

3.3 Product Performance

EPA requires scientific evidence that registered products sold to control pests known to impact

public health, such as those that vector diseases, are effective against the target pest (40 CFR 158.1709). Effectiveness refers to a product's ability to control the specific target pest when the product is applied in accordance with the label directions, precautions, and limitations of use. Subpart R in 40 CFR part 158 includes requirements for generating species-specific data for label claims for public health pesticides. Labeling statements are based on the performance standards and utilize tests in OPPTS Test Guidelines Series 810. Suggested performance standards are usually expressed as percentages of pest control, or percentages of other intended responses, calculated from measurements made on treated plots compared with those made on untreated control plots. These standards, however, are not always absolute or inflexible. On a case-by-case basis, data requirements identified in Subpart R may be adjusted for new technologies or atypical use patterns that would make a particular data requirement inappropriate and not useful in the Agency's evaluation of the product.

3.3.1 Data and Information Assessed for Product Performance

Wolbachia-based pesticides, such as the end-use product WB1 Males, present a different mode of action when compared to other biopesticide or conventional mosquito controls (e.g., *Bt israelensis*, methoprene, naled; see Section 4.2 below). Rather than directly killing exposed larvae or adults, *Wolbachia* products create a cytoplasmic incompatibility between gametes resulting in inviable offspring. Infected male mosquitoes are the delivery system for the pesticide, which is then spread to the wild female population via mating. Given the uniqueness of this approach, the Agency has not yet developed test guidelines under Series 810 for such products. Therefore, when MosquitoMate applied for an Experimental Use Permit to test WB1 Males in the field, the Agency reviewed submitted product performance test protocols with the objective of assessing the capacity of the product to suppress a percentage of wild *Ae. aegypti* populations in treated plots as compared to untreated control plots. The Agency advised that properly replicated, single release point application studies should be conducted using a standard release rate over the time of year with peak *Ae. aegypti* population abundance with consistent release time points to establish baseline coverage of the product.

Since 2015, WB1 Males have been assessed for percent population suppression of wild *Ae. aegypti* populations under Experimental Use Permit (89668-EUP-3) through caged trials and field test sites in California and Florida. MosquitoMate conducted areawide, multiple release point studies with variable release rates over inconsistent coverage areas using different application methods within and across studies (see Mains et al. 2019, Crawford et al. 2020). The variable release rate was intended to measure the overflooding ratio (i.e., number of WB1 Males per one *Ae. aegypti* wild type male present in the coverage area) that achieves various percent population suppression levels of *Ae. aegypti* in public health settings. These studies demonstrated that WB1 Males are capable of suppressing 68-95% of the wild *Ae. aegypti* population when weekly release rates are maintained at an overflooding ratio of 10:1 on a per acre-basis across large coverage areas when applied via hand containers or continuous vehicle release application methods. In the submitted studies, the discrete weekly application rate varied between 400-2,200 WB1 Males released per acre per week over 20-170 acres to maintain a 10:1 overflooding ratio.

While no single study conducted by MosquitoMate measured a discrete application rate over a minimum coverage area to assess product efficacy, a preponderance of evidence suggests that *w*AlbB will reduce the population of *Ae. aegypti* mosquitos at levels exceeding 68% when a 10:1 overflooding ratio is maintained (Mains et al. 2019, Crawford et al. 2020). The product label allows for two application methods: 1) discrete application rate of 1,200 WB1 Males per acre per week over a minimum of a 20-acre contiguous application area, and 2) variable release rate if the applicator successfully maintains an overflooding ratio of > 10:1. The label includes restrictions on the personnel who may apply WB1 Males in commercial settings as a means to assure consistent product application when using the overflooding ratio release method. The directions for use on the product label state that WB1 Males are only to be released by MosquitoMate, persons directly contracted by MosquitoMate, or federal, state, tribal, or local government officials for public health or vector control and further, such individuals must calculate the overflooding ratio weekly by comparing pre- and post- release average male trap counts in the treatment area or by molecular methods.

3.3.2 Product Performance Conclusions

The Agency concurs that WB1 Males will reduce populations of *Ae. aegypti* mosquitoes over the treatment area (20 or more continuous acres), when applied by trained personnel under the direction of MosquitoMate. However, given the difficulty in evaluating live mosquito release products such as WB1 Males, the specific percentage of population suppression that may be expected based on the labelled release method has not been established. Accordingly, the product label for WB1 Males does not provide claims as to a specific level of population reduction. Therefore, the EPA considers the database of studies supporting the product performance of WB1 Males complete, provided the product label limits the type of personnel who may apply the product in commercial settings. The efficacy assessments and label supporting this decision can be found in the associated regulatory docket (search for "EPA-HQ-OPP-2020-0028" at www.regulations.gov).

