Attention: Mosquito Control Project Kaua'i Department of Land and Natural Resources U.S. Fish and Wildlife Service

22 July 2023

Aloha,

This testimony is in regards to draft Kaua'i Environmental Assessment (EA) completed in June 2023.

I am **strongly opposed** to the request for Anticipated Finding of No Significant Impact (AFONSI) for the release of lab bred Wolbachia southern house mosquitoes in Kaua'i and all Hawaiian Islands for numerous reasons documented in this extensive testimony. There is insufficient detail for the public to properly evaluate the EA as being safe for the environment. The Kaua'i draft EA requires much more study on secondary impacts with no less than a full scope Environmental Impact Statement (EIS) since mosquitoes are a vector of disease.

The Incompatible Insect Technique (IIT) is being promoted in the EA as a mosquito control method to help save endangered birds from avian malaria. Page 9 of the EA states this technique has been successfully implemented in 14 countries and 4 cities in the United States, but fails to list the countries and projects that are connected. The only Wolbachia program that has been implemented worldwide at this scale is the World Mosquito Program funded by The Bill and Melinda Gates Foundation. This is a different method involving Aedes *aegypti* males and females released into urban areas for population replacement to control Dengue fever, a human disease. The World Mosquito program chart on its website lists difference between the methods used worldwide. The IIT method proposed for Maui and Kaua'i "relies on the continuous production and release of male mosquitoes and is, therefore, more expensive than the World Mosquito Program's method. There is no field evidence that it can reduce the risk of mosquito-borne diseases."

https://www.worldmosquitoprogram.org/en/learn/how-our-method-compares

The IIT method has never been used for conservation purposes or with the species Culex *quinquefasciatus* (southern house mosquito) anywhere worldwide. This is an experiment based on several unsound justifications and references. Federal documents admit the outcome is unknown. The public has already voiced numerous concerns about the release of lab bred mosquitoes in response to the Maui EA which is now being challenged in environmental court to seek a ruling to require an Environmental Impact Statement. No further actions should be taken to release biopesticide mosquitoes anywhere Hawai'i while the need for further study of the risks is actively being litigated.

Since spring 2022, as a veteran in National Security and Investigations for over 30 years, I have personally studied the science in depth behind the use of Wolbachia for mosquito control. After reviewing thousands of pages of scientific papers, environmental assessments, government documents, videos, interviews, funding, and grants related to

Wolbachia; as well as consulting with experts regularly; what stands out from all this research is that Wolbachia bacterium strains are still being discovered and its impacts are yet to be fully understood. Its influence on other life forms; including humans, native birds, arthropods and filarial worms' reproductive cycle and pathogen infection (either to block or promote) is **still in process** of being vetted.

Science is still grasping the mechanisms of Wolbachia as documented on page 32 of Evaluation of Existing EFSA Guidelines for their Adequacy for the Molecular Characterization and Environmental Risk Assessment of Genetically Modified Insects with Synthetically Engineered Gene Drives. "The mechanism of Wolbachia-induced pathogen-blocking is not well understood (Marshall et al., 2019). Yet, this feature, along with the gene drive-like inheritance pattern of Wolbachia, has been harnessed in replacement strategies to limit disease transmission by mosquito populations." http://www.ask-force.org/web/EFSA/EFSA-GMO-Panel-Gene-drive-document-forconsultation-20200129.pdf

We are awaiting results of grants researched out of Penn State University thru NIH including WOLBACHIA-INDUCED ENHANCEMENT OF HUMAN ARBOVIRAL PATHOGENS. "A SOBERING REMINDER THAT THE PATHOGEN INHIBITORY EFFECTS RESULTING FROM WOLBACHIA INFECTION IN SOME INSECTS CANNOT AND SHOULD NOT BE GENERALIZED ACROSS VECTOR-PATHOGEN SYSTEMS. UNDERSTANDING THE GENERAL ARE CRITICAL FOR ESTIMATING HOW LIKELY WOLBACHIA-BASED CONTROL STRATEGIES ARE TO FAIL OR MAKE THINGS WORSE, FOR IDENTIFYING POTENTIAL POINTS WHERE WOLBACHIA-BASED CONTROL IS LIKELY TO BREAK DOWN IN THE FIELD, AND FOR PLANNING RISK MITIGATION STRATEGIES IN HE CASE OF UNFORESEEN HARMFUL OUTCOMES. IN THIS RESEARCH, WE WILL INVESTIGATE THE HYPOTHESIS THAT WOLBACHIA-INDUCED MODULATION OF THE MOSQUITO HOLOGENOME CAN LEAD TO INCREASED ARBOVIRUS INFECTION/TRANSMISSION IN SOME VECTOR-PATHOGEN SYSTEMS OF HUMAN IMPORTANCE."

