Request for Exemption of Federal and State Agencies for Use of a Pesticide Under Emergency Conditions

Section 18 of FIFRA Specific Exemption

Department of Agriculture
STATE OF HAWAII

1428 South King St.
Honolulu, Hawaii 96814

DQB Males
Wolbachia pipientis*, wAlbB, contained in live adult male *Culex quinquefasciatus
EPA Reg. No. PENDING

To Control Mosquitos (*Culex quinquefasciatus*), the vector of avian malaria, for Conservation Purposes in Hawaii

with registrant Verily Life Sciences LLC

OCTOBER, 28, 2022

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2022 FIFRA Section 18 Specific Exemption for DQB Males in Hawaii

General information requirements of §40 CFR 166.20(a) in an application for a Specific Exemption

TYPE OF EXEMPTION BEING REQUESTED

✓ SPECIFIC

QUARANTINE

PUBLIC HEALTH

SECTION 166.20(a)(1): CONTACT PERSON(S) AND QUALIFIED EXPERTS

i. This application is to the Administrator of the Environmental Protection Agency (EPA) for a Specific Exemption to authorize the use of *Wolbachia pipientis*, wAlbB , contained in live adult male *Culex quinquefasciatus* (DQB Males, EPA Registration Number is pending) to control mosquitos (*C. quinquefasciatus*), the vector of avian malaria, for conservation uses in Hawaii by the Hawaii Department of Agriculture (HDOA). Any questions related to this request should be addressed to:

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ii. The following qualified experts are available to answer questions:

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SECTION 166.20(a)(2): DESCRIPTION OF PESTICIDE REQUESTED

- **Common Chemical Name (Active Ingredients):** *Wolbachia pipientis*, wAlbB (DQB strain)

- **Trade Name:** DQB Males
  EPA Reg. No.: Unregistered

- **Confidential Statement of Formula:** Attached to this submission

- **Formulation:**
  wAlbB contained in live adult male *Culex quinquefasciatus* mosquitoes (DQB strain)
  active ingredient < 0.3%*
  *percent (w/w) of adult male mosquitoes

- **Mosquito and Wolbachia source:**

The DQB line of mosquitoes was developed through transfection of *Wolbachia pipientis* wAlb*B* isolated from *Ae. albopictus* KLP strain mosquitoes originating from Kuala Lumpur, Malaysia into *Culex quinquefasciatus* Palmyra strain mosquitoes originating from Palmyra Atoll. Prior to transfection, the naturally occurring wPip infection was removed from the Palmyra strain through antibiotic treatment using tetracycline and rifampicin as described in Pike & Kingcombe 2009 following the feeding protocol outlined in Dobson and Rattanadechakul 2001. Methods for DQB line generation are substantively similar to those outlined in MRID 51788911 with non-significant changes to account for Culex egg morphology. The DQB line was not created using genetic modification and the mosquitoes are not genetically modified organisms.

Table 1. Taxonomic designation of the *Wolbachia* present in the DAB line of *Ae. aegypti.*

Kingdom	Bacteria
Phylum	Proteobacteria
Class	Alphaproteobacteria
Order	Rickettsiales
Family	Rickettsiaceae
Genus	*Wolbachia*
Species	Pipientis
Clade	Supergroup: B
Strain	DQB: (**D**ebug) (*Culex* ***q****uinquefasciatus*) (wAlb**B**) DQB contains

	wAlbB *Wolbachia* isolated from *Aedes albopictus* originating from Kuala Lumpur, Malaysia (KLP strain).

Prior to release of the DQB strain on Hawai'i, the original line of mosquitoes (Palmyra mosquito genetic background) will be backcrossed into a locally appropriate *Culex quinquefasciatus* strain mosquitoes (from Maui or Kauai) cleared of wPip as outlined above. Wolbachia phenotypes pertinent to incompatible male releases (cytoplasmic incompatibility, maternal transmission), are not affected by mosquito genetic background as exemplified by the use of the WB1 and WB2 strains of wAlbB Wolbachia in *Ae. aegypti* of different geographical origins in published IIT trials (Mains *et al.* 2019 [WB1 in Florida strain], Crawford *et al*. 2020 [WB1 in a Fresno-Clovis strain], Beebe e*t al.* 2021[WB2 in Innisfail strain], Ng *et al.* 2021 [WB2 in Singapore strain]).

Backcross and QC protocols on backcrossed lines are detailed in the DQB males manufacturing process (MRID 51991801).

SECTION 166.20(a)(3): DESCRIPTION OF PROPOSED USE

i. **Sites to be Treated:** State, Federal and Private wildlife conservation areas that contain *Cx. quinquefasciatus* mosquitoes throughout the State of Hawaii.

ii. **Method of Application:** Point releases by hand or aerial releases.

iii. Rate of Application: Initial absolute rates of release are at least 150 males/acre/week, which may be adjusted upwards or downwards after a Mark Release Recapture in the proposed treatment area as this will establish baseline mosquito populations in the treatment area and the ecology, field longevity and other factors used to estimate release rates (number of males/acre/week) sufficient to achieve and maintaining the "overflooding ratio" of ≥ 10:1 DQB males:wild type (WT) male *Cx. quinquefasciatus* in adult traps in the release area as described on the DQB label. If wild type populations are significantly suppressed release rates may be lowered while still achieving ≥ 10:1 overflooding DQB:WT males.

We estimate that ~150 males/acre/week will be required as an initial minimum release rates for effective suppression (see Attachment B) based on published data of female *Cx.*

quinq. populations in Hawaiian forest reserves. Trapping data in treatment areas or appropriate proxy locations will be used to adjust release rates upwards or downwards as required to maintain an overflooding of 10:1 DQB males:WT males and/or to compensate for higher levels of WT Cx. quinq.

A program will be set up by DLNR and USFWS to monitor mosquito populations throughout the year in treatment sites. Samples of collected mosquitoes will undergo molecular testing (using methods similar to those outlined in Crawford et al. 2020) to differentiate recaptured *w*AlbB DQB males from Wild Type males and to estimate the overflooding ratio across and within the treatment area. The frequency and distribution of collections and molecular testing will be included in the monitoring program plan, and may be adjusted throughout the treatment program so as to adequately sample the treatment site, and identify seasonal variations.

iv. **Maximum Number of Applications:** *156 applications per release site per year based on an anticipated maximum of 3 releases per week.* At every treatment location release may be for up to a year, with the intention of having multiple releases per week. If strong suppression is achieved releases may be reduced in frequency at a given location with releases starting at a new location at the same cadence. Thus the total number of application days is up to 3x52 = 156 during the year. If the permit is extended then a similar rate of releases is expected to occur.

v. **Total Acreage to be Treated:** Up to 20,000 acres of State, Federal and Private wildlife conservation areas in the State of Hawaii. The actual treatment area will be determined by DLNR and USFWS based on conservation priorities, access logistics and the supply of mosquitoes available.

No State lands under the authority of the Department of Hawaiian Homelands will be included in proposed locations from which mosquitoes will be released (treatment areas).

vi. **Total Amount of Pesticide to be Used:**

Maximum amount of DQB Males to be applied per year: Up to 3,000,000 males per week = 156,000,000 males/year.

Maximum amount of *Wolbachia pipientis*, wAlbB to be applied per year: Up to ~1.83g/week = 95g/year

vii. Restrictions and Requirements Concerning the Proposed Use ~~(not on labeling)~~

- HDOA Plant Quarantine Branch added *Cx. quinquefasciatus* to the List of Restricted Animals (Part A) allowing for inoculated male mosquitoes to be imported into the State of Hawaii. As such, an import permit from the Plant Quarantine Branch must be obtained prior to importation.

- A microbial permit for *Wolbachia pipientis* will be required from HDOA Plant Quarantine Branch prior to importation.

- Applicators are required to notify the HDOA Pesticides Branch at least seven (7) days prior to application, this may be done via a weekly or monthly schedule. Notification information must include the name of the applicator(s), employer's name, phone number, e-mail address (if applicable), location via address, GPS coordinates, or Tax Map Key (TMP), estimated amount of mosquitos to be released, and estimated date of application. Notifications will be-submitted via email to: HDOA.Sec18@hawaii.gov or via mail to Hawaii Department of Agriculture Pesticides Branch, 1428 S. King Street, Honolulu, HI 96814.

- Applicators are required to keep records of each application. A weekly cumulative report may be submitted for each treatment area. Use records will be recorded on forms provided by the HDOA Pesticides Branch and signed or signed electronically. These records must be submitted to the Branch within sixteen (16) days following each application or following the last weekly application. Records of application must be submitted via email to: HDOA.Sec18@hawaii.gov or via mail to Hawaii Department of Agriculture Pesticides Branch, 1428 S. King Street, Honolulu, HI 96814.