4. Benefits, Alternatives and Public Comment

4.1 Benefits

By definition, biopesticides, including microbial pesticides, are favorable when compared to currently registered conventional alternatives because biopesticides are naturally occurring with a history of exposure to humans and the environment demonstrating minimal toxicity and a non-toxic mode of action to the target pest(s). Benefits of biopesticides as compared to conventional pesticides typically include lower toxicity profiles for humans and non-target organisms, and faster degradation in the environment.

Biopesticides may have the following benefits:

- Usually are inherently less harmful than conventional pesticides.

- Generally, affect only the target pest and closely related organisms, in contrast to broad-spectrum conventional pesticides that may affect many different organisms (e.g., birds, insects, and mammals).
- Can greatly decrease the use of conventional pesticides while crop yields remain high, when used as a component of integrated pest management programs.

Like other microbial pesticides, *w*AlbB is expected to align with some of the potential benefits above in that the active ingredient is species-specific to *Aedes aegypti*, poses no risks to non-target organisms, and has no toxicity or adverse effects to humans. Further, *w*AlbB could fill specific pest control needs in areas where mosquitoes are a public health pest.

As a public health tool, *w*AlbB may reduce the spread of vector-borne diseases through population suppression, represent an additional means of control where many extant tools have decreasing efficacy due to resistance, and target cryptic breeding sites that conventional pesticides cannot access. Mosquitoes are found throughout the world, and many transmit pathogens which may cause disease. *Ae. aegypti* is an invasive species of mosquito that is non-native to the continental United States; however, it has established in many locations in the Southern and Western regions of the country and distribution continues to spread further. *Ae. aegypti* is capable of vectoring pathogens of public health concern including yellow fever, chikungunya, dengue, and Zika viruses. Control of *Ae. aegypti* is increasingly challenging as this species has developed a level of resistance to all extant conventional insecticides (Asgarian et al. 2023). Accordingly, there is an increasing need for new vector control tools that would facilitate an integrated pest management approach, including a suite of management options to optimize protection against disease-carrying mosquitoes and maximize sustainability. The approval of *w*AlbB will provide an additional control measure for use by mosquito control districts nationwide to suppress populations of this invasive, disease-vectoring pest. Uniquely, when compared to conventional mosquitocide applications, released *Ae. aegypti* will seek out females even in locations inaccessible to conventional chemicals. These locations (called cryptic breeding sites) are areas that insecticide applications may miss. Given the anthropophilic nature of *Ae. aegypti*, many cryptic breeding sites occur near homes in containers that may collect standing water (e.g., potted plants, tires, tree holes, puddles, etc.) and may support mosquito breeding in as little as a ¼ inch of standing water. The potential to reach cryptic breeding sites is a key benefit of *w*AlbB over conventional mosquito oversprays that cannot be applied with consistency to these niche locations.

4.2 Alternatives

Mosquitoes are primarily managed through adulticides (aerially or ground applied), the application of larvicides to standing bodies of water, and the utilization of integrated pest management strategies, which include minimizing mosquito breeding grounds.

A list of registered alternative active ingredients to *w*AlbB is provided in Table 1. The list is not meant to be exhaustive, but rather provides examples of different types of pesticides, such as biopesticides (microbial and biochemical pesticides) and conventional pesticides, that are

present in registered commercial products currently available on the market. It should be noted that the WB1 Males product is specific to *Aedes aegypti*; by contrast, every compound listed in Table 1 is efficacious against multiple mosquito species. However, *Ae. aegypti* has developed measurable resistance to all of the listed mosquitocides below (Asgarian et al 2023).

Table 1. Active Pesticide Ingredients in Registered Alternatives to *w*AlbB for Population Suppression of wild *Ae. aegypti*

Ingredient Name	Pesticide Type
Methoprene	Biochemical
S-Methoprene	Biochemical
Bacillus thuringiensis israelensis	Microbial
Bacillus sphaericus	Microbial
Malathion	Conventional
Naled	Conventional
Pyrethrins/pyrethroids	Conventional
Carbamates	Conventional
Organophosphates	Conventional

4.3 Public Comments

In the Federal Register of March 27, 2020, EPA published a Notice of Receipt (NOR) for this new active ingredient. The NOR provided an opportunity for public comment and no comments were received.

Because the *Wolbachia w*AlbB strain as contained in male *Ae. aegypti* is a new active ingredient, EPA opened a subsequent 15-day public comment period on the proposed decision beginning on February 21, 2024. EPA undertook action in accordance with a policy, first implemented in October 2009, designed to provide a more meaningful opportunity for the public to participate in major registration actions. During the open comment period, the EPA received 17 comments that were all supportive of the proposed registration. Of the comments received 15 were from mosquito control districts, one was from a state health department, and the final comment was from a national professional association. Of the comments from mosquito control districts two were from Florida and the remaining 13 were from various California counties (see Table 2).