https://govtribe.com/award/federal-grant-award/project-grant-r01ai116636

Wolbachia Potential to Increase Pathogen Infection

The Southern House Mosquito can transmit Avian Malaria, Avian Pox, Western Equine Encephalitis, West Nile Virus, Canine Heartworm, Lymphatic Filariasis/Elephantiasis, St. Louis Encephalitis and is a potential vector of Zika virus. There are Wolbachia studies that have shown it to increase pathogen infection in some instances

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"Mosquitoes infected with the bacteria Wolbachia are more likely to become infected with West Nile virus and more likely to transmit the virus to humans, according to a team of researchers." "The results suggest that caution should be used when releasing Wolbachia-infected mosquitoes into nature to control vector-borne diseases of humans." https://www.sciencedaily.com/releases/2014/07/140710141628.htm

Wolbachia Enhances West Nile Virus (WNV) Infection in the Mosquito Culex tarsalis https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0002965

Wolbachia Can Enhance Plasmodium Infection in Mosquitoes: Implications for Malaria Control? https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4154766/

Antibiotic Resistance

Page 12 of Kauai EA states: "To produce the incompatible male southern house mosquitoes for this project, a laboratory line of Hawai'i mosquitoes was generated with the wAlbB strain of Wolbachia. This was accomplished through a multi-step process that involved rearing Hawai'i mosquitoes in the lab and removing the wPip Wolbachia from their bodies with **common antibiotics**. The wAlbB strain of Wolbachia was then transferred into the eggs of these Wolbachia-free Hawai'i mosquitoes."

Use of this method over time with constant releases can lead to antibiotic resistance with unknown effects on the environment and can cancel out effectiveness of treatment for diseases in which Wolbachia is implicated in humans which is highly concerning.

The endosymbiont Wolbachia rebounds following antibiotic treatment https://pubmed.ncbi.nlm.nih.gov/32639986/

Previous mosquito control projects in California and Cayman Islands using Genetically Modified (GM) mosquitoes (which also uses antibiotics during lab rearing) have not renewed contracts. "Cayman Island officials were set to renew their contract. But data from the trials indicated serious problems, leading the territory's environmental health minister to tell the Edmonton Journal, the scheme was not getting the results we were looking for. There was further concern that the <u>released mosquitoes could be spreading antibiotic resistance or make mosquito-borne diseases worse by lowering individual immunity."</u>

Modified Mosquitoes Fail to Beat Malaria https://www.pressreader.com/canada/edmonton-journal/20181126/281951723871847

"British biotechnology company Oxitec is withdrawing its application to release billions of genetically engineered mosquitoes in California, according to a recent update from the California Department of Pesticide Regulation."

https://beyondpesticides.org/dailynewsblog/2023/05/efficacy-and-health-issues-stop-release-of-genetically-engineered-mosquitoes-in-california-florida-continues/

There are parallels between GM and Wolbachia techniques. Biologically Wolbachia lab infected mosquitoes are not GM mosquitoes, but the study designs, math, and adherence to protocol apply to both situations. The main biological difference is there is slower horizontal transfer of mutations of the GM mosquito than with horizontal transfer of Wolbachia. This means Wolbachia as a natural gene drive has the potential to have **greater unknown impact** on the environment, which necessitates the need for a full scope Environmental Impact Statement (EIS).

Horizontal Spread, Vertical Transmission, and Wolbachia as Gene Drive

"The evidence of horizontal spread of Wolbachia shows that the bacteria go not only to sexual cells, but also to somatic cells (non-sexual cells of the body). Wolbachia can also live outside of the intra-cellular systems for several months." Wolbachia Horizontal Transmission Events in Ants: What Do We Know and What Can We Learn? https://pubmed.ncbi.nlm.nih.gov/30894837/

Horizontal Gene Transfer Between Wolbachia and the Mosquito Aedes *aegypti* https://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-10-33

This document submitted by Oxitec to the EPA in 2015 outlines numerous legitimate and studied issues regarding the use of Wolbachia. https://downloads.regulations.gov > EPA-HQ-OPP-2015-0374-0018 > attachment_1.pdf

"Wolbachia is a bacterium residing within the cells of insects, and is passed through vertical transmission from mother to offspring. Even a single Wolbachia infected female could lay hundreds of eggs that would invade the wild population, rendering the Incompatible Insect Technique ineffective and spreading a new strain of Wolbachia into the environment. Modelling has shown that conditions of lower competition can favour infected females [6-8]. In other words, as a mosquito population is reduced, or if a population is already low, the chances of Wolbachia invading the wild population are increased."

