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Attorneys for Plaintiffs
Hawai‘i Unites and Tina Lia

IN THE CIRCUIT COURT OF THE FIRST CIRCUIT

STATE OF HAWAI‘I

HAWAI‘I UNITES, a 501(c)(3) nonprofit corporation; TINA LIA, an individual,

Plaintiffs,

v.

BOARD OF LAND AND NATURAL RESOURCES, STATE OF HAWAI‘I, and DEPARTMENT OF LAND AND NATURAL RESOURCES, STATE OF HAWAI‘I,

Defendants.

Civil No. 1CCV-23-0000594 (JMT)
(Environmental Court)

DECLARATION OF DR. LORRIN W. PANG

DECLARATION OF DR. LORRIN W. PANG

I, DR. LORRIN W. PANG, under pain of perjury and law, do hereby state and declare as follows:

1. I am a resident of the County of Maui in the State of Hawai‘i.

2. I am over the age of eighteen (18).

3. I believe that the intent to save rare birds is sound and if the action called “Suppression of Invasive Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui” (“the Action”) goes as planned, this would be a valuable tool for future interventions. However, with new life forms coming to the islands, there is too much potential for unexpected, dangerous, irreversible “evolutionary” events. This is especially true when the new organisms cannot be contained to their target ecosystem.

4. I have been compiling studies documenting horizontal *Wolbachia* bacterial spread, and I’m concerned about the potential for significant adverse outcomes of the Action.

5. The evidence of horizontal spread of *Wolbachia* bacteria (documented in peer-reviewed studies) 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 intra-cellular systems for several months. Horizontal transmission of the *Wolbachia* bacteria can occur through mating, shared feeding sites, and serial predation of larva in standing water breeding sites. Studies that downplay the possibility of horizontal transmission based on aedes aegypti mosquitoes are flawed references because aedes aegypti are resistant to *Wolbachia*.

6. Peer-reviewed studies have shown *Wolbachia* bacteria in mosquitoes to cause increased pathogen infection and to cause mosquitoes to become more capable of spreading diseases such as avian malaria and West Nile virus. West Nile virus can infect birds and humans. This project has the potential to cause the extinction of endangered native birds, and it could impact human health.

7. The final EA for the Action failed to address biopesticide wind drift – the movement of biopesticide mosquitoes through wind to unintended areas. Mosquitoes carried on the wind into and out of the release sites of the project area have not been factored into the math model or the overall plan.

8. The final EA for the Action failed to adequately address the potential for the release of biopesticide mosquitoes to cause unexpected evolutionary events and population replacement.

AUTHENTICATION

1. Attached hereto as **Exhibit 9** is a true and correct copy of my CV/Resume.

2. Attached hereto as **Exhibit 10** is a true and correct copy of a statement further detailing my concerns regarding the action called "Suppression of Invasive Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui".

3. Attached hereto as **Exhibit 11** is a true and correct copy of an undated draft research article that I co-authored with other scientists entitled "Barriers with valve mechanisms are predicted to protect crops from Rat Lungworm disease transmitted by slug hosts" highlighting how population changes are often determined by pathways set up in parallel, not just sequentially; that models must be set up by the initial assumptions with the math derivations of the formula to follow; that the models must predict intuitively the changes in populations when extreme limits are reached (steady state and non-steady state); that tracking units of the parameters of the math expression is a very useful practice in complicated models. Because this draft article is awaiting publication and the copyright does not belong to me, I asked that it be filed under seal.

FURTHER DECLARANT SAYETH NAUGHT

This Declaration is based upon my personal knowledge or as otherwise indicated, and I am competent to testify as to the truth of the statements contained herein.

DATED: Wailuku, Hawai'i, June 18, 2023.