Table 2. Comments Received During the Proposed Registration Decision Period

Commenter	Document ID
Anastasia Mosquito Control District	EPA-HQ-OPP-2020-0028-0010
West Valley Mosquito and Vector Control District	EPA-HQ-OPP-2020-0028-0011
Sacramento-Yolo Mosquito and Vector Control District	EPA-HQ-OPP-2020-0028-0012
Fresno Mosquito and Vector Control District*	EPA-HQ-OPP-2020-0028-0013
Placer Mosquito and Vector Control District	EPA-HQ-OPP-2020-0028-0014
Florida Keys Mosquito Control District	EPA-HQ-OPP-2020-0028-0015

Commenter	Document ID
San Gabriel Valley Mosquito and Vector Control District	EPA-HQ-OPP-2020-0028-0016
Fresno Westside Mosquito Abatement District	EPA-HQ-OPP-2020-0028-0017
American Mosquito Control Association	EPA-HQ-OPP-2020-0028-0020
Mosquito and Vector Control Association of California	EPA-HQ-OPP-2020-0028-0021
Delano Mosquito Abatement District	EPA-HQ-OPP-2020-0028-0022
Ventura County Vector Control	EPA-HQ-OPP-2020-0028-0023
Fresno Mosquito and Vector Control District*	EPA-HQ-OPP-2020-0028-0024
Northwest Mosquito and Vector Control District	EPA-HQ-OPP-2020-0028-0025
Greater Los Angeles County Vector Control District	EPA-HQ-OPP-2020-0028-0026
Alameda County Mosquito Abatement District	EPA-HQ-OPP-2020-0028-0027
State of Hawaii, Vector Control Branch, Department of Health	EPA-HQ-OPP-2020-0028-0028

* The Fresno Mosquito and Vector Control District commented twice on the proposed registration decision. Both comments were from the same author and were substantively similar including different information about the geographical nature of Fresno (e.g., number of cemeteries per square mile) or budgetary constraints for the district.

All comments received were in favor of the registration of WB1 Males. Overall, commenters were supportive of a new tool for *Ae. aegypti* control and noted the perceived safety and efficacy of WB1 Males. Many commenters also provided information related to the prevalence of *Ae. aegypti* in different geographies, lack of effective pesticides for control of this species due to resistance, and rising number of dengue cases in different parts of the United States. Both the Northwest Mosquito and Vector Control District and Greater Los Angeles County Vector Control District noted the utility of WB1 Males to control populations of mosquitoes that may be exposed to dengue from those travelling to and from the United States from countries with higher rates of this disease. The American Mosquito Control Association underlined that there are only two modes of action currently available for control of *Ae. aegypti* and both extant MoAs have resistance concerns. The association further summarized the need for the WB1 Males product as follows:

> "Traditional mosquito control strategies show variable and often limited effectiveness against *Aedes aegypti* as they exploit small, cryptic water sources and have shown resistance to many commonly used insecticides. Hence, there is a critical need for new and innovative mosquito control strategies to integrate into vector management programs around the United States. The WB1 sterile insect technique is unlike available mosquito control products and offers a much needed novel approach."

A comment was also received from the Hawaiian Department of Health (HDOH), Vector Control Branch. While in support of the technology, HDOH urged EPA to allow use in Hawaii as part of the registration. EPA's approval of WB1 Males product does allow for use in all U.S. states and territories, including Hawaii.

5. Registration Decision

The risk assessment for *Wolbachia w*AlbB strain in male *Ae. aegypti* has a complete database comprised of studies and information that meet the data requirements and support the labeled use. Therefore, EPA is granting the registration of one end use product, WB1 Males, under FIFRA Section 3(c)(5).

EPA is registering the *Wolbachia w*AlbB strain in male *Ae. aegypti* for use in population suppression of *Ae. aegypti* in public health settings nationwide. To mitigate potential exposure to the active ingredient the following terms of registration are included:

- The quality control procedures that are part of the manufacturing process must: 1) ensure the released mosquito gender ratio of no more than 1:250,000 (female *w*AlbB-infected mosquitoes to male *w*AlbB-infected mosquitoes), and 2) test for the fitness and viability of the WB1 Males that are maintained in the laboratory colony. As part of the quality control procedures, MosquitoMate must:

    - Submit to EPA bi-annual reports on the results of these quality control procedures.

    - Report to EPA any incidences where batches of WB1 Males exceed the 1:250,000 mosquito gender ratio and any reduction in WB1 Males fitness and viability, within 30 days of when first detected.

    - Halt the distribution of any batches of WB1 Males that do not pass the standards of the approved manufacturing process or the quality control procedures.