- Applicators agree to be subject to at least one pesticide inspection for Section 18 Exemption use.

- Current mosquito population status of WT *Cx. quinquefasciatus.* and released DQB males along with overflooding will be determined by the monitoring program set up by DLNR and USFWS and the Pesticides Branch will be informed of how the monitoring program will operate including frequency of sampling and the frequency and volume of molecular testing.

- Any adverse effects resulting from the use of DQB Males under this emergency exemption must be immediately reported to the HDOAPesticides Branch at 808-973-9402.

viii. **Duration of the Proposed Use:**

One year, beginning from the date of issue of the Specific Emergency Exemption.

Applications of DQB Males are expected throughout the year.

Mosquitoes in Hawaii breed throughout the year due to Hawaii's warm climate.

ix. **Earliest Possible Harvest Date:**

Not applicable to this application.

x. **Restrictions and Requirements:**

- The sites to be treated are conservation areas that have known populations of *Culex quinquefasciatus.*
- This product is for use only for conservation agencies and their designated representatives.
- The target pest is the wild type mosquito (*Culex quinquefasciatus*).
- DQB Males must be applied as soon as possible, at least within 72 hours of receipt.
- DQB Males will be applied at a rate of up to 3,000,000 males per week (up to ~1.83g /week) at a ≥ 10:1 overflooding ratio of DQB males:WT *Cx. quinquefasciatus* males.

SECTION 166.20(a)(4): ALTERNATIVE METHODS OF CONTROL

Several organizations including USFWS, NPS, University of Hawaii, and HDOA have spent the past 7 years reviewing various vector control options and methods for direct control of Avian Malaria. None of these methods meet the requirements of potential efficacy, operational feasibility and appropriateness for use in a conservation area.

i. Registered Alternative Pesticides ("Emergency Status and Alternative Considered, 2022) :

Area-wide application of conventional pesticide products

1. At least six conventional pesticide spray formulations are registered in Hawaii and labeled for control of mosquitoes in non-agricultural areas, however none of the products are labeled for use against mosquitoes in conservation areas, forests, bogs or waterways. Two products, Sevin© Brand XLR Plus Carbaryl Insecticide and Sevin© RP4 Carbaryl Insecticide contain the active ingredient Carbaryl. The other four products, Fyfanon© ULV Mosquito Insecticide, Fyfanon© 57% EC, Fyfanon© EW Insecticide and Malathion 5EC© contain Malathion.

2. The use of pesticide products containing Carbaryl, Malathion or other active ingredients with modes of action known to impact arthropods are unacceptable for application in natural areas due to the presence of endemic and rare native arthropod species in the areas proposed for application. There are over 1400 described arthropod species which are endemic to the island of Kauai and over 1700 described arthropods endemic to the island of Maui. The natural areas which serve as the last refuges for the native forest birds also support a diversity of the remaining endemic arthropods, including federally listed endangered and threatened species.

a. Fourteen Hawaiian picture wing flies (*Drosophilia sp.*), are listed as endangered by USFWS. These species are endemic to the Hawaiian islands, and many are found primarily in upper elevation montane rainforests; the refugia of native Hawaiian forest birds and the proposed treatment area.
   i. Examples:
      1. *Drosophila musaphilia*: Endemic to the island of Kauai. Its host plant, Acacia koa, is fairly common and stable within, and surrounding, its known range on Kauai. Critical habitat currently identified on Kauai: Kokee (794,321 acres within the proposed application area)
      2. *Drosophila neoclavisetae*: endemic to the island of Maui. Host plants are reported to be from Cyanea sp. and occur in native, upper elevation montane rainforests, within the proposed application area.
      3. *Drosophila ochrobasis*: is endemic to mesic and wet montane habitats on the island of Hawaii, within the proposed application area.

b. Six Hawaiian damselflies (*Megalagrion sp.*) are listed as endangered by USFWS. These species are endemic to the Hawaiian islands, and while none are extant in the proposed treatment areas, their reliance on aquatic habitat/streams/surface habitat with connectivity in the project areas would preclude application of chemical insecticides due to risk of non-target pesticide drift and run-off.
   i. Examples:
      1. Flying earwig Hawaiian damselfly (*Megalagrion nesiotes*): Terrestrial or semi-terrestrial naiads may occur in damp leaf litter, moist leaf axils of plants up to several feet above ground, or within moist soil or seeps between boulders in suitable habitat. Primarily occurs on Maui Nui.
      2. Pacific Hawaiian damselfly (*Megalagrion pacificum*): Pacific Hawaiian damselflies are now believed to be limited to the islands of Maui and Molokai and one population found in 1998 on Hawaii island.

c. Blackburn's sphinx moth (*Manduca blackburni*): is listed as endangered by USFWS. It is Hawaii's largest native insect, with a wing span of up to 5 inches (12 centimeters). It was originally found throughout the Hawaiian islands, but is now restricted to populations on Maui Nui and Hawaii (Big Island). It was originally found from sea level to 5000 ft.

3. Potential for non-target impacts on other insects and therefore birds

a. These upper elevation forest bird refugia are the most pristine native Hawaiian rainforests currently in existence. They host a myriad of native insect species that are critical to the ecosystem in which the native forest birds exist/thrive, and as food sources to the native forest birds.
   i. Examples
      1. Maui creeper (Maui ‘alauahio) (*Paroreomyza montana*): Insectivore endemic to Maui Nui. Occurs above 900 meters.
      2. crested honeycreeper (Akohekohe) (Palmeria dolei): nectarivorous, and insectivorous endemic to Maui Nui. Occur primarily above 1,100 meters.
      3. Kauai akialoa (honeycreeper) (*Akialoa stejnegeri*): nectarivorous, and opportunistic insectivore endemic to Kauai. Occurred throughout the islands.
      4. `I`iwi (*Drepanis coccinea*): `I`iwi are widely recognized as one of the most spectacular and iconic of the extant Hawaiian forest birds. They are nectarivorous,and opportunistic insectivores endemic to all major Hawaiian islands, but relegated to Hawaii, Kauai and Maui.
      5. ‘Apapane (*Himatione sanguinea*): Small, crimson and primarily nectarivorous honeycreeper that occurs in upper elevation forests (above 1,250 meters) on all major Hawaiian islands.

4. The remote native montane rainforest refugia of endemic Hawaiian forest birds are inaccessible and challenging to operate in. Locating specific larval mosquito habitat across this remote and challenging terrain is extremely difficult due to both the structure and slope of the forest. Larval habitat occurs throughout the montane rainforest in tree fern cavities and the rock holes and pools of intermittent stream beds. Also, the extensive rainfall causes swamping of many possible standing water sites, reducing the ability to locate cryptic standing water sources as well as decreasing the efficacy of chemical applications.
5. Insects can develop resistance to chemical insecticides
6. Chemicals can run-off into waterways, and the area proposed for application is within Kauai and Maui watersheds. https://www.hawaiiwatershedatlas.com/watersheds/kauai/24004.pdf))
   a. Could impact groundwater/drinking water resources.
   b. Stream invertebrates and freshwater fish
   c. Downstream impacts on marine invertebrates/systems

Broadcast constrained larvicide products

7. There are at least 13 products registered in Hawaii and labeled for control of mosquitoes in the larval stage. VectoBac Primary Powder©, VectoBac DT Biological Larvicide©,

VectoBac G Biological Larvicide Granules©, VectoBac 12AS Aqueous Suspension Biological Larvicide©, Bonide Mosquito Beater WSP© and MosquitoDunks© contain the active ingredient Bacillus thuringiensis israelensis (Bti). VectoMax FG Biological Larvicide©, Gnatrol WDG Biological Larvicide, and Terro No Mess Mosquito Larvicide Pouches© contain Bti and Bacillus sphaericus (Bs). VectoLex WSP Biological Larvicide©, VectoLex FG Biological Larvicide©, VectoLex WDG Biological Larvicide©, which contain Bs. Two products, Metalarv S-PT Mosquito Growth Regulator Spherical Pellets© and Metalarv XRP Mosquito Growth Regulator Extended Release Pouch© contain insect growth regulator (S)-Methoprene as the active ingredient.

8. The broadcast constrained biopesticides and growth regulators are unlikely to achieve the level of larval suppression needed in rainforests aquatic habitat given the significant rainfall the rainforests on Maui and Kauai receive.
9. Biopesticides Bti and Bs have poor efficacy in controlling later instar larvae.
    a. Biopesticides and growth regulators are unlikely to effectively treat areas with high canopy, or cryptic standing water/larval breeding sites using an aerial broadcast strategy in conservation areas.
    b. Remote, isolated, and treacherous terrain definitive of these native Hawaiian rainforests makes it virtually impossible to treat cryptic Culicid larval habitat using hand or even aerial broadcast techniques in conservation areas.