Signed: _____

Dr. Lorrin W. Pang

CURRICULUM VITAE 2018

Name:		Lorrin Wayie Pang
Military Rank:		Lt. Colonel, Medical Corp (Retired) Walter Reed Army Institute of Research
Date/Birthplace:		30 March 1953 Honolulu, Hawaii
Wife's Name:		Kathleen K. Shida Pang
Children		Two daughters
Education/Training:	1971-75	Princeton University, BS Chemistry, Cum Laude
	1975-79	Tulane Medical School, MD
	1976-79	Tulane School of Public Health MPH in Tropical Medicine
	1979-80	Federal University of Brazil; Recife, Pernambuco, Post Graduate Studies in Pathology and Infectious Diseases
	1980-81	Letterman Army Hospital, San San Francisco, CA, Medicine Intern
	1981-82	Walter Reed Army Institute of Research, Washington DC, Preventive Medicine Residency
Positions Held:	1982-87	Epidemiologist, AFRIMS (Walter Reed Inst. Overseas Laboratory) Bangkok, Thailand
	1987-90	Chief, Preventive Medicine Service, Tripler Army Medical Center, Honolulu, Hawaii
	1987-89	Clinical Associate Professor, School of Public Health, University of Hawaii, Honolulu, Hawaii
	1990-92	Medical Officer, Malaria Unit, World Health Organization, Geneva, Switzerland.

EXHIBIT 9

1992-97	Clinician/Epidemiologist, Walter Reed Institute of Research Overseas Laboratory, Brazil.
1994-95	Adviser to Pan American Health Organization (Meningitis Vaccine)
1985-05	Adviser to World Health/UNDP Organization (Tropical Disease Research Unit: Chagas Disease, Leishmaniasis, Malaria, Clinical Trials), 2000 malaria program changed to United Nations Global Fund (for work in Central America)
1997-00	Chief, Department of Bacteriology and Molecular Genetics, AFRIMS, Walter Reed Institute of Research Overseas Laboratory, Bangkok, Thailand.
1997-00	Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.
2000-Present	District Health Officer, Maui County State of Hawaii
2002-04	Consultant Glaxo Smith Kline Pharmaceuticals-
2013	Consultant DNDI (Drugs for Neglected Disease Initiative, affiliate of Doctors without Borders)
2013	Visiting Professor of Medicine, Federal University of Brasilia, Brazil
2013-Present	Reviewer of grants for US Congress (CDMRP, Congress Directed Medical Research Program)

Awards: Army Achievement Medal, 1982, 1996
Army Research and Development Medal, 1987
Army Meritorious Service Medal, 1990, 1997

Certification: Medical License State of Louisiana, 1980- 2000
Hawaii State License, 2000-present
Board Certification in Preventive Medicine, 1990

2002 Discovery Channel feature covering dengue outbreak and eradication on Maui

2006-8 listed on Americas Best Doctors list (3% of nation's doctors)

Publications Peer Reviewed Journals:

1. Lemon SM, Miller RN, Pang LW, Prier RE, Bernard KW. Failure to achieve predicted antibody responses with intradermal and intramuscular human diploid cell rabies vaccine. Lancet 1984;19:1098-1100.

2. Webster HK, Boudreau EF, Childs GE, Yongvanitchit, Pang LW. Antimalarial drug susceptibility testing of *P. falciparum* in Thailand using a microdilution radiosotope method. Am J Trop Med Hyg 1985;34(2):228-35.

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16. Childs GE, Boudreau EF, Milhous WK, Wimonwattratee T, Pooyindee N, Pang LW, Davidson DE. A comparison of the *in vitro* activities of amodiaquine and desethylamodiaquine against isolates of *Plasmodium falciparum*. Am J Trop Med Hyg 1989;40:7-11.

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18. Pang LW. Chemoprophylaxis and treatment of malaria (letter). NEJM 1989;320:1561.

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doi:10.4269/ajtmh.21-1053
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76. Pre-procedural testing improves estimated COVID-19 prevalence and trends
View ORCID ProfileGenevieve C. Pang, View ORCID ProfileAmy T. Hou, Krizhna L. Bayudan, Ethan A. Frank, Jennifer Pastiglione, Lorrin W. Pang doi:
https://doi.org/10.1101/2022.04.13.22273200https://www.medrxiv.org/content/10.1101/2022.04.13.22273200v1.