- As part of the procedures to monitor for establishment of the *w*AlbB strain in *Ae. aegypti* in the environment, MosquitoMate must adhere to the following monitoring protocol:

    - Monitor for *w*AlbB-infected offspring in the environment on a quarterly basis starting six weeks from initial releases in each of the new climate zones added to this registration as defined by the IECC Climate Zone Map (2012). Monitoring is required only in those zones where WB1 Males are actively being released.

    - Collect a representative sample of 93 *Ae. aegypti* mosquitoes collected in the field as eggs or larvae using ovitraps, larval collection using dippers or suction devices, or adults using BG/CDC traps (or collect all *Ae. aegypti* samples if fewer than 93 are collected across sites). Sampling must occur using a minimum of ten traps within a 10 km radius of the release area.

    - Perform PCR or LAMP assays for the *Wolbachia w*AlbB strain in the sampled *Ae. aegypti* mosquitoes as described in the *Wolbachia* infection Quality Control

document. If *w*AlbB-infected *Ae. aegypti* offspring are detected, sampling will be increased to monthly in the detection area.

- If 10% of *Ae. aegypti* eggs or larvae sampled from a site per visit are confirmed positive for *w*AlbB in two consecutive monthly tests, then cessation of releases within 3 km of the positive site must occur. Releases may resume if an additional mosquitocide is used or once <10% of *Ae. aegypti* eggs or larvae are positive for *w*AlbB during subsequent monthly monitoring. Once no WB1-infected *Ae. aegypti* eggs or larvae are detected at the positive site during monthly monitoring, quarterly monitoring may resume.

- Submit to EPA bi-annual reports on the results of environmental monitoring.

- Report to EPA any occurrence of *w*AlbB-infected offspring in the environment within 30 days of when they are first detected.

In addition to the above terms of registration for the WB1 Males registration, label language intended to ensure the product performance of *w*AlbB is required as follows:

- WB1 Males are only to be released by MosquitoMate, persons directly contracted by MosquitoMate, or federal, state, tribal, or local government officials for public health or vector control.

- If a variable release rate based on a 10:1 overflooding ratio is utilized, then weekly population monitoring to maintain the ratio must be conducted.

The registration is time-limited and will expire on December 31, 2028. Given that *w*AlbB is a emerging technology for use as a pesticide, this time limitation will provide an opportunity for considering any new information relevant to its risk assessments that may arise during this period before extending the registration. The time limitation is consistent with EPA's approach to other emerging technology pesticide products.

The risk assessments and label supporting this decision can be found in the associated regulatory docket (search for "EPA-HQ-OPP-2020-0028" at www.regulations.gov).

6. References

Additional references are provided in the risk assessment documents available in the docket for this action (search for "EPA-HQ-OPP-2020-0028" at www.regulations.gov).

Asgarian TS, Vatandoost H, Hanafi-Bojd AA, and Nikpoor F., 2023. Worldwide Status of Insecticide Resistance of *Aedes aegypti* and *Ae. albopictus*, Vectors of Arboviruses of Chikungunya, Dengue, Zika and Yellow Fever. J Arthropod Borne Dis. 17(1):1-27. doi: 10.18502/jad.v17i1.13198. PMID: 37609563; PMCID: PMC10440498.

Environmental Protection Agency (EPA). 2022. Science review of product performance data for registration for the product WB1 Males (EPA File Symbol: 89668-I) containing the *Wolbachia pipientis* WB1 strain in male *Aedes aegypti*. Data and information were provided in support of a Section 3 Registration. Memorandum from K. Welch to M. Weiner through A. Reynolds and M. Mendelsohn. Dated January 4, 2022.

Environmental Protection Agency. 2023. Efficacy claims for the product WB1 Males (EPA File Symbol: 89668-I) containing the *Wolbachia pipientis* WB1 strain in male *Aedes aegypti*. Data and information were provided in support of a Section 3 Registration. Memorandum from K. Welch to M. Weiner through A. Reynolds. Dated May 17, 2023.

Environmental Protection Agency. 2023. Environmental Risk Assessment for the Product WB1 Males (EPA File Symbol: 89668-I) Containing the *Wolbachia* pipientis *w*AlbB strain in male *Aedes aegypti*. Data and Information Were Provided in Support of a FIFRA Section 3 Application. Memorandum from A. Pierce to M. Mendelsohn and M. Weiner through G. Sinclair. Dated July 17, 2023.

Environmental Protection Agency. 2023. Wolbachia WB1 strain (*w*AlbB) in *Ae. aegypti* (WB1 Males): Human Health Risk Assessment for a FIFRA Section 3 Registration Request for Use to Suppress *Ae. aegypti* mosquitoes. Memorandum from W. Striegel to M. Mendelsohn and M. Weiner through A. Pierce. Dated November 15, 2023.