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Perspective

Commander's Introduction

MG Steve Jones

Time for a Revolution in AMEDD Doctrine

When the Army Medical Department was organized on July 27, 1775, it was formed as a team. The act of Congress establishing a hospital for an Army of twenty thousand appointed the following officers and attendants: one director general and chief physician, four surgeons, one apothecary, twenty surgeon's mates, one clerk, two storekeepers and one nurse for every ten sick. Army Medicine had been serving our Soldiers for almost a year when our nation declared independence—we are the country's first, and arguably best healthcare team. Our accomplishments over the past 240 years have been significant and have been the result of a team effort. Major Walter Reed discovered the mosquito was the vector that carried Yellow Fever, but it was Colonel William Gorgas who led the campaign to control mosquitoes that allowed construction of the Panama Canal. Today, casualty survival rates are at historic levels because of the teamwork between AMEDD staff, operational commanders, Soldiers, and Families.

AMEDD doctrine has traditionally described ten medical battlefield operating systems; the major healthcare functions that are employed to provide care in theater. This was a useful concept for planners and helped ensure critical functions were not forgotten. It was aligned with Army doctrine which described battlefield operating systems as the elements of combat power, but created an artificial distinction between care in theater and care in garrison. It does not portray the fact that casualty care occurs every day in our fixed hospitals, and that every member of the AMEDD makes important contributions to the effort. It also does not adequately reflect recent changes in how we provide healthcare while deployed and at home.

The February 27, 2008, edition of *Field Manual 3-0: Operations* was a fundamental departure from prior versions. Just as the 1976 edition of *Field Manual 100-5: Operations* brought the Army from the rice paddies of Vietnam to the battlefields of Western Europe, the 2008 edition took the Army into 21st century conflicts among people. It replaced the older battlefield operating systems with six warfighting functions as the elements of combat power:

Mission Command	Fires
Movement and Maneuver	Sustainment
Intelligence	Protection

A seventh warfighting function, Engagement, has since been added. Warfighting functions are defined as a group of tasks and systems (people, organizations, information, and processes) united by a common purpose that commanders use to accomplish missions. It is a useful concept that reinforces the ideas of mutual support and fighting as combined arms teams.

Field Manual 4-02: Army Health System lists the ten medical functions:

- Medical Mission Command
- Medical Treatment
- Hospitalization
- Medical Evacuation
- Dental Services
- Preventive Medicine Services
- Combat and Operational Stress Control
- Veterinary Services
- Medical Logistics
- Medical Laboratory Services

It defines two missions for the AMEDD: Health Service Support and Force Health Protection. The Health Service Support Mission comprising casualty care, medical evacuation, and medical logistics falls under the Sustainment Warfighting Function. Force Health Protection comprising preventive medicine, veterinary services, preventive aspects of combat and operational stress control, dental and services, and laboratory services falls under the Protection Warfighting Function.

The full breadth of AMEDD support to Unified Land Operations and the continuum of casualty care are far greater than what is described in AMEDD doctrine. Efforts to keep Soldiers healthy and fit allow them to perform better while deployed and recover more quickly if injured. Medical treatment facilities at home station

provide specialty care through reach-back support and telemedicine. Casualty care does not stop after evacuation to the continental United States, but continues with definitive care and rehabilitation of the wounded, the operation of Warrior Transition Units, and the support provided to families. Our approach to healthcare has changed significantly since Vietnam. Primary healthcare is now provided in Soldier-Centered Medical Homes who partner with line unit staff and commanders. The Brigade Healthcare Team now includes a physical therapist and behavioral health provider and nurse in addition to medics, physician assistants, dentists, and surgeons. The Combat Lifesaver and 68W (Health Care Specialist) programs, tactical combat casualty care, damage control resuscitation, and damage control surgery have significantly improved prehospital care. Critical care flight paramedics and nurses on tactical medevac aircraft have increased survival rates of critically injured patients. These patients no longer spend weeks in theater hospitals but are evacuated to large medical centers within days, with care continuing in hospitals along the way. Patient outcomes are monitored closely and data collected by the Joint Theater Trauma System forms the basis of a robust performance improvement system. Regular after action conferences with returning units and research teams also provide information that is used to improve care. This approach to care and the hard work of the entire AMEDD team has raised the survival rate of casualties wounded on the battlefield from 76% in Vietnam to 92% today.