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78. Summary article airport testing.

79. Rat Lung barrier valve effect.

Statement: Dr. Lorrin Pang, Private Citizen

BACKGROUND

I am a tropical disease and vector expert speaking as a private citizen on this matter. I've authored over 75 publications in peer-reviewed medical journals covering a broad range of studies such as malaria, dengue, rabies, rat lungworm, and COVID. I've been an advisor and voting member of the U.S. Congress Medical Research Program for the past several years, serving on committees for infectious diseases – many of which are mosquito-borne. From 1985-2005, I worked with the World Health Organization (WHO) and Walter Reed Institute's Malaria Program, focusing on global malaria control efforts through interventions combining diagnostics, chemotherapeutics, vector control, and vaccine development. As a public health leader on the islands, I've mitigated mosquito-borne illnesses – including dengue and Zika – for over two decades. I was honored for my life-saving intervention in Hawaii's dengue fever outbreak.

I've attached a confidential submission for publication which highlights some of the items discussed below: That population changes are often determined by pathways set up in parallel, not just sequentially; that models must be set up by the initial assumptions with the math derivations of the formula to follow; that the models must predict intuitively the changes in populations when extreme limits are reached (steady state and non-steady state); that tracking units of the parameters of the math expression is a very useful practice in complicated models.

(CV attached; confidential research article attached)

CONCERNS

<u>Horizontal Transmission: Non-Sexual</u>

A primary concern is non-sexual horizontal transmission of the introduced *Wolbachia* strains between the introduced biopesticide mosquitoes and the existing wild mosquitoes. Imported *Wolbachia* bacterium strain *w*AlbB has been disclosed as the strain for use in this biopesticide. Additional strains *w*AlbA and *w*Pip4 are also planned for import in connection with this project. These newly introduced strains (referred to here as "X") are not currently present within the corresponding *Culex quinquefasciatus* species of Hawaii's established mosquito population.

I have been compiling studies documenting horizontal *Wolbachia* bacterial spread, and I'm concerned about the potential for significant adverse outcomes of the state's proposal. The intent to save rare birds is sound. If the project goes as planned, this would be a valuable tool for future interventions. However, with new life forms coming to the islands, there is too much potential for unexpected, dangerous, irreversible "evolutionary" events. This is especially true when the new organisms cannot be contained to their target ecosystem. Already there are published papers pointing out the real threat of horizontal spread of the novel *Wolbachia* beyond the male *Culex* mosquito. The papers cover two general areas – the widespread detection of *Wolbachia* across so many diverse types of insects, and more recently, the growing number of reports of mechanisms of how this might occur. First, we all must agree that unintended horizontal spread of the imported strain(s) to, say, female *Culex*, *Aedes* mosquitoes, or other insect vectors of diseases

EXHIBIT 10

would be a catastrophe, and probably irreversible. Hawaii has a bad history of invasive species entering and spreading unabated, including their spread of infectious diseases.

A recent study out of Singapore[1] describes *Wolbachia* bacteria strain "evolutionary associations" between mosquito hosts. The results of this mechanism widespread into diverse insect populations are not known. It may start with a few horizontal transfers to female mosquitoes. After that, the mating *Wolbachia*-X-compatible pair will quickly produce viable X offspring and spread the X bacteria strain (the term for this is "sweep"). If that were to happen here, the full capacity of those offspring to transmit disease would be unknown. This type of spread and sweep could also affect other insects, not just the targeted mosquito.

The possibility of unintentionally producing X-infected females in the wild has not been adequately addressed. The introduced *Wolbachia* strain can spread horizontally as a life form to other mosquitos (including *Aedes* – vectors of human disease) and perhaps create that *Wolbachia* female *Culex,* which everyone is bending over backwards to avoid via lab contamination.