"The Wolbachia is an endosymbiont on the cytoplasm of the cell so over a thousand new genes are introduced into the insect cells, some or all of which have the potential to randomly integrate into the insect's nuclear genome with unknown consequences. Moreover, the possible persistence of Wolbachia mosquitoes themselves is a significant concern. For the reasons set forth below, each new strain of mosquito, or indeed any artificially Wolbachia infected insect needs to be treated as a new strain and thoroughly tested in the laboratory before any field releases."

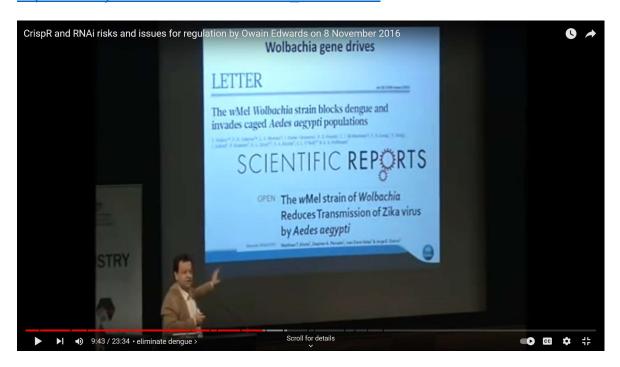
"The whole genome of Wolbachia can transfer to a host genome, meaning a host mosquito could be transformed with over one thousand new genes with unpredictable results [2-5]."

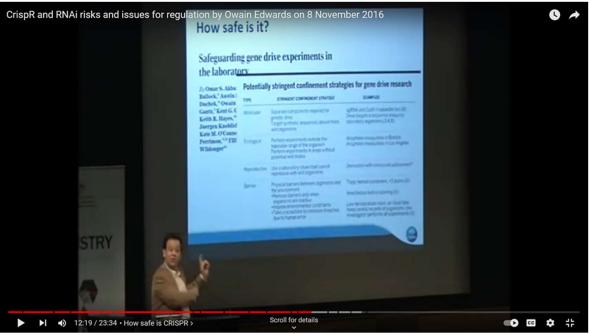
"It has already been shown that horizontal gene transfer (HGT) can transfer genes between Wolbachia and its host in Aedes aegypti [12] and several other mosquito species [13]. Therefore, **Wolbachia can genetically transform** its host with functional genes with currently unknown consequences."

"Horizontal transmission between unrelated host species is a proven phenomenon in Wolbachia [25]. Studies have demonstrated that genetic sequences, ranging in size from Horizontal transmission between unrelated host species is a proven phenomenon in Wolbachia [25]. Studies have demonstrated that genetic sequences, ranging in size from single genes to entire bacterial genomes, have been transferred from Wolbachia to

many of their insect hosts [2-5], and its effect on disease transmission is variable and potentially dangerous."

Owain Edwards of CSIRO in Australia (Commonwealth Scientific and Industrial Research Organisation) was involved in the Aedes *aegypti* trial around Innisfail (Beebe et al 2021) that was funded by Verily Life Sciences. Dr. Edwards refers to Wolbachia as a type of natural gene drive during his 2016 presentation for APVMA. https://www.youtube.com/watch?v=Lm WS9eXYIU





Dr. Edwards elaborates there are limitations on the use of Wolbachia application over time which can lead to limited choice of genes and for the Wolbachia technique to remain effective at suppressing mosquito population, a variety of natural strains are needed. The next step in the process is explained using CRISPR technology - synthetic gene drives. Dr. Edwards emphasizes while working on synthetic gene drives, "it requires double and triple containment to make sure these don't get out of the laboratory." This is concerning since page 32 of EA says, "DLNR is also exploring future options for establishing a state-run mosquito-rearing facility in Hawai'i; mosquito sources could also originate from a similar but state-run mosquito-rearing facility in the future. Should DLNR pursue this option, the appropriate regulatory permits and documentation (environmental reviews and facility compliance) would be necessary."