Updating our doctrine will allow the AMEDD to better communicate the many ways it supports Unified Land Operations every day. It will lead to a better understanding of new concepts in casualty care, the contributions of all members of the team, and of how units and leaders combine capabilities across medical functions to accomplish their mission. It should start with the premise that casualty care begins with the maintenance of a healthy and fit force, extends from the point of injury through definitive care and rehabilitation, and includes the operation of Warrior Transition Units. After action reviews from our fixed hospitals should be regularly conducted and included in lessons learned programs. Gaps in the provision of definitive care and rehabilitation should be identified and included in the requirements process to drive development of new capabilities. Medical functions should be updated to reflect how we provide care rather than listing capabilities, and should include:

- Medical Mission Command
- Primary Care
- Prehospital Care
- Evacuation and En Route Care
- Hospital Care
- Definite Care and Rehabilitation
- Force Health Protection
- Medical Sustainment
- Medical Engagement
- Medical Information
- Research and Innovation

Several of these functions are new. The addition of Medical Engagement as a function recognizes the significant support the AMEDD provides Combatant Commanders in shaping the environment. Including Research and Innovation recognizes the important contributions of these members of the team. Medical Information includes not only health threats but capabilities, treatment considerations, military, political, economic, and cultural considerations that affect healthcare operations. It includes the information needed to develop and sustain a high degree of situational understanding while operating in complex environments against determined, adaptive enemy organizations and emerging health threats.

Like conflict, healthcare is a complex, risk-filled human endeavor. Army healthcare is more complex, has greater risk, and is filled with even more uncertainty and emotion. We often deliver care under austere and extreme conditions, sometimes under fire, and always under the scrutiny of Congress and the media. We understand we are part of a bigger team with a bigger mission because we serve our nation. Our mission is to keep Soldiers healthy and ready to fight, giving them confidence with our presence on the battlefield and comfort knowing we are caring for their families back home. We should reflect this reality in our doctrine.

Baseline Susceptibility to Pyrethroid and Organophosphate Insecticides in Two Old World Sand Fly Species (Diptera: Psychodidae)

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Abstract

Phlebotomine sand flies are blood-feeding insects that transmit *Leishmania* parasites that cause various forms of cutaneous and visceral leishmaniasis and sand fly fever viruses (*Phlebovirus*; Bunyaviridae) in humans. Sand flies pose a significant threat to US military personnel deployed to *Leishmania*-endemic and sand fly fever endemic regions which include Europe, the Mediterranean basin, Middle East, Central Asia, Southwest Asia, and Africa. A research project supported by the Department of Defense Deployed Warfighter Protection Program was initiated to evaluate the susceptibility of 2 Old World sand fly species, *Phlebotomus papatasi* and *P duboscqi*, to a number of commonly used pyrethroid and organophosphate insecticides. A new glass vial bioassay technique based on the CDC bottle assay was developed for this study. The exposure time-mortality relationship at a given insecticide concentration was determined for each insecticide, and their relative toxicity against the 2 sand fly species was ranked based on bioassay results. This study validated the new bioassay technique and also generated baseline insecticide susceptibility data to inform future insecticide resistance monitoring work.