There is a big difference between the standard Sterile Insect Technique (SIT) strategies used in the past that were based on radiation or chemicals, and the relatively new Incompatible Insect Technique (IIT). The mathematical models may be similar for estimating threshold criteria to affect mosquito population dynamics, but standard methods of sterility are not bacterial life forms that might escape horizontally and amplify in other ecological niches. While sterility models can predict the thresholds needed to exterminate a species (in this case insects), the radiation sterility factor (standard SIT) does not behave the same as a life form (*Wolbachia* bacteria). There may be different modeling between radiation and *Wolbachia* "sterility" for the male mosquitoes, depending on male mosquito fitness – but more importantly, for the unintended female *Culex* to which the *Wolbachia* X spreads horizontally. How is this supposed to be self-contained? Horizontal spread has the potential to be a disaster that cannot be recalled. The bacterium is a life form, and you might not be able to turn back the clock by simply shutting off the male mosquito "fountains."

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 intra-cellular systems for several months.[2] Two additional studies clearly document widespread horizontal transmission of *Wolbachia*. The first focuses on predatory wasps spreading the bacteria through contaminated mouth parts when feeding serially on target insects such as aphids[3]. More research into which predators, like the damselfly and dragonfly, sequentially feed on both male and female mosquitoes is needed to determine how this may affect Maui's ecosystems. This scenario might play out in either the predator of adults feeding on adult mosquitoes (X-infected and wild), or the X-infected predator of larvae feeding on wild mosquito larvae in common breeding sites. The second study looks at ant colonies spreading *Wolbachia* through the gastrointestinal (GI) tract when the ants feed on their fungus gardens.[2] What about shared sugar feeding sites for X-infected male and wild adult male and female mosquitoes? The sparser the sugar sites, the more communal interaction they will have. I find these studies of horizontal transfer across species of insects highly concerning. Even if this project achieved miraculous blocking of avian malaria to the native birds, what else would it do?

Studies that downplay the possibility of horizontal transmission based on *aedes aegypti* mosquitoes are flawed references because *aedes aegypti* are resistant to *Wolbachia*.

Horizontal Transmission: Venereal

An additional concern is the possibility of sexual/venereal horizontal transmission of the introduced bacteria through mosquitoes mating. This pathway is not well published and requires more study[4], specifically with the *Culex q*. mosquitoes planned for use in this project. While the proponents of the project claim that horizontal transmission only occurs on an evolutionary scale with a very slow spread, there are examples showing that it may occur during a short duration of weeks and then months. X-infected male mosquitoes may transmit the introduced strain to wild females through blood, mucous, and semen during mating. Granted, if this occurs via venereal route in the wild female mosquito, the first half of their life their matings will be sterile. However, after this their matings will produce offspring of both sexes and soon will "sweep" the population with the introduced *Wolbachia* strain.

There is a paper in *Nature*[5] by Frydman et al. studying a fruit fly lab model of entry into the germline from somatic tissue in about a week or two. Germ cells just touching somatic cells within the female mosquito's body can pick up the bacteria. Whether or not accidentally released X-infected females and wild males would have sterile mating outcomes would become irrelevant at this stage. Even if the introduction of X-infected males was stopped, the initial mating with females X-infected through horizontal transmission (and compatible through entry of the bacteria into the female germline) would produce viable offspring of both sexes of X-infected mosquitoes.

Vertical Transmission Suppressed: Horizontal Transmission Increased

When vertical transmission of the *Wolbachia* bacteria is suppressed, horizontal transmission of the bacteria can increase.[6] The *Wolbachia* is trying to survive. If vertical transmission is blocked, the bacteria maintain horizontal transmission until the host is fertile again, then they return to vertical transmission. During the time that the bacteria is challenged by the inability to transmit vertically, it may spread horizontally to other mosquitoes, spiders, fruit flies, and other insects (including insect vectors of disease).