Federal documents state plans for future tools to include synthetic gene drives, next generation tools, synthetic biology control tools, novel technology deployment, and precision-guided Sterile Insect Technique (pgSIT) (CRISPR technology) in Hawai'i. While "technology for this approach is not available for near-term implementation," development and deployment of these tools appear to be a long-term goal at the federal level." U.S. Department of the Interior Strategy for Preventing the Extinction of Hawaiian Forest Birds –

https://www.fws.gov/sites/default/files/documents/DOI%20Strategy%20for%20Preventing%20the%20Extinction%20of%20Hawaiian%20Forest%20Birds%20%28508%29.pdf

Lack of Bio-Security

There has been no documentation offered to the public outlining risk analysis conducted on the security vulnerabilities for lab bred mosquitoes that can be utilized as bioweapons against a population (intended) nor details of quality control mechanisms for accidental transmission of pathogens (unintended). This includes failure to discuss how they will deal with accidental female escape, wind drift, or how male lab bred culex q. mosquitoes released into the wild can pass pathogen to biting females thru mating and shared feeding/water sources. The public has no idea how these lab mosquitoes will be quality controlled and tested.

Intended entomological warfare involves infecting insects with a pathogen and then dispersing the vectors over target areas. Invasive insects can also be deployed into a country en masse to take out crops and cripple a food supply. In New York the Plum Island lab was involved in the development of offensive bioweapons that led to Lyme's disease outbreaks. Japan's biological warfare unit (Unit 731) was deployed against China during World War II. The unit deployed plague-infected fleas and cholera-infected flies to take out the Chinese. https://citizens.news/694097.html

"We recommend careful invigilation of the international borders, airports, and seaports by the trained scientists to identify any accidental and/or deliberate import of alien arthropod vectors. Therefore, it is well advised to take seriously the possibility that arthropod could be used to attack people. Moreover, future research priorities should also includes high-throughput molecular diagnostics of diseases, identification of vectors, phylogenetic studies to understand the origin and distribution of the pathogen and vector strains. A rapid action team of trained scientist and health workers equipped with modern sophisticated diagnostic tools and suitable vector extinguishers should be appointed by the state and/or central health authorities to counter act any such emergency". Bioterrorism on Six Legs by Dr. Manas Sarkar.

A patent was developed in 2014 involving drones that transport and release mosquitoes. It mentions in the patent these drones can be co-opted for bio-weapons military programs. https://patents.google.com/patent/US8967029B1/en

Page 23 of the EA states "By contrast, male's proboscises are adapted to primarily feed on plant nectar and secretions, and do not feed on blood (Mullen and Durden 2009). Therefore, male mosquitoes cannot transmit disease." **This is incorrect and misleading to the public** since we come to find male lab bred mosquitoes can pass pathogens to wild biting females thru mating and shared feeding/water sources. Venereal Transmission of St. Louis Encephalitis Virus by Culex *quinquefasciatus* Males (Diptera: Culicidae) – Donald A. Shroyer (Journal of Medical Entomology, 5/1990) https://academic.oup.com/jme/article-abstract/27/3/334/2220754?login=false

There is no mention in the Kaua'l EA on how lab batches will be quality controlled or tested for unintended pathogens upon arrival to Hawai'i or if lab employees in contact with these mosquitoes will go thru security clearance screening and training. No documented assurances have been made to the public that lab suppliers will be testing mosquitoes for human, equine, canine, or avian diseases to ensure that they are pathogen-free prior to shipping to Hawai'i.

The science and tech industry in the United States, to include Silicon Valley and Academia, has been heavily infiltrated by the Chinese Communist Party (CCP) and non-government organizations such as Davos and the World Economic Forum whom have been strongly pushing Agenda 2030 thru climate change initiatives. Due to the deterioration of relations between the US and China, among other adversaries, mosquito control releases should not move forward until sound security protocols are adequately implemented. https://www.justice.gov/opa/pr/harvard-university-professor-and-two-chinese-nationals-charged-three-separate-china-related

The Bill and Melinda Gates Foundation (Gates), also connected to the above-mentioned entities, are strong proponents of climate agenda and have openly discussed support of human depopulation. This is the same foundation that has been funding ongoing research of Wolbachia (World Mosquito Program and numerous grants) and GM mosquitoes including Oxitec since 2002. Gates has also funded research developing anti-malaria vaccines using mosquitoes as a delivery system which is highly concerning.