Phlebotomine sand flies are vectors of *Leishmania* species protozoan parasites that cause various forms of leishmaniasis and the sand fly fever viruses (*Phlebovirus*; Bunyaviridae) in humans and other animals in tropical and subtropical regions of the world.[1] *Leishmania* parasites are transmitted by the bite of infected female sand flies.[2] An estimated 1.3 million new human cases of leishmaniasis and 20,000 to 30,000 deaths occur worldwide annually, which represents a major public health problem in affected regions including the Mediterranean basin, Central Asia, Southwest Asia, Southeast Asia, East Africa, Afro-Eurasia, and the Americas.[3] This sand fly-borne disease with no approved vaccines and lengthy and costly treatment causes significant social and economic burden to civilian populations in endemic regions. Phlebotomine sand flies also pose a significant threat to US military personnel who are deployed to those regions, particularly the Middle East, Southwest Asia, and Africa where sand fly *Leishmania* vectors are endemic.[4] During Operation Iraqi Freedom, the US military reported over 1,000 confirmed cases of cutaneous leishmaniasis among deployed personnel between May 2003 and November 2004.[5] Various insecticides and application technologies were tested at a US airbase in southern Iraq during that period with only limited success in reducing sand fly populations.[6] The reasons for failure in completely eliminating or substantially reducing sand fly populations by frequent insecticide applications remain poorly understood.[6]

Chemical insecticides have been used for indoor and outdoor residual applications and bed net treatments to reduce sand fly populations in endemic regions.[1,7-10] The insecticide dichlorodiphenyltrichloroethane (DDT) has been used to control sand flies after World War II.[11,12] DDT resistance was reported in *Phlebotomus papatasi* (Scopoli) populations in Iran in a field survey conducted during 1985-1988, although the use of DDT had been discontinued since 1969.[13] DDT resistance was similarly reported in both *P papatasi* and *P argentipes* (Annandale and Brunetti) in India, Nepal, and Sri Lanka.[10,14-17] Populations of *P papatasi* were shown to be susceptible to pyrethroid and organophosphate insecticides in laboratory studies of field-collected sand fly samples from North Africa and the Middle East.[18,19] However, multiple resistances to DDT, organophosphate, and pyrethroid insecticides were detected in India and more recently in Sudan.[14,20] One *P papatasi* population collected from the Surogia village in Sudan demonstrated significant resistance to malathion and propoxur in laboratory bioassays, and the observation was also supported by reduced inhibition of acetylcholinesterase by these organophosphate

insecticides.[20] Compared to mosquitoes and other vector species, there is a general lack of understanding of prevalence of insecticide resistance in sand flies.[1,21] Only a few studies have been reported in the literature on possible biochemical or molecular mechanisms of insecticide resistance in sand flies.[21-23]

Previous sand fly insecticide susceptibility/resistance tests were carried out following a World Health Organization (WHO) standard procedure that was originally designed for mosquitoes in which insects were exposed to an insecticide-impregnated filter paper [24,25], or the Centers for Disease Control and Prevention (CDC) bottle assay [3,26] that exposes adult sand flies to an insecticide-treated surface inside the glass bottle. The WHO assay requires sand flies to be exposed to insecticide for one hour and subsequent transfer of sand flies to clean containers for incubation under appropriate temperature and humidity conditions for 24 hours before mortality can be assessed. The CDC bottle assay is more rapid and the time-mortality relationship of an insecticide concentration can be established by checking mortality every 15 minutes during a 2-hour exposure period.[3,21] In most sand fly studies, one or two diagnostic concentrations were tested for resistance detection.[18,19,27,28] Difficulties in collecting sufficient numbers of live sand flies for bioassays and the lack of a standard sand fly bioassay technique may have impeded progress in insecticide resistance studies in sand flies.

In this study we developed a simplified glass vial bioassay technique based on the CDC bottle assay to test exposure time–mortality relationships at selected insecticide concentrations. This test used fewer sand flies and allowed rapid determination of sand fly susceptibility. The objective of this study was to evaluate this new bioassay technique and determine baseline susceptibility to a number of commonly used pyrethroid and organophosphate insecticides in 2 sand fly species, *P papatasi* and *P duboscqi* (Neveu-Lemaire), which are relevant to US military operations in the Middle East and Africa.