Other products considered

10. According to the Hawaii Department of Agriculture, Pesticides Branch there are currently 1,621 products registered in Hawaii which contain language on the label indicating their application has an adverse impact on mosquitoes or the sites listed are not applicable to conservation areas.
    i. Of the products available, 1,521 are miticides, disinfectants, repellants, feeding depressants, sunscreens, or products for pets and/or areas with domesticated livestock. The remaining products are general insecticides (sprays, drenches, foggers or termiticides) for control of ants, roaches, bedbugs, spiders, scorpions, dust mites, lice, biting flies, etc. for interior of commercial or residential structures, exterior barriers to structures, lawns, gardens, stables, greenhouses and golf courses.

Biological control using Toxorhynchites sp. mosquitoes

11. Multiple species in the state: *T. brevipalpi, T. theobaldi*
12. Released in 1929 and again in 1950 to control Aedes sp.
13. These biological control agents are present across the state and are not effective in suppressing mosquito populations.

Ungulate removal and control

14. In studies of native forest plots where feral ungulates (including pigs) were removed by trapping and other methods, researchers have demonstrated a correlation in the abundance of *Culex spp.* mosquitoes when comparing pigfree, fenced areas to adjacent sites where feral pig activity is unmanaged (Hess et al. 2006).
15. Management of feral pigs may be strategic to managing avian malaria and pox, particularly in remote Hawaiian rain forests where studies have documented that habitats created by pigs are the most abundant and productive habitat for larval mosquitoes (USGS 2006).
16. The consequences of feral pig activities thus further exacerbate the impacts to iiwi from avian malaria and avian pox, by creating and enhancing larval habitats for the mosquito vector, thereby increasing exposure to these diseases.
17. The Hawai`i Department of Land and Natural Resources has maintained liberal public hunting seasons to minimize forest damage caused by feral pigs and goats within the Alaka`i Wilderness Preserve for several decades. Unfortunately, public hunting succeeds only in the more accessible areas of the preserve, and ungulate populations in more remote areas remain quite high.
18. Aerial reconnaissance and shooting of feral goats and pigs has been attempted in the most remote regions, but is not effective in inaccessible forest bird refugia.
19. Even in the absence of feral goats and pigs there would still be larval and breeding habitat available in these native rainforest refugia.
20. Management of feral ungulates is a part of any Integrated Pest Management strategy when managing Hawaii's native forest resources.

SECTION 166.20(a)(5): EFFECTIVENESS OF PROPOSED USE

Overview

The use of an Incompatible Insect Technique (IIT) by releasing male mosquitoes with a Wild Type incompatible *Wolbachia pipientis*, has been identified by Hawaiian conservation agencies and partners as the fastest environmentally appropriate option for suppressing Avian Malaria mosquito vectors in Hawaiian bird refugia at a landscape-scale. *Wolbachia pipientis* is a naturally occurring bacteria present in ~60% of arthropods, including many endemic to Hawaii, including *Aedes albopictus* mosquitoes (which is the source of the wAlbB strain in these DQB *C. quinquefasciatus*) and also in Hawaiian WT *C. quinquefasciatus* mosquitoes. When male mosquitoes carrying one strain of *Wolbachia pipientis* breed with other mosquitoes with a different strain of *Wolbachia pipientis*, they produce non-viable eggs leading to population suppression.[1] The DQB line was not created using genetic modification and the mosquitoes are not genetically modified organisms.

Rationale for expected efficacy and label

The label for DQB males (wAlbB *Wolbachia pipientis* in male *C. quinquefasciatus*) states that:

- Releases of male mosquitoes are to be performed at least weekly at a suggested minimum initial treatment rate of 150 males/acre/week. Male mosquitoes are released to the air and fly away to mate with indigenous females. If multiple containers are used, mosquito releases should be distributed evenly over the treatment area with release points spaced <1 km apart to ensure consistent coverage within the treatment area. To ensure highest possible efficacy adhere to this regimen until the end of the mosquito season.
- Trapping data in treatment areas or appropriate proxy locations (as reviewed by the Hawaii Department of Land and Natural Resources) should be used to adjust release rates as required to maintain desired overflooding ratio of DQB:Wild type male *Cx. quinquefasciatus* of >10:1 and to compensate for estimated higher levels of *Cx. quinquefasciatus* in treatment areas as appropriate.
- Overflooding ratio is determined by comparing the pre- and post-release average male trap counts in treatment areas or by using molecular methods on males sampled from treatment areas (to differentiate wAlbB males from Wild Type see Crawford et al 2020 for similar methods). For treatment areas inaccessible for regular trapping the Hawaii Department of Agriculture may approve appropriate proxies.

We estimate (see Attachment B) a minimum effective release rate for DQB males into primary endangered Hawaiian bird forest refugia to be 150 males/acre/week. Trapping data in treatment areas or appropriate proxy locations (as determined by DLNR and USFWS) will be used to adjust release rates as required to maintain an overflooding of 10:1 DQB males:WT males and to compensate for higher levels of WT *Cx. quinquefasciatus* as necessary.

These release strategies and overflooding rates are in line with published studies with successful suppression results from incompatible male *Culex* and *Aedes Wolbachia pipientis* programs (IIT programs, see Table 2 below), and are supported for DQB males QC assays demonstrating that DQB males possess the phenotypic properties required for a successful IIT suppression program. We note that while we provide, as required, a minimum application rate, the application rate is best calculated as that required to achieve a ratio to the wild type so that incompatible matings outnumber compatible matings, leading to population suppression, as the rate of wAlbB males per week per acre will vary because of the variability of wild-type mosquito populations in the treatment areas (conservation areas).

We outline below the pesticidal mode of action, and summarize data and field studies that demonstrate successful Wild Type mosquito suppression when overflooding is achieved with healthy incompatible male mosquitoes. More details are presented in Table 1 and other documents submitted along with this application (Attachment E, including the manufacturing process outlining quality controls.)

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[1] USFWS Information Memorandum. March 26, 2021. “Preventing Extinction of Hawaii’s Endangered Forest Birds through Landscape-Scale Mosquito Control”

Mode of action and phenotype of *Wolbachia pipientis* active ingredient is equivalent for DQB wAlbB in *C. quinquefasciatus* and published wAlbB in *Aedes aegypti* field studies.

The modes of action for incompatible *Wolbachia pipientis* use as a pesticide in *Culex* and *Aedes* mosquitoes are the same: sustained high levels of incompatible matings by incompatible *Wolbachia*-males leads to fewer viable offspring by WT females and each succeeding generation of WT mosquitoes will be smaller, leading to population suppression of the WT population. This mode of action and the efficacy of male releases is a result of three factors, all of which are true for DQB as well as for published studies using other *w*AlbB strains in *Aedes* mosquitoes:

1. presence of *w*AlbB *Wolbachia* in released *w*AlbB *C. quinquefasciatus* males, which leads to demonstrated Cytoplasmic Incompatibility between released *w*AlbB males and WT *C. quinquefasciatus* females infected with *w*Pip *Wolbachia pipientis*
2. released wAlbB males are healthy leading to field survival and mating efficacy
3. sufficient, and appropriately located, released males to ensure that most matings of WT female mosquitoes are incompatible, leading to wild females laying eggs that do not hatch resulting in population decline of the target, and potentially disease-carrying mosquitoes.

This mode of action is identical to that of other incompatible *Wolbachia pipientis* male mosquito products including those already reviewed by the EPA (specifically WB1 and ZAP) and those used successfully internationally (e.g WB2 in Australia and Singapore). This mode of action is demonstrated by a limited number of key phenotypes and processes, all of which hold true for DQB and support the label claims. Table 1 (below) summarizes the phenotypes and requirements necessary for the mode of action of DQB males and outlines how the manufacturing process (MRID 51991801) ensures these standards are met.

Table 1. Phenotypes and requirements for DQB male mode of action.