https://www.npr.org/sections/goatsandsoda/2022/09/21/1112727841/a-box-of-200-mosquitoes-did-the-vaccinating-in-this-malaria-trial-thats-not-a-jo

Wolbachia Has Been Implicated in Human Disease

Wolbachia is NOT harmless to humans. It effects filarial worms that cause human disease such as river blindness and is implicated in Elephantiasis. These diseases effect millions of people each year. According to the CDC website, "There is a promising treatment using doxycycline that kills the adult worms by killing the Wolbachia bacteria on which the adult worms depend in order to survive".

https://www.cdc.gov/parasites/onchocerciasis/treatment.html

"For decades, people have blamed a parasitic nematode worm for a disease that has blinded at least 250,000 people now living in Africa and South America. But the real culprit may be the ubiquitous Wolbachia, bacteria that colonize many hundreds of species, including the worm indicted in river blindness. Researchers now report that Wolbachia stimulate the severe immune system response that slowly robs people of their vision". https://www.science.org/content/article/worms-may-not-act-alone-river-blindness

Anti-Wolbachia therapy for onchocerciasis & lymphatic filariasis: Current perspectives https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6755775/

Efficacy of 2- and 4-week rifampicin treatment on the Wolbachia of Onchocerca volvulus https://pubmed.ncbi.nlm.nih.gov/18679718/

The Kauai EA's assertion that released mosquitoes pose no risk to human health is based on unsound science. On page 25 of the EA it says "Wolbachia cannot live within vertebrate cells and cannot be transferred to humans even through the bite of a mosquito that carries it (Popovic et al. 2010). "

In contrast we know science is recently **discovering detection of Wolbachia genes in humans**: Detection of Wolbachia genes in a patient with non-Hodgkin's lymphoma https://www.clinicalmicrobiologyandinfection.com/article/S1198-743X(14)00040-8/fulltext

"Wolbachia 16S rRNA and fbpA genes were twice detected over 5 days in the blood of a patient with high fever. The patient was given fluoroquinolones and the fever resolved. Four weeks later, he was diagnosed with non-Hodgkin's lymphoma and received R-CHOP (Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Prednisolone) treatment resulting in complete remission. This is the first report of detection of Wolbachia genes from the blood of human patients with non-Hodgkin's lymphoma."

The 2010 article by Popovici et al. cited in the EA has been discredited by the EPA. The EPA Human Studies Review Board met in 2018, and the following question was posed:

"Is the research described in the published article 'Assessing key safety concerns of a Wolbachia-based strategy to control dengue transmission by Aedes mosquitoes'

scientifically sound, providing reliable data for the purpose of contributing to a weight of evidence determination in EPA's assessment of the risks to human health associated with releasing Wolbachia-infected mosquitoes?"

The Board's response states: "The Board concluded that the research described in the article by **Popovici et al. was not scientifically sound** and does not provide reliable data to contribute to a weight of evidence determination for assessment of human health risks due to release of Wolbachia-infected mosquitoes."

Inconsistent Climate Data and Mosquito Population Trends

The EA states, "Some climate change models suggest that the mean temperatures in Hawai'i may increase by 3°–4°C by 2100 (Hayhoe et al. 2018). The effects of climate change have been found to result in increased stress to natural systems through altered temperatures and rainfall patterns (Alexander et al. 2016). Increases in mean temperatures, for example, have facilitated the spread of mosquitoes and avian malaria into habitats where cool temperatures very recently limit mosquito presence and transmission of malaria to highly susceptible endemic forest birds (Atkinson et al. 2014)."

Contrary to the above claims, from 1978 to 2017 (0 to 1600 meters) Kagawa and Giambelluca 2019, Spatial Patterns and Trends in Surface Air Temperatures and Implied Changes in Atmospheric Moisture Across the Hawaiian Islands, 1905–2017. Researchers summarized data from weather stations on several islands pooled together. They extended the range of observations to the year 2017. Daytime cooling was noted at upper elevation below the trade wind inversion that is consistent with observed cooling of -0.2 to -0.8° C/decade at multiple high elevation stations during 1988–2013 (960–2,990 m; Longman, Giambelluca, et al., 2015). https://agupubs.onlinelibrary.wiley.c