Materials and Methods

Sand Flies. Adult male *P papatasi* and *P duboscqi* were used in this study. Adult *P papatasi* were from a laboratory colony maintained at the US Department of Agriculture Agricultural Research Service Knipling-Bushland US Livestock Insects Research Laboratory, Kerrville, TX. The colony was established using pupae from a long-established Israeli strain of *P papatasi* maintained at the Division of Entomology, Walter Reed Army Institute of Research, Silver Spring, MD. Adult females were blood-fed using an in vitro membrane feeding system (Figure 1A). Larvae were fed with a sand fly larval diet consisting of a composted mixture of rabbit feces and rabbit food.[29] Male and female sand flies in the cage were fed daily with 30% sucrose water after emergence and maintained at 26°±2°C and a relative humidity of 85%±2% in an environmental chamber. Adult *P duboscqi* were from a laboratory colony maintained at the US Army Medical Research Unit-Kenya (USAMRU-K) field station located at the Kenya Agricultural Research Institute Marigat Field Station.

Insecticides. Technical-grade permethrin (92.2% active ingredient) and coumaphos (97.4% active ingredient) were obtained from FMC (Philadelphia, PA) and Bay Vet (Shawnee, KS), respectively. All other technical-grade insecticides were obtained from Chem Service (West Chester, PA). Formulated etofenprox (Zenivex E4 RTU, 4%) was a product of Wellmark International (Schaumburg, IL). Dilutions of technical insecticide in acetone were made to generate test concentrations ranging from 0.0001% to 0.1% (Figure 1B). To treat the inside surface of glass vials with insecticide, a volume of 0.2 mL of the test solution was added to a 20 mL glass scintillation vial (Fisher Scientific, Pittsburgh, PA), which was placed on a hotdog roller (Figure 1C) for 1 hour to allow evaporation of solvent and uniform insecticide coating of the glass surface. The treated vials were recapped and stored at room temperature after drying and used within 2 days for study of *P papatasi* or 4 to 7 days for study of *P duboscqi* to allow for travel to the Kenya field location. Dilution of formulated etofenprox was made using bottled water at the field station in Kenya. Etofenprox-treated vials were set at ambient room temperature (≈25°C to 30°C) with frequent rolling by hand for 3 hours to allow solvent to evaporate. Three vials were prepared for each test concentration.

Bioassays. All bioassays with *P papatasi* were done using adult males of mixed ages (3 to 10 days) for reasons noted below under normal laboratory conditions (Temperature 23°±2°C). Five to 7 treated vials, each representing one concentration, and the control vial that was treated with acetone only were placed on the counter (Figure 1D, 1E). Adult male sand flies were directly aspirated from the holding cage using a mouth aspirator and briefly knocked down with CO_2 before being placed in the vials (10 sand flies/vial). Flies woke up in about 30 seconds. Fly mortality in each vial was checked every 10 minutes, according to the order in which flies were added to each vial, for up to 3 hours, or until all flies in each vial were dead (Figure 1F). Each experiment was repeated 3 times so each test concentration had a total of 3 replicates. Bioassays with *P duboscqi* were conducted following the same protocol using adult males of mixed ages (≈3 to 10 days) kept at room temperature in the