A related topic is beginning to be examined in the science community. Human pathogens (i.e., Zika and dengue viruses) used to be thought of as "dead-end" infections in male mosquitoes who may have become infected through horizontal transmission mechanisms. Males don't bite humans to expand the virus in human hosts. However, it is now of concern that these viral pockets in male mosquitoes, though relatively rare, serve as back-up reservoirs to reinoculate females when the female-mosquito/human cycle breaks down. For example, if all humans get a virus and mass immunity puts the cycle on "hold" until enough non-immune humans are born or transient immunity is lost, the female mosquitoes will tap into the viral back-up reservoir via horizontal transmission.

Proponents of the use of this biopesticide argue that horizontal transmission, venereal or otherwise, is so rare that it can be ignored in the math models. They extrapolate from the models

where cytoplasmic incompatibility (CI) is not operating for sterilization of matings, for example in the sweep model. Much of the spread of *Wolbachia* is vertical (V). Let us say that for every *Wolbachia* spread, 99.9% are via vertical spread and only 0.1% are horizontal (H). Let us pick a unit population where 100K new mosquitoes become infected with *Wolbachia* via sex. Of this 100K, only 100 would have become infected with the bacteria through H and the rest through V. But in the CI application, none get it through V and still females are inoculated somatically through the H mechanism of mating. This 100 may move quickly to the germline cells, but does the bacteria remain only in 100, or can it expand through lack of competition to fill the niche that the V transfers would have occupied? Was there competition between V and H descending lines; and if there is no longer a V line (due to CI), will H females expand?

Even when there is successful vertical transmission, there is horizontal transmission to non-germ cells such as neural tissue, which changes the behavior of the host to support what is in the germ cells. The bacterium is altruistic and helps sister cells to dominate as a safety backup, sacrificing the individual self for the sake of the larger group. This is useful because the larger group does have some common genes which the individual shares. When the two systems (V and H) are running predominantly vertical transmission with some relatively minor horizontal transmission, the horizontal is a back-up system to reinoculate the vertical system if the vertical system ever fails. This horizontal system is relatively small compared to the vertical system but is rather important in many systems including *Wolbachia*. If the CI process shuts down vertical transmission, the horizontal system is still running, and it may grow because that lineage does not compete with a vertical system which has been blocked by sterility.

With the *Wolbachia*, when the vertical production is plentiful, the horizontal movement will seem inconsequential, often a dead end; but once the vertical system collapses (through CI or through natural sterility of the insect – for instance, through mosquito HIV), the horizontal system in males will still be there and can restart the vertical system again when it moves to the germ cells in females. With the use of this biopesticide, the sterility might not stop completely, but the horizontal system will still act to save the *Wolbachia* line in males by making females that will expand the strain line vertically. All of the sacrificed horizontal transmission when the vertical system operates will be useful to reinoculate the system when CI passes or is “low.”

Math Model: Choke Points and Rate Limiting Step

The math model for this project does not seem to account for choke points. If only a certain number of larvae from compatible mosquitoes will survive due to availability of, say, food sources in standing water breeding sites, then any reduction in viable offspring due to incompatibility may not significantly affect the number of surviving larvae. The viable larvae will compete for microbes to eat, and only a specific number of larvae will have enough food to survive (rate determining step). That number may remain relatively constant based on volume of food-source microbes, and the non-viable offspring of incompatible mosquitoes may have no effect, or limited effect, on the survival rate of larvae in the breeding site overall.

Determination of efficacy of the biopesticide might be based on a flawed set up of the math model. The question is, do things affecting a population occur in sequence or in parallel; and if we treat them like resistors on an electrical circuit, isn’t the rate limiting step like a capacitor

somewhere in the circuitry? A very restrictive rate limiting step such as the paucity of microbial food in breeding water severely limiting the number of larvae reaching the adult stage would cause the reproduction/sterility interventions to be ineffective. Even if the proportions of X-infected male mosquitoes released were increased, there would be very little impact.