Phenotype/Requirement	**DQB Quality Controls during manufacturing process (MRID 51991801)**	**Summary and Notes**
Presence of *w*AlbB in released males: • 100% maternal transmission of *w*AlbB • Confirmation of *w*AlbB presence in release batches	100% maternal transmission is confirmed in each strain of DQB developed DQB *w*AlbB colony QC is performed throughout	Regular sampling confirms maintenance of *w*AlbB in the *Cx. quin.* DQB colony.

	releases.	
Cytoplasmic Incompatibility of released males with WT females	Bidirectional CI is confirmed in each strain of DQB developed. Sufficiency (>90%)of bidirectional CI is confirmed using DQB strain for release and suitable wild type colonies.	Bidirectional CI testing is performed as part of standard QC after generation of new backcross lines. CI is also confirmed against wild type colonies from areas proposed for male releases.
Healthy released males (longevity)	Minimum 2 days median longevity of males at point of release.	Consistent with *w*AlbB in *Ae. aegypti* studies and WB1 EUP label. Note that the lab longevity of *Cx. quinquefasciatus* is lower than *Aedes aegypti*.
Minimal *w*AlbB female contamination	<1:250,000 female contaminants.	Consistent with WB1 and WB2 field studies, EUPs (89668-EUP-3) and labeling. Note that for EUP-89668-EUP-3 the WB1 manufacturing process of Verily was reviewed by EPA (EPA decision #525795 published as EPA-HQ-OPP-2017-0392-0004). This Verily QC process is substantively identical to that previously provided to EPA (e.g. MRID 51991801). As described in (Crawford et al 2020) Verily may optionally perform additional quality control checks including additional independent machine learning image reviews and human reviews which significantly further reduce female contamination in the released product. For DQB males these steps result in the expectation of a significantly lower rate during ongoing operations, estimated to be <1:2,000,000 and continually improved.
Sufficient males in the correct location	See proposed DQB label (Attachment A) for dosing and distribution methods in treatment area.	DQB Label is based on evidence from published studies showing suppression when *w*AlbB males are reared according to Verily's manufacturing method (or similar) and released according

		to the proposed DQB label (see Attachment A). Target is to achieve ratio of at least 10:1 DQB males to wild male so incompatible matings outnumber compatible matings.

Table 2 below contains a summary and references to published field studies with incompatible *Wolbachia pipientis* males that demonstrate the same pesticidal phenotypes and mode of action as that of DQB males. These studies are relevant data that establish field efficacy and safety of male incompatible *W. pipientis* in mosquitoes when reared using Verily's mosquito manufacturing process or an equivalent process. These data demonstrate that WB1 and WB2 wAlbB strains, and hence DQB releases, conducted in accordance with the proposed DQB label (≥10:1 incompatible:WT male) are sufficient to achieve effective suppression across a range of environments. We also include literature from a historical incompatible *Cx. pipiens* (noting that *Cx. quinquefasciatus* is a part of the *Cx. pipiens* species complex) field trial showing that the release of a sufficient quantity of incompatible male *Culex* can achieve suppression and local elimination.

Table 2. Timeline of published studies demonstrating field efficacy of Insect Incompatibility Technique using incompatible *Wolbachia* male releases in mosquitoes.

Year	Mosquito Species	*Wolbachia* Strain	Location	Summary	Reference
1967	*Culex pipiens fatigans*	*w*Pip	Okpo, Myanmar	Natural incompatibility of a French (Paris) strain of *Cx. pipiens* carrying *w*Pip was leveraged to control local population through daily releases of 5000 incompatible males over 12 weeks in 1967. During the 12th week, 100% inviable egg rafts were recorded. Overflooding rates were not recorded.	Laven 1967
2010	*Ae. polynesiensis*	B clade *Wolbachia* from *Aedes riversi*	Toamaro, Uninhabited 'motu' island, French Polynesia	Natural bidirectional incompatibility of *Ae. riversi Wolbachia* (Clade B) with wild *Ae. polynesiensis* carrying Clade A *Wolbachia* was exploited through introgression of *Ae. riversi* Clade B *Wolbachia* into *Ae. polynesiensis* background after antibiotic clearing, to create the released CP strain. 30 weeks of male releases across ~ 10 acres averaging 3,800 CP males/week resulted in ~20% reduction in egg hatch rate, attributed to CI. [~44% reduction in female trap counts]	O'Connor *et al.* 2012
2014	*Ae. albopictus*	*w*Pip	Lexington, Kentucky	A transfected strain of *Ae. albopictus* naturally cleared of wAlbA/B before wPip introduction was employed under EPA EUP in	Mains *et al.* 2016

				Lexington. 10,000 incompatible males per week were released over 17 weeks into a ~30 acre treatment site. Compared to an untreated control area, a peak female adult suppression of 66% suppression was recorded. Overflooding ratios were not recorded, though male trap counts in treatment areas were ~2x control areas.	
2015 - 2017	*Ae. albopictus*	*w*Pip	Guangzhou, China	Triple-*Wolbachia* infection employed with wPip transfected into *Ae. albopictus* strain naturally infected with wAlbA and wAlbB. Additional radiation treatment used to sterilize residual females to avoid population replacement through accidental female release. Yearly female adult reductions of 83%-100% recorded across two release sites (80 Acres) compared to three matched controls after three male releases per week (between 24.3k - 48.1k males per acre per week; male overflooding ratio of 9:1 to 16:1 Wb:WT male overflooding)	Zheng *et al.* 2019
2016	*Ae. aegypti*	*w*AlbA & *w*AlbB (ThAB)	Nong Satit, Thailand	Superinfected *Ae. aegypti* line generated with *w*AlbA and *w*AlbB infection. Before male releases, sex separated male pupae were irradiated to sterilize residual females and avoid population replacement through accidental female release. For 6 months, 10,000-25,000 males were released weekly into a 160 acre treatment site. Compared to control areas, an 84% reduction in hatch rate and 97.3% reduction in mean number of females trapped per household was recorded.	Kittayapong *et al.* 2019
2017 - 2018	*Ae. aegypti*	*w*AlbB (WB1)	Fresno, California	wAlbB transfected *Ae. aegypti* line (WB1) male releases resulted in >95% suppression of female *Ae. aegypti* population based on BG trap collections in 2018 season across three treatment sites (724 acres) compared to matched controls after daily releases of males at a minimum rate of 671 males/acre/week (48:1 Wb:WT male overflooding, achieved by starting releases prior to, and suppressing, mosquito population growth in treatment areas).	Crawford *et al.* 2020
2018	*Ae. aegypti*	*w*AlbB (WB1)	Miami, Florida	*w*AlbB transfected *Ae. aegypti* line (WB1) male releases for 6 months into 170 acres treatment site. Three to five releases per week were performed totalling 75,000-375,000 males per week. Post intervention, a maximum reduction in females of 78% was recorded when comparing the center of the release site to the control area.	Mains *et al.* 2019
2018	*Ae. aegypti*	*w*AlbB (WB2)	Innisfail, Australia	Males from *w*AlbB transfected *Ae. aegypti* line (WB2) backcrossed into local strain (wAlbB2-F4)were released across three treatment sites totalling 478 acres for 20 weeks at a rate of 75-161 males/acre/week (6:1 to 18:1 Wb:WT male overflooding ratio depending on treatment site, which resulted in 78-97% suppression based on female trap counts compared to three matched control areas.	Beebe *et al.* 2021
2019	*Ae. aegypti*	*w*AlbB (WB2)	Singapore	The WB2 wAlbB transfected *Ae. aegypti* line backcrossed into local Singapore strain was	Ng *et al.* 2021

				employed in releases over 3 years in high-rise apartments neighborhood (Yishun & Tampines) at ~1500 males/acre/wk and a Wb:WT male overflooding ratio of 30:1. Due to the high-rise nature of the landscape, acre estimates are inaccurate. Overall a 93-98% reduction in Ae. aegypti and 71-88% reduction in dengue incidence was recorded.	

SECTION 166.20(a)(6): DISCUSSION OF RESIDUES FOR FOOD USES

Not applicable to this application.

SECTION 166.20(a)(7): DISCUSSION OF RISK INFORMATION

Risks to Human Health

WoIbachia pipientis is an obligate intracellular bacterium, occurring naturally in many invertebrates as a part of their microbiome, including, insects, isopods, ticks and filarial worms of humans and other animals (Casiraghi et aI., 2005; Saridaki & Bourtzis 2010). *WoIbachia pipientis* is present in up to 65% of insect species (Hilgenboecker et al. 2008, Kittayapong et al. 2008, Ruang-Areerate et al. 2003.) including many mosquitoes, including both *Cx. quinquefasciatus* and other related mosquitoes in the *Culex pipiens* species complex, and *Aedes albopictus* (the source of the *Wolbachia pipientis* strain used in DQB males,) resulting in a long history of environmental exposure of humans to *Wolbachia pipientis* in mosquitoes.

The human health risks of male mosquitoes with incompatible *Wolbachia pipientis* have been reviewed by the EPA in the context of other products (see U.S. EPA 2017a, and U.S. EPA 2017c for example) and the Agency found "**negligible exposure to humans resulting in a negligible human health risk and therefore meets the standard of no unreasonable adverse effects.**" (U.S. EPA, 2017a)

Similar to previously published EPA reviews the rationale for applying this finding of negligible risks of harm to human or environmental health to DQB males is (in summary):

- For DQB male mosquito releases, exposure of humans to *Wolbachia pipientis* is extremely low as released male mosquitoes do not bite, do not vector disease, and do not survive for more than a few days in the wild.
- In the context of releases of DQB *Cx. quinquefasciatus* in Hawaiian forest reserves as a part of a bird conservation program, the human exposure will be even lower as there is minimal human habitation in the treatment areas.