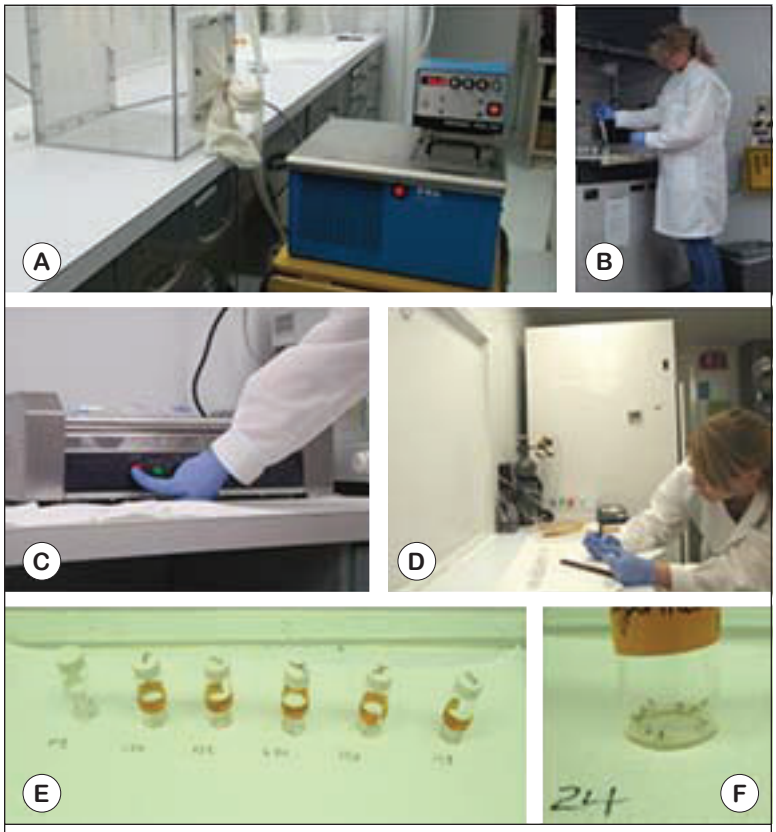

Figure 1: (A) Membrane feeding of adult sand flies; (B,C) treatment of glass vials with insecticide; (D,E,F) determination of sand fly mortality.

USAMRU-K field station in Kenya (Figure 2). Room temperature in the laboratory ranged between 25°C and 30°C. All glass vials were pretreated with insecticides at the USDA laboratory in Texas, except for etofenprox which was prepared on site.

Data Analysis. Probit analysis of time-mortality data for each insecticide and concentration tested were conducted using POLO PLUS software.[30] The LT_{50} and LT_{90} (exposure time at which 50% and 90% flies died) were generated and used to compare the relative toxicity of insecticides tested in this study.

Results and Discussion

Results with *P papatasi.* Adult males began to die after 30 to 40 minutes of exposure to the lowest concentration (0.00001% or 0.5 ng/cm^2) of pyrethroid insecticides (Figure 3), and it took over 120 minutes to reach 100% mortality. Both the time at which sand flies started to die and the time when 100% mortality was reached decreased with increasing insecticide concentrations (0.0001, 0.001, and 0.01%). The time (minutes) it took to kill 50% of the treated flies (LT_{50}) for each of 3 concentrations of 6 pyrethroid insecticides tested are listed in Table 1. Based on LT_{50} data, prallethrin and λ-cyhalothrin were most toxic to sand flies, followed in order by deltamethrin, cyfluthrin, cypermethrin, and permethrin. Compared to pyrethroid insecticides, organophosphate insecticides were generally less toxic (Figure 4). At the lowest concentration (0.00001%) tested, organophosphate insecticides were slow in killing sand flies. Sand flies started to die only after over 60-minute exposure (diazinon) or even after 120 minutes (chlorpyrifos). Similarly, higher concentrations of organophosphate insecticide caused flies to die more rapidly. Based on LT_{50} data listed in Table 2, the order of relative toxicity of the 4 organophosphate insecticides was diazinon ≥ chlorpyrifos > coumaphos > dichlorvos.

Results with *P duboscqi.* Toxicity bioassay results for 6 pyrethroid and 3 organophosphate insecticides against adult males are listed in Table 2. Prallethrin was again the most toxic pyrethroid insecticide. Susceptibility of *P duboscqi* sand flies to permethrin was similar to that of *P papatasi*. The order of relative toxicity was: prallethrin > λ-cyhalothrin > deltamethrin > cypermethrin > permethrin > etofenprox. A limited data set was obtained for the 3 organophosphate insecticides tested against *P duboscqi* sand flies. Chlorpyrifos appeared to be more toxic than malathion and carbaryl (Table 2).

Figure 2. Sand fly bioassays were conducted at the US Army Medical Research Unit-Kenya field station in Marigat, Kenya.