Math Model: Biopesticide Wind Drift

Further diluting the math model basic assumptions is the factor of wind drift. Mosquitoes carried on the wind into and out of the release sites of the project area have not been factored into the math model or the overall plan. Lowland male (and female) wild mosquitoes can travel by wind drift up from lowlands to the project area and dilute the intervention mating pool, affecting the efficacy goal of 90% lab-reared male matings. This rapid drift could dilute the proportion of novel *Wolbachia*-infected male mosquitoes.

Considering these factors, the mark-release-recapture study to estimate whether more or less mosquitoes would be released could be open to interpretation. In human trials, empirical data from feasibility analysis precedes formal studies. We go over numbers from human subjects and use the control group to draw conclusions. If this biopesticide mosquito project is to draw on historical controls, the cause-and-effect interpretation will have many ecological confounders and will risk the ecologic fallacy. If this possibility is inevitable, these conditions should be stated now.

Superinfection: Multiple Strains

Mosquitoes and other insects can be infected with more than one strain of *Wolbachia* bacteria at the same time. This is called "superinfection.[7]" *Culex q*. mosquitoes are very susceptible to many strains of *Wolbachia*. Superinfection in *Culex q*. has not been studied for this project. Superinfection could affect cytoplasmic incompatibility, horizontal transmission, evolutionary events, and population replacement.

Wolbachia: Increased Pathogen Infection and Disease-Spreading Capability

Peer-reviewed studies have shown *Wolbachia* bacteria to cause increased pathogen infection in mosquitoes[8] and to cause mosquitoes to become more capable of transmitting both avian malaria[8] and West Nile virus (avian and human)[9]. More study is needed in this area, specifically study of the *Culex quinquefasciatus* mosquito and the *w*AlbB, *w*AlbA, and *w*Pip4 *Wolbachia* strains, along with any combinations (superinfections) of bacteria strains planned for use in this project. Increased pathogen infection and increased disease-spreading capability could be detrimental to the endangered native bird populations, other animals, insects, humans, and subsequently the ecosystems as a whole.

Novel Experiment

This biopesticide mosquito release is an experiment. *Culex q*. has never been used for cytoplasmic incompatibility stand-alone field release. Scientists advising on this project have not studied horizontal transmission or movement of *Wolbachia* from somatic cells to germ cells in

Culex q. *Wolbachia*-infected mosquitoes are more widely released globally for population replacement, not suppression. Efficacy studies are focused on the population replacement method. The population suppression method has not been sufficiently studied. The potential collateral damage from the use of this biopesticide is unknown.

Alternatives: Not Considered

Alternative approaches to mitigating avian malaria have not been considered, including treatment of avian malaria in the mosquito phase through antimalarial drug feeding (i.e., primaquine and ivermectin) in rabbits and/or battery-powered warm artificial blood packs containing the antimalarial drugs. The range of blood-feeding females is a lot wider than extrapolated from sugar feedings of males.

CONCLUSION

While I have chosen to address each mechanism separately, all mechanisms interact with each other. There has been insufficient study in each area of concern and in the combination of mechanisms. The precautionary principle calls for further study of the probability of efficacy and the potential for collateral damage. The use of this novel biopesticide requires a feasibility study, independent of the proposal itself, analyzing and considering all of the critical aspects of the proposed project in order to determine the likelihood of it succeeding. Though I have been presented with the math model, I would like to see the basic assumptions factored in prior to the derivation of the actual expressions/conclusions. I would like to see incorporation of choke points and rate limiting factors, wind drift and expansion of horizontal transfer reservoirs if/when vertical transmission is blocked. Mitigation measures must be established to assure that side-effects would be contained. Detailed study in each area of concern, separately and together, is needed.

Proponents may be right that this intervention will save the native birds in the short-term, but long-term consequences to other island ecologies and to these same native birds may ultimately be detrimental. When one realizes the latter, the damage may be impossible to recall or repair, like the effects we've seen with so many other invasive species in Hawaii.

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