Additional skepticism to global warming trend is gaining momentum among the scientific community. The World Climate Declaration – There is no Climate Emergency was signed by over 1580 vetted scientists and continues to grow. https://clintel.org/wp-content/uploads/2021/03/WCD-A4version09202013.pdf

Greenpeace Founder Patrick Moore Says Climate Change Based on False Narratives https://www.theepochtimes.com/science/exclusive-former-greenpeace-founder-patrick-moore-debunks-the-false-narratives-of-climate-change-4709568?rs=SHRDHWFRF

Climate Activists Invest in Property on Beaches They Say are Disappearing https://www.washingtonexaminer.com/politics/climate-activists-invest-property-beaches-climate-change-sea-rise

In 2013 Lisa Crampton and Anouk Glad conducted a study of Plasmodium *relictum* infection in Culex *quinquefasciatus*. The rate of capture of adult mosquitoes and Plasmodium *relictum* percentage was extremely low at Alakai Plateau of Kaua'i. https://onlinelibrary.wiley.com/doi/pdfdirect/10.1111/jvec.12157

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"The infection rate of Plasmodium *relictum* is also essential to understanding the transmission rate to birds on the Alakai Plateau. We screened 17 mosquitoes caught at Halepa'akai and 16 mosquitoes caught at Kawaikoi in October and November for P. *relictum* infection using PCR. One mosquito from Halepa'akai tested positive for infection. We dissected 33 mosquitoes caught at Kawaikoi (winter and spring); none of them tested positive for infection by P. *relictum* (neither oocysts nor sporozoites were observed). Only three mosquitoes caught at Halepa'akai (January) were dissected, and none of them were infected (neither oocysts nor sporozoites were observed). Thus, the **prevalence rate of P.** *relictum* in our study is 1.45% (n=69)."

Page 34 of EA uses mosquito estimates documented over 10 years ago from Hawai'i Island. "Estimates range from an abundance of approximately 600 mosquitoes per acre near sea level on Hawai'i Island where monthly temperatures average 70–75° F, to an abundance of five mosquitoes per acre at an elevation of approximately 4,000 feet where temperatures average 55–60° F (Samuel et al. 2011, Atkinson et al. 2014)."

Page 19 of EA states "Mosquito populations and avian malaria have recently expanded into higher elevation habitat, which is the last refugia for these endangered avian species." I could not find a reference study proving the mosquitoes are invading higher elevations in the proposed release areas in Kaua'i or recent documentation on the prevalence rate of Plasmodium relictum since the Crampton and Glad study in 2013.

Verily Life Sciences and Rhodamine B

Verily's registrant representatives are listed in the Department of Agriculture Import Application - https://hdoa.hawaii.gov/wp-content/uploads/2018/05/HDOA-Mosquito-Request-PA_Final-6.8.21.pdf - and are co-authors of Mark Release Recapture of Male Aedes aegypti use of **Rhodamine B** to Estimate Movement, Mating and Population Parameters for an Incompatible Male Program

https://www.researchgate.net/publication/345648051_Title_Mark-release-recapture_of_male_Aedes_aegypti_Diptera_Culicidae_use_of_rhodamine_B_to_estima te_movement_mating_and_population_parameters_in_preparation_for_an_incompatible_male_program

The EA mentions no use of the toxin Rhodamine B. Will Rhodamine B be used in Kauai's MMR studies? Is there potential ongoing use of Rhodamine B could have implications on land and aquatic lifeforms?

https://www.sciencedirect.com/science/article/abs/pii/S0045653521025522

Rhodamine B (RhB) is among the toxic dyes due to the carcinogenic, neurotoxic effects and ability to cause several diseases for humans. https://pubmed.ncbi.nlm.nih.gov/33857893/

In Summary I am opposed to request for approval of the draft Kauai Environmental Assessment for the numerous reasons documented in this testimony. Sections of the EA lack sufficient detail, contain outdated references and EPA discredited sources. It is unfortunate this project is being fast tracked and in hindsight, a full scope EIS should have been completed years ago.

I am additionally concerned the use of Wolbachia IIT as a mosquito suppression method to save the birds will not have the intended outcome, according to the World Mosquito Program "there is no field evidence it can reduce the risk of mosquito born diseases". The logical next step already in discussion in Federal documents would be a segway into controversial and potentially dangerous synthetic gene drive technology in which corporate gain will be at the expense of the Hawaiian ecosystem.

Respectfully,

Donna Thompson Kamuela, HI sharkgss@protonmail.com