The bioassay technique involving a glass vial to measure exposure time-mortality relationships has been used previously to assess sand fly susceptibility to a number of insecticides.[28] Based on data obtained in this study, we verified that our modified version of the CDC bottle bioassay technique was sensitive and reliable. We were able to demonstrate a time-mortality relationship at different concentrations of the same insecticide, as well as compare sand fly susceptibility to different insecticides at particular test concentrations. Based on LT_{50} data, we were able to rank relative toxicity of pyrethroid and organophosphate insecticides to both *P papatasi* and *P duboscqi* sand fly species. The susceptibility data obtained from this study will help military entomologists in the field with the selection of insecticides for sand fly control. The results from this study could also be used as baseline susceptibility data for comparative purposes in resistance monitoring of field-collected sand fly populations. Additionally, having reference susceptibility data to several conventional pyrethroid and organophosphate insecticides facilitates future work involving the screening of new insecticides, including essential oils and other natural products[31] for sand fly control.

Like mosquitoes, only female sand flies are blood feeders. Many insecticide studies use females as test subjects. Because we were at the early stage of establishing the *P papatasi* colony, females were reserved for maintaining and propagating the sand fly colony. Therefore, we used adult males for toxicity bioassays. Male sand flies are slightly smaller than females, and are known to be more sensitive than females to insecticides.[32] However, our results indicate that relative toxicity of insecticides against sand flies could be assessed using males in bioassays. Depending on the collection technique employed in the field, sand fly samples can include adult males and females. Therefore, future laboratory studies will involve experiments to ascertain if males are as susceptible to insecticides as females.

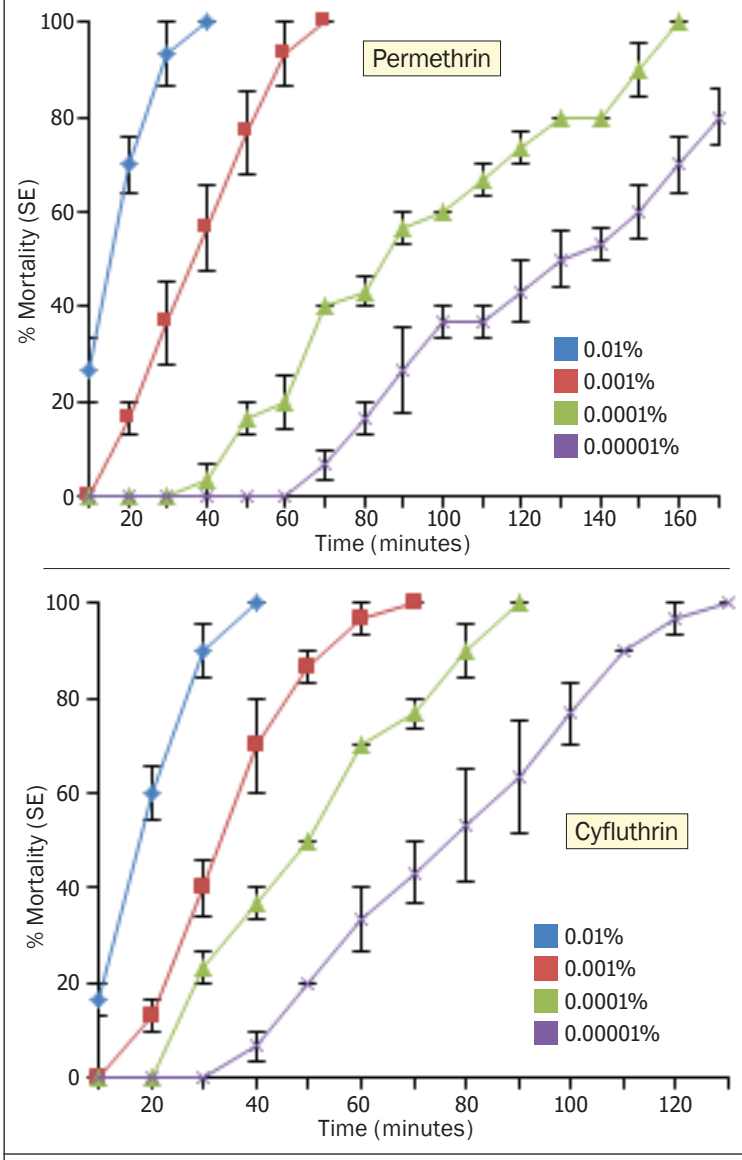

Figure 3. Exposure time-mortality responses of male *P papatasi* to different concentrations of representative pyrethroid (permethrin, cyfluthrin) insecticides.