- There is a long evolutionary and ecological history of environmental exposure of humans, plants and animals to organisms carrying *Wolbachia pipientis*, specifically *Culex* and *Aedes* mosquitoes and other invertebrates, with no recorded infection of *Wolbachia* in non-arthropod animals.
- As an obligate endosymbiont, *Wolbachia pipientis* cannot survive for any significant time outside the cells of its host arthropod As a result, there is no expected infective or direct exposure to humans and non-target organisms beyond what is already present due to ongoing environmental interactions.

In its "Final Registration Decision of the New Active Ingredient *Wolbachia pipientis* ZAP (wPip) strain in *Aedes albopictus* PC Code: 069035" (U.S. EPA, 2017a, published as EPA-HQ-OPP-2016-0205-0034), the EPA concluded that "Under the guidance of the National Academy of Sciences (NAS), EPA's Office of Pesticide Programs conducts assessments to evaluate the risks associated with the use of a pesticide. These assessments often evaluate the risk in the context of hazard and exposure using the following relationship:

Risk = Hazard x Exposure

Based on this equation, it follows that a small intrinsic hazard posed by the pesticidal substance and low levels of exposure both contribute to the reduction of the overall risk associated with the use of a pesticide. For the purposes of this [FIFRA Section 3 registration for ZAP mosquitoes], the *Wolbachia* ZAP strain will only be used in male *A. albopictus* mosquitoes. Male mosquitoes do not bite and do not feed on blood, and therefore do not significantly contribute to the exposure of humans to the *Wolbachia* ZAP strain. On the other hand, female mosquitoes bite to take blood meals to support the energy-intensive process of egg production." The EPA review goes on to state "The expected accidental release rate of 1 ZAP-infected female for every 250,000 ZAP-infected males is considered negligible exposure to humans resulting in a negligible human health risk and therefore meets the standard of no unreasonable adverse effects."

As described in publications (Crawford et al 2020), the Verily male mosquito manufacturing process includes multiple sex separation steps including 1) pupal separation based on the sexual dimporphism of male and female pupe whereby male pupae are typically smaller than female pupae, 2) visual adult sorting on eclosed adults whereby multiple photographs are taken of each individual mosquito and evaluated by machine vision for male and female characteristics. Together with Quality Control assays in the manufacturing process, these steps ensure <1:250,000 female:male in the released product, which is the female contamination required in the ZAP registration (EPA-HQ-OPP-2016-0205-0034). As described in (Crawford et al 2020), Verily may perform additional quality control checks including additional independent machine learning-based image reviews and human reviews, which significantly further reduce female contamination in the released product. For DQB males, these steps result in the expectation of a female contamination rate significantly lower than 1:250,000 during ongoing operations, with the process undergoing continual improvement.

We note that the Verily mosquito manufacturing process for DQB ensures that Mosquitoes are reared according to EPA and CDC guidelines so that colonies and released males will not be infected with human or animal pathogens. The manufacturing process MRID 51991801 provides further details. In addition we note that released male mosquitoes do not, and cannot bite, and so they cannot transmit any such pathogens, and that releases will occur in conservation areas without human habitation

Risks to the Environment

Risks of removal of *Cx. quinquefasciatus*

None of the six species of biting mosquitoes present in Hawaii are native to the islands, and the Hawaii Department of Land and Natural Resources (DLNR) confirms that native wildlife do not rely on mosquitoes as a prey base (Winchester and Kapan, 2013). Hawaii's native fauna evolved over millions of years as constituents in a diverse community assemblage. In contrast, mosquitoes are comparatively recent introductions, having invaded Hawaii less than 200 years ago (Nishida 1994). *Culex quinquefasciatus* was introduced to the island of Maui in 1826 when a whaling vessel which traveled from Mexico docked at the Port of Lahaina (Van Dine, 1904). And, their subsequent slow spread to additional islands and habitats means their ecological interactions with native wildlife in some locations is even more limited in duration. Native taxa, which are generalist insectivores, do feed opportunistically on mosquitoes, however there are thousands of other arthropod food resources in the environment, including well over one hundred native and non-native aquatic Dipterans. The endangered Hawaiian hoary bat, one of only two mammals native to Hawaii, primarily eats larger-bodied prey such as beetles and moths (Pinzari et al, 2019). Native damselflies, including rare and endangered species, can persist in habitat where mosquitoes are present, but also thrive in habitat where mosquitoes are not yet present and/or are only observed in low abundance (Polhemus and Asquith, 1996). **As such, there are no anticipated repercussions of a diminished or eradicated population of *Cx. quinquefasciatus.***

Risks of Active ingredient or unintentional contaminants

As noted above, *WoIbachia pipientis* is an obligate intracellular bacterium, occurring naturally in many invertebrates, including, insects, isopods, ticks and filarial worms of humans and other animals (Casiraghi et aI., 2005; Saridaki & Bourtzis 2010). This includes up to 65% of insect species (Hilgenboecker et al. 2008, Kittayapong et al. 2008, Ruang-Areerate et al. 2003). Thus there is a long evolutionary and ecological history of environmental exposure of plants and animals to *Wolbachia pipientis,* including to the wAlbB in DQB males.

In its "Final Registration Decision of the New Active Ingredient *Wolbachia pipienti*s ZAP (wPip) strain in *Aedes albopictus* PC Code: 069035" (U.S. EPA 2017a published as EPA-HQ-OPP-2016-0205-0034), the **EPA concluded that "there is a history of ubiquitous environmental exposure to the bacterium and that, in conjunction with the data provided, no adverse effects to the environment, including endangered species, is expected as a consequence of the release."** The ecological risk assessments for the ZAP EUP and WB1 wAlbB in *Ae. aegypti* EUP made similar conclusions for low risk of adverse effects in non-target organisms. The Agency also concluded that "none of the potential effects [of *Wolbachia pipientis* outside of the target species] have either been documented to occur or are likely to occur within a timeframe relevant to the ZAP-infected mosquitoes' life-span." which would also apply to male DQB mosquitoes.

The "Ecological risk Assessment for *Wolbachia pipientis* wAlbB *Ae. aegypti* EUP 89668-EUP-3 extensions/amendment Decision #525796, DP #438208"(U.S. EPA 2017b published as EPA-HQ-OPP-2017-0392-0002) summarized earlier Agency reviews of incompatible *Wolbachia pipientis* in male *Ae. aegypti* (and specifically to wAlbB *Wolbachia pipientis* which is the subtype present in DQB males) and stated that "Adverse effects are not expected for birds or wild mammals as a result of the release of mosquitoes infected with *W. pipientis* wAlbB strain" and separately stated that the "rationale [provided for earlier reviews of risks of *Wolbachia pipientis* to non-target organism] was acceptable for the EUP and section 3 of the ZAP strain and are thus considered acceptable for wAlbB *Aedes aegypti* [...]" Additionally they stated that "EPA's conclusion that male Ae. aegypti mosquitoes carrying the wAlbB strain of W. pipientis will not cause adverse effects to non target organisms is independent of the number of males released and release locations." This same reasoning should apply to *Wolbachia pipientis* strain wAlbB in DQB, and we likewise conclude that no adverse effects are likely to occur in non-target plants or animals from the labeled release of DQB male adult *Ae. aegypti* mosquitoes.

SECTION 166.20(a)(8): COORDINATION WITH OTHER AFFECTED FEDERAL OR STATE AGENCIES

The following state/federal agencies were notified of the Hawaii Department of Agriculture's (HDOA's) actions to submit an application for a Specific Exemption, under Section 18 of FIFRA, to EPA:

- Hawaii Department of Health: Environmental Management Division
- University of Hawaii Pesticides Program
- Hawaii Department of Land and Natural Resources: Division of Forestry and Wildlife
- United States Fish and Wildlife Service: Pacific Islands Fish and Wildlife Office
- United States National Park Service: Biological Resource Division
- United States Geological Survey

SECTION 166.20(a)(9): ACKNOWLEDGEMENT BY REGISTRANT

Verily Life Sciences LLC has been notified of HDOA's intent regarding this application (see attached letter of support, submitted as an attachment with this packet). They have also provided a copy of a label with the Specific Exemption use directions.

SECTION 166.20(a)(10): DESCRIPTION OF PROPOSED ENFORCEMENT PROGRAM

Hawaii Department of Agriculture has the authority to regulate the distribution, storage, sale, use and disposal of pesticides in Hawaii. In addition, the EPA/HDOA grant enforcement agreement provides the Department with the authority to enforce the provisions of FIFRA, as amended, within the State. Therefore, HDOA will make at least one random, unannounced call per week on applicators participating in the Section 18 Specific Exemption program for Male DQB to check for compliance with provisions of the Specific Exemption. If violations are discovered appropriate enforcement action will be taken.

Notification and contact information from the previous seven (7) days will be placed into a sorted spreadsheet by island. Applicators will all be assigned a random number via RAND function and sorted highest to lowest. The contact information of the applicator at the top of the list will then be provided to credentialed inspectors to contact for inspection scheduling. If the inspector cannot contact the applicator within one (1) business day the inspector will request a new applicator from the Pesticides Program Manager. This process will be continued for at least the first three months after exemption approval to ensure appropriate use.

SECTION 166.20(a)(11): REPEATED USES

This is the first time that HDOA has applied for this Specific Emergency Exemption.

SECTION 166.25(b)(2)(ii): PROGRESS TOWARDS REGISTRATION

Verily Life Sciences LLC is working towards a Section 3 registration for Male DQB. The Section 3 registration is expected to be submitted in 2023 if the Section 18 Specific Emergency Exemption is approved. As such Section 3 approval is anticipated by the end of calendar year 2024.

The Section 3 label is expected to be submitted in 2023 if the Section 18 specific exemption is approved. Section 3 approval is anticipated by the end of calendar year 2024.

SECTION 166.20(b)(1): NAME OF PEST

Culicid Mosquito, Wild Type *Culex quinquefasciatus.*

Culex quinquefasciatus is a non-native Culicid that invaded Hawai'i ~100 years ago from ship ballasts (Samuel et al. 2011). In Hawai'i, densities of Cx. quinquefasciatus are correlated with temperature and standing water (generally eutrophic) availability (Matthew E. Reiter and LaPointe 2009). As Hawai'i is a mountainous tropical archipelago in the Pacific Ocean with a number of native rain forests at both lower and upper elevations, variance of Cx. quinquefasciatus densities in Hawai'i are largely associated with elevational (i.e. temperature) gradients (Samuel et al. 2011; M.E. Reiter and LaPointe 2007). As Cx. quinquefasciatus females must take a blood meal to complete a gonotrophic cycle, and they feed on a wide array of animals, including humans, they are implicated in a number of zoonotic diseases (e.g. west nile virus, japanese encephalitis etc.), as well as diseases of agricultural (e.g. dog heartworm) and conservation (e.g. avian malaria) significance (please see Attachment C, Table 1). Only avian malaria, avian pox, and dog heartworm, diseases caused by pathogens of agricultural and conservation concern, are transmitted by Cx. quinquefasciatus in Hawai'i (Samuel et al. 2018; Ash 1962). For a detailed review of the ecology and biology of Cx. quinquefasciatus in Hawai'i, as well as an overview of pathogens Cx. quinquefasciatus transmits, please see Attachment C.

SECTION 166.20(b)(2): DISCUSSION OF EVENTS WHICH BROUGHT ABOUT THE EMERGENCY CONDITION

Avian malaria, introduced into the Hawaiian islands in the 19th century and spread by a non-native mosquito (*Culex quinquefasciatus*), has resulted in devastating effects to Hawaii's endemic and iconic forest birds. Already threatened by habitat loss and predation by invasive predators, avian malaria reduced the diverse forest bird assemblage from more than 50 species known historically down to only 23 remaining extant species, 15 of which are listed as threatened or endangered. The remaining species are now restricted to the highest elevations of Hawaii's mountains, where it is too cold for mosquitoes and avian malaria to persist. However, as climate change increases temperatures, mosquitoes are ascending into the highest elevations of remaining forest bird habitat; resulting in further range restriction and population declines. Areas previously thought safe for forest birds are now inundated with mosquitoes, including Haleakala National Park, Hawaii Volcanoes National Park, and numerous State-owned lands managed for forest bird conservation.

Despite ongoing efforts to support Hawaii's forest bird recovery through an array of long-standing partnerships, there are no current management tools to address the mosquito/disease threat. Without a means of mosquito control at a landscape-level, the survival and recovery of Hawaii's few remaining forest birds are at imminent risk. Updated information collected in early 2021 indicates that the extinction of four or more of the remaining species is likely to occur within the next few years due to avian malaria.

Over the last six years, the State of Hawaii, the Pacific Islands Fish and Wildlife Office (PIFWO), and our conservation partners have worked to identify and develop conservation strategies to address this crisis. After thorough review, the use of an Incompatible Insect Technique (IIT), Wolbachia infected mosquitoes, has been identified as the fastest option for suppressing mosquitoes at a landscape-scale. Wolbachia is a naturally occurring bacteria; when mosquitoes carrying the bacteria breed with other mosquitoes, they only produce non-viable eggs leading to population suppression. While this tool has been successfully deployed in urban areas for public health around the world, it has never been attempted for a conservation application in a remote forest setting.

The conservation partners have established a Birds, Not Mosquitoes Steering Committee to lead the collaborative effort in developing and implementing Wolbachia IIT in Hawaii. Initial implementation of the tool is expected in 2023; however, there are numerous compliance, infrastructure, financial and technical milestones that must be met to support this timeline. Effective implementation is not likely to come in time for some forest bird species; therefore, PIFWO continues to work with our partners to identify interim actions to prevent their extinction (USFWS memo, 2021).

Additional support for this program and intervention is provided by the following resolutions:

- Hawaii Invasive Species Council adopted Resolution 17-2 in 2017, supporting research and evaluation of landscape-scale control technologies for mosquitoes, and encouraging researchers to approach this research in a way that could potentially benefit both native wildlife and human health in Hawaii https://dlnr.hawaii.gov/hisc/files/2013/02/HISC-Reso-17-2-Mosquitoes.pdf
- House Resolution (HR) 297 passed the Hawaii State House in 2019, and directed "DOA to review the Aedes aegypti mosquito with Wolbachia bacteria, including Aedes aegypti mosquitoes originating from Hawaii stock that could be imported for landscape scale mosquito control, and render a determination to place it on the appropriate animal import list. Requires DOA, DOH, and DLNR to collaborate on a report to the Legislature with recommendations for appropriate vector control programs. https://www.capitol.hawaii.gov/session2019/bills/HB297_SD1_.htm
- House Resolution (HR) 95 passed the Hawaii State House in 2021 urging DLNR, DOA, DOH and UH to implement a mosquito control program using Wolbachia to reduce mosquito population levels throughout the state. https://www.capitol.hawaii.gov/session2021/bills/HR95_HD1_.htm

Areas affected (counties of geographical locations).

The State of Hawaii counties of Honolulu, Hawaii, Kauai, Niihau, and Maui.

SECTION 166.20(b)(4)(i, ii, iii): DISCUSSION OF ANTICIPATED ECONOMIC LOSS

Not applicable to this application.

SECTION 166.20(b)(3): DISCUSSION OF ANTICIPATED RISKS TO ENDANGERED OR THREATENED SPECIES, BENEFICIAL ORGANISMS, OR THE ENVIRONMENT

Endangered and Threatened Species in Hawaii

Overview

Due to the biological nature of *Wolbachia*, the proposed application of DQB Males in forested and non-crop areas in Hawaii is unlikely to adversely affect federally listed T&E species. The use of DQB Males to control mosquitoes in forested and non-crop areas is intended to reduce the abundance of *Cx. quinquefasciatus* mosquitoes which are known to vector avian malaria to T&E bird species.

Furthermore, no harm to listed plants, invertebrates or birds is expected to occur as a result of IIT application. The DLNR-DOFAW, and designated agents of the State, have the authority to conduct the conservation and management activities outlined in this application, including accessing State lands, conducting helicopter operations, and completing invasive species research, management, and control. Staff and partners will use existing trails to access field sites. To prevent the spread of invasive insects, plants and plant diseases all equipment will be washed and decontaminated between field excursions. In the event that helicopters are used to transit to field sites, they will only land on preexisting designated landing zones, therefore causing no additional negative impacts from downwash and noise, and no additional impacts to native vegetation. DLNR has a staff entomologist, malacologist, botanist, and forest bird biologist who are included in planning, site selection and project implementation. Final treatment areas will be selected in cooperation with USFWS PIFWO staff.

Listed invertebrate species present:

Maui – Blackburn's sphinx moth (*Manduca blackburni*) may be present within the proposed treatment areas, however we do not expect field activities will have any positive or negative impact on the species. *Manduca blackburni* is not typically present in closed canopy wet forest areas as its host plants are native to mesic and dry forest habitat. Nonetheless, staff will be trained to identify the species and its associated host plants.

Kauai - Endemic picture-wing fly *Drosophila musaphilia* is present within the proposed treatment areas. The DLNR-DOFAW entomologist will ensure that staff and partners are aware of locations of existing populations and can recognize known host plants. As a result, we do not expect to have any positive or negative impacts on the species.

Hawaii Island - While no populations are known to be extant, endemic picture-wing flies *D. ochrobasis* and *D. heteroneura* may be present within the proposed treatment areas. The DLNR-DOFAW will ensure that staff and partners can recognize known host plants. As a result, we do not expect to have any positive or negative impacts on the species.

The DLNR-DOFAW Hawaii Invertebrate Program and the Snail Extinction Prevention Program have not identified additional sensitive locations of existing T/E insect or snail populations within the proposed treatment areas but will be consulted if additional populations are detected. As a result, we do not expect to have any positive or negative impact to any additional listed invertebrate species.

Listed bird species present:

Several threatened and endangered waterbird, forest bird and seabird species are confirmed or may be present and breeding in the project areas. To prevent negative impacts to these species we will use existing trails, camps and helicopter landing zones to access treatment areas. Mosquito suppression will have a positive impact on forest bird species. We do not anticipate negative impacts.

Maui -

Kiwikiu or Maui parrotbill, *Pseudonestor xanthophrys*

ʻAkohekohe, *Palmeria dolei*

ʻIʻiwi, *Drepanis coccinea*

ʻUaʻu or Hawaiian petrel *Pterodroma sandwichensis*

'A'o or Newell's shearwater, *Puffinus newelli*

ʻAkeʻake or Band-rumped storm-petrel, *Hydrobates castro*

Nēnē, *Branta sandvicensis*

Kauai -

ʻAkikiki, *Oreomystis bairdi*

ʻAkekeʻe, *Loxops caeruleirostris*

ʻIʻiwi, *Drepanis coccinea*

Puaiohi, *Myadestes palmeri*

ʻUaʻu or Hawaiian petrel *Pterodroma sandwichensis*

‘Ake‘ake or Band-rumped storm-petrel, *Hydrobates castro*

Nēnē, *Branta sandvicensis*

Hawaii Island -

‘Akiapola‘au, *Hemignathus wilsoni*

Hawai‘i ‘akepa, *Loxops coccineus*

Palila, *Loxioides bailleui*

‘I‘iwi, *Drepanis coccinea*

‘Alawī or Hawai‘i creeper, *Loxops mana*

‘Ua‘u or Hawaiian petrel *Pterodroma sandwichensis*

‘Ake‘ake or Band-rumped storm-petrel, *Hydrobates castro*

Nēnē, *Branta sandvicensis*

Listed mammal species present:

Kaui, Maui and Hawaii Island – The Hawaiian bat (*Lasiurus cinereus semotus*) is present in the proposed treatment areas. The Hawaiian hoary bat is an insectivore, however it evolved in the absence of mosquitoes, and its diet primarily consists of larger-bodied prey such as beetles and moths. As such, we do not expect the reduced abundance of *Cx. quinquefascatius* to have any positive or negative impacts on bat populations.

Listed plant species present: (better to insert tables after editing)

To prevent negative impacts to rare, threatened and endangered plants we will use existing trails and helicopter landing zones to access treatment areas. No vegetation will be cleared or disturbed. Between field sites all gear will be decontaminated to prevent the spread of invasive plant seeds and diseases. To prevent the spread of rapid ohia death, no field gear used on the Big Island will be used on Maui or Kauai. DLNR-DOFAW island botanists, as well as staff from the Plant Extinction Prevention Program, have identified listed plants known to occur within the treatment area and will be consulted if additional populations are detected. As a result, we do not expect to have any positive or negative impact to any listed plant species.

Maui Plant Taxon Name	Fed Status

Asplenium peruvianum var. insulare	Endangered
Bidens campylotheca subsp. pentamera	Endangered
Bidens campylotheca subsp. waihoiensis	Endangered
Calamagrostis expansa	Endangered
Clermontia samuelii subsp. hanaensis	Endangered
Clermontia samuelii subsp. samuelii	Endangered
Cyanea asplenifolia	Endangered
Cyanea copelandii subsp. haleakalensis	Endangered
Cyanea hamatiflora subsp. hamatiflora	Endangered
Cyanea horrida	Endangered
Cyanea kunthiana	Endangered
Cyanea maritae	Endangered
Cyanea mceldowneyi	Endangered
Cyrtandra ferripilosa	Endangered
Deparia kaalaana	Endangered
Diplazium molokaiense	Endangered
Gardenia remyi	Endangered
Geranium arborerum	Endangered

Geranium hanaense	Endangered
Geranium multiflorum	Endangered
Joinvillea ascendens subsp. ascendens	Endangered
Melicope balloui	Endangered
Melicope ovalis	Endangered
Menisciopsis boydiae	Endangered
Microlepia strigosa var. mauiensis	Endangered
Nothocestrum longifolium	Endangered
Ochrosia haleakalae	Endangered
Peperomia subpetiolata	Endangered
Phlegmariurus mannii	Endangered
Phyllostegia bracteata	Endangered
Phyllostegia brevidens	Endangered
Phyllostegia haliakalae	Endangered
Phyllostegia pilosa	Endangered
Plantago princeps var. laxifolia	Endangered
Platanthera holochila	Endangered
Schiedea diffusa subsp. difusa	Endangered

Wikstroemia villosa	Endangered

Kauai Plant Taxon Name	**Federal Status**
Adenophorus periens	Endangered
Alectryon macrococcus var. macrococcus	Endangered
Asplenium dielpallidum	Endangered
Astelia waialealae	Endangered
Bonamia menziesii	Endangered
Brighamia insignis	Endangered
Canavalia napaliensis	Endangered
Cyanea recta	Endangered
Cyanea rivularis	Endangered
Cyrtandra kealiae subsp. kealiae	Endangered
Cyrtandra paliku	Endangered
Dryopteris glabra var. pusilla	Endangered
Dubautia latifolia	Endangered
Dubautia pauciflorula	Endangered
Dubautia waialealae	Endangered

Euphorbia halemanui	Endangered
Euphorbia halemanui	Endangered
Euphorbia remyi var. kauaiensis	Endangered
Euphorbia remyi var. remyi	Endangered
Euphorbia remyi var. remyi	Endangered
Exocarpus luteolus	Endangered
Geniostoma helleri	Endangered
Geranium kauaiense	Endangered
Hibiscadelphus distans	Endangered
Hibiscus clayi	Endangered
Ischaemum byrone	Endangered
Isodendrion longifolium	Threatened
Joinvillea ascendens subsp. ascendens	Endangered
Joinvillea ascendens subsp. ascendens	Endangered
Keysseria helenae	Endangered
Lobelia niihauensis	Endangered
Lysimachia daphnoides	Endangered
Lysimachia pendens	Endangered

Melicope degeneri	Endangered
Melicope Haupuensis	Endangered
Melicope pallida	Endangered
Melicope paniculata	Endangered
Melicope puberula	Endangered
Melicope rostrata	Endangered
Melicope rostrata	Endangered
Myrsine fosbergii	Endangered
Myrsine knudsenii	Endangered
Myrsine linearifolia	Endangered
Myrsine mezii	Endangered
Nothocestrum latifolium	Endangered
Nothocestrum peltatum	Endangered
Peucedanum sandwicense	Threatened
Phyllostegia helleri	Endangered
Phyllostegia renovans	Endangered
Pittosporum napaliense	Endangered
Platanthera holochila	Endangered

Poa mannii	Endangered
Poa sandvicensis	Endangered
Poa siphonoglossa	Endangered
Polyscias racemosa	Endangered
Pritchardia viscosa	Endangered
Psychotria grandiflora	Endangered
Psychotria hobdyi	Endangered
Pteralyxia kauaiensis	Endangered
Ranunculus Mauiensis	Endangered
Remya kauaiensis	Endangered
Schiedea helleri	Endangered
Schiedea lychnoides	Endangered
Schiedea membranacea	Endangered
Schiedea spergulina	Endangered
Schiedea viscosa	Endangered
Sesbania tomentosa	Endangered
Solanum sandwicense	Endangered
Spermolepis hawaiiensis	Endangered

Stenogyne kealiae	Endangered
Wilkesia hobdyi	Endangered
Xylosma crenatum	Endangered

Hawaii Island Plant Taxon Name	**Federal Status**
Abutilon menziesii	Endangered
Achyranthes mutica	Endangered
Adenophorus periens	Endangered
Argyroxiphium kauense	Endangered
Argyroxiphium sandwicense subsp. sandwicense	Endangered
Asplenium dielerectum	Endangered
Asplenium peruvianum var. insulare	Endangered
Bidens hillebrandiana subsp. hillebrandiana	Endangered
Bidens micrantha subsp. ctenophylla	Endangered
Bonamia menziesii	Endangered
Calamagrostis expansa	Endangered
Cenchrus agrimonioides var. agrimonioides	Endangered
Clermontia drepanomorpha	Endangered

Clermontia lindseyana	Endangered
Clermontia peleana subsp. peleana	Endangered
Clermontia peleana subsp. singuliflora	Endangered
Clermontia pyrularia	Endangered
Colubrina oppositifolia	Endangered
Cyanea copelandii subsp. copelandii	Endangered
Cyanea hamatiflora subsp. carlsonii	Endangered
Cyanea marksii	Endangered
Cyanea platyphylla	Endangered
Cyanea shipmanii	Endangered
Cyanea stictophylla	Endangered
Cyanea tritomantha	Endangered
Cyperus fauriei	Endangered
Cyrtandra giffardii	Endangered
Cyrtandra nanawaleensis	Endangered
Cyrtandra tintinnabula	Endangered
Cyrtandra wagneri	Endangered
Delissea argutidentata	Endangered

Deparia kaalaana	Endangered
Dracaena konaensis	Endangered
Exocarpos menziesii	Endangered
Festuca hawaiiensis	Endangered
Flueggea neowawraea	Endangered
Gardenia brighamii	Endangered
Gardenia remyi	Endangered
Gouania vitifolia	Endangered
Haplostachys haplostachya	Endangered
Hibiscadelphus giffardianus	Endangered
Hibiscadelphus hualalaiensis	Endangered
Hibiscus brackenridgei subsp. brackenridgei	Endangered
Ischaemum byrone	Endangered
Isodendrion hosakae	Endangered
Isodendrion pyrifolium	Endangered
Joinvillea ascendens subsp. ascendens	Endangered
Kadua cookiana	Endangered
Kadua coriacea	Endangered

Kokia drynarioides	Endangered
Melicope remyi	Endangered
Melicope zahlbruckneri	Endangered
Mezoneuron kavaiense	Endangered
Microlepia strigosa var. mauiensis	Endangered
Neraudia ovata	Endangered
Nothocestrum breviflorum	Endangered
Ochrosia haleakalae	Endangered
Ochrosia kilaueaensis	Endangered
Phlegmariurus mannii	Endangered
Phlegmariurus stemmermanniae	Endangered
Phyllostegia brevidens	Endangered
Phyllostegia floribunda	Endangered
Phyllostegia parviflora var. glabriuscula	Endangered
Phyllostegia racemosa	Endangered
Phyllostegia stachyoides	Endangered
Phyllostegia velutina	Endangered
Phyllostegia warshaueri	Endangered

Pittosporum hawaiiense	Endangered
Plantago hawaiensis	Endangered
Plantago princeps var. laxifolia	Endangered
Portulaca sclerocarpa	Endangered
Portulaca villosa	Endangered
Pritchardia lanigera	Endangered
Pritchardia maideniana	Endangered
Pritchardia schattaueri	Endangered
Ranunculus hawaiensis	Endangered
Ranunculus mauiensis	Endangered
Sanicula sandwicensis	Endangered
Scaevola coriacea	Endangered
Schiedea diffusa subsp. difusa	Endangered
Schiedea diffusa subsp. macraei	Endangered
Schiedea hawaiiensis	Endangered
Sesbania tomentosa	Endangered
Sicyos albus	Endangered
Sicyos macrophyllus	Endangered

Silene hawaiiensis	Threatened
Silene lanceolata	Endangered
Solanum incompletum	Endangered
Solanum nelsonii	Endangered
Spermolepis hawaiiensis	Endangered
Stenogyne angustifolia	Endangered
Stenogyne cranwelliae	Endangered
Tetramolopium arenarium subsp. arenarium	Endangered
Vicia menziesii	Endangered
Vigna o-wahuensis	Endangered
Wollastonia venosa	Endangered
Zanthoxylum dipetalum var. tomentosum	Endangered
Zanthoxylum hawaiiense	Endangered

Benefits of action to Threatened and Endangered species

- The number of endemic forest bird passerines of Hawaii has declined from 58 described species to only 22 known to be alive in the wild today. Of these 22 species remaining in the wild, 14 are listed as threatened or endangered, including 11 honeycreeper species. The remaining honeycreeper species are now restricted to upper elevation refuges from avian malaria and its vector *Cx. quinquefasciatus*. Mosquitoes are now invading these last remaining native refugia due to climate change induced temperature increases. Projections of recently obtained data predict the extinction of four or more of the remaining species within the next few years. There are no current management tools compatible with Hawaii's topographically complex and remote native refugia that can effectively address the mosquito threat across the landscape. To preserve Hawaii's forest birds for future generations an efficient and effective landscape level tool must be applied to control *Cx. quinquefasciatus*, the primary vector of avian malaria. Current models indicate that in the absence of avian malaria the potential habitable range for native forest birds would increase significantly. The use of IIT to suppress mosquito reproduction and population size in forest bird habitats, potentially preserving and expanding existing refugia, would help to reduce the probability of a threatened or endangered species going extinct in the near future. If conducted in an efficient and timely manner the application of IIT will act as a stop-gap until the implementation of broadly expansive climate action.

- Hawaii's forest birds act as seed dispersers and pollinators and are ecologically significant to the ecosystem in which they inhabit. The extinction of Hawaiian forest birds will directly impact native ecosystems and forests such that their loss may beget a cascading effect throughout the ecosystem, indirectly causing the decline and extinction of other threatened or endangered species.

- These native ecosystems are also predominantly watersheds. Extensive modifications of these ecosystems could cause drastic reductions in water uptake and distribution for natural and anthropogenic needs (e.g. drinking water, agriculture, T&E stream inhabitants etc). As Hawaii is isolated from any other fresh water sources, the preservation of these native watersheds is of the utmost importance.

Cultural benefits

- The recent report produced by U.S. Geological Survey, U.S. Fish and Wildlife Service, and U.S. Office of Native Hawaiian Relations provided the most up-to-date scientific and biocultural information available. From the report:

  > "*A majority of Native Hawaiian participants viewed management decisions around ʻakikiki, ʻakekeʻe, kiwikiu, and ʻākohekohe akin to making end of life choices for members of their ʻohana. While most participants thought immediate steps should be taken to prevent the extinction of these species, extinction was not always considered the worst-case scenario. Instead, the likelihood of success; welfare of individual birds; and social, biocultural, and cultural connection of the birds to their natural environment were significant considerations when evaluating management options. If these considerations could not be fully realized, some participants considered it more appropriate to allow the birds to go extinct in their natural environment without further intervention, akin to allowing a member of their ʻohana to breathe their last breath in their "one hānau," or birthplace.*"
  >
  > (Eben, et. al. 2022)

- Hawaii's forest birds have been revered, honored and deified by Native Hawaiians for centuries, this is definitive of the deep kinship that Native Hawaiians and Hawaiian forest birds share (Eben et al. 2022).

- Effectively implementing IIT in a timely manner will enable Hawaii's forest birds to maintain and potentially expand their populations in their native ecosystems, thereby reducing and/or removing any impact that captive rearing may have on these culturally and ecologically significant resources.

Direct Economic Benefits

- As noted previously many Hawaiian forest birds are seed dispersers and pollinators, and thus the ecosystem in which they inhabit directly benefits from their presence. Hawaiian forest bird refugia are primarily watersheds which maintain the freshwater aquifers on all of the main Hawaiian islands. A single watershed on Oahu was estimated to provide groundwater drinking services of around $14 billion (Kaiser et al., 2008); there are ~437 watersheds on the main eight Hawaiian islands (Atlas of Hawaiian watersheds, 2008). Modifications to these ecosystems caused by the removal of a seed disperser and pollinator may have a down-stream detrimental effect on these native watersheds, and thus aquifers. These modifications may reduce fresh water uptake and cause excessive erosion, detrimentally affecting agricultural yields and other anthropogenic uses (e.g. drinking water, tourism, cost-of-living, food prices etc.).

- No direct estimate of the annual contribution of bird watching (birding) to Hawaii's economy exists, but national and regional projections can be used as a proxy to estimate local contribution minimums. Haleakalā (Maui) and Hawaii Volcano (Island of Hawaii) National Parks hosted 2,160,975 visitors in 2018, generating an estimated $161,559,000 for the local economy in the State of Hawaii (Thomas et al., 2021). These parks include both spectacular scenery and an expansive array of native species, including many native forest birds. In the United States the outdoor recreation economy was estimated to generate ~ $800 billion annually (Roberts 2017), and birding in 2011 was estimated to have generated ~$40 billion (of that ~$800 billion), or ~5% of the outdoor recreation economy (USFWS 2013). Applying this proportion to the visitor spending in and around these two Hawaii National Parks predicts that birding generates at minimum ~$8 million annually in the State of Hawaii. If these numbers are broadly applied to both Oahu and Kauai (i.e. ~$4 million each) then the projected annual income generated from birders in Hawaii would be ~$16 million. Maintaining these Federally protected natural heritage resources will allow the continued economic influx from birding into Hawaii's economy.