Although *P duboscqi* is one of the major sand fly species transmitting leishmaniasis in Kenya and has been the target of control efforts,[33,34] little is known about insecticide susceptibility in this sand fly species. To the best of our knowledge, this is the first report of laboratory testing of *P duboscqi* susceptibility to commonly used pyrethroid and organophosphate insecticides.

Due to the difficulty in collecting large numbers of live sand flies in the field, having enough flies to run a dose-response bioassay, which requires a large number of sand flies, is impractical. While the standard CDC bottle assay uses 250 ml Wheaton glass bottles, the 10 ml glass vials we used in this study were smaller, required fewer sand flies to run a test, and are amenable for field deployment. Treatment of glass vials with technical insecticides in a resource-constrained environment may be difficult. Pretreatment of glass vials at a standard laboratory equipped with a simple roller and similar devices would allow the use of pretreated glass vials in field locations at least for 7 days posttreatment, as demonstrated in this study. Diagnostic concentrations are recommended for a number

of insecticides against mosquitoes.[25] Although several concentrations have been tested in susceptibility studies, no standard diagnostic insecticide concentrations are available for most insecticides to detect resistance in sand fly species.[19,28] Collaboration among sand fly researchers is necessary to coordinate efforts to develop standards for insecticide resistance monitoring in sand flies. This study provides baseline susceptibility data that can inform future insecticide resistance monitoring in sand flies.

Table 1. Results of glass vial bioassays of pyrethroid and organophosphate insecticides against the Israeli strain of *P papatasi* maintained at USDA-ARS-KBUSLIRL in Kerrville, Texas.

	Solution Concentration (%)	Surface Concentration	Slope	χ^2 (n)	LT_{50} (95% CI)
Pyrethroids					
Cypermethrin	0.0001	5 ng/cm²	6.5	13.9 (14)	30.0 (26.9-32.9)
	0.001	50 ng/cm²	4.4	17.4 (10)	21.1 (16.1-26.2)
	0.01	0.5 µg/cm²	/	/	<20
Deltamethrin	0.0001	5 ng/cm²	2.6	19.6 (21)	29.9 (24.8-35.0)
	0.001	50 ng/cm²	/	/	<20
	0.01	0.5 µg/cm²			n/t
Cyfluthrin	0.0001	5 ng/cm²	6.1	7.4 (17)	32.1 (28.9-35.2)
	0.001	50 ng/cm²	4.9	4.9 (9)	16.5 (14.0-19.)
	0.01	0.5 µg/cm²			n/t
λ-cyhalothrin	0.0001	5 ng/cm²	2.4	6.5 (14)	12.8 (8.1-16.5)
	0.001	50 ng/cm²	/	/	<10
	0.01	0.5 µg/cm²			n/t
Permethrin	0.0001	5 ng/cm²	5.0	11.6 (45)	85.9 (80.1-91.8)
	0.001	50 ng/cm²	5.1	13.1 (17)	34.2 (30.5-37.8)
	0.01	0.5 µg/cm²	4.4	6.9 (8)	14.2 (11.6-16.7)
Prallethrin	0.0001	5 ng/cm²	4.9	4.1 (8)	13.8 (11.4-16.0)
	0.001	50 ng/cm²	/	/	<10
	0.01	0.5 µg/cm²			n/t
Organophosphates					
Chlopyrifos	0.001	50 ng/cm²	8.7	26.1 (21)	53.1 (49.1-57.2)
	0.01	0.5 µg/cm²	7.5	5.5 (14)	28.3 (25.6-30.9)
	0.1	5 µg/cm²			n/t
Coumaphos	0.001	50 ng/cm²	12.2	16.3 (28)	77.6 (74.3-81.2)
	0.01	0.5 µg/cm²	5.4	6.5 (17)	34.7 (31.2-38.2)
	0.1	5 µg/cm²			n/t
Diazinon	0.001	50 ng/cm²	12.1	13.7 (20)	53.4 (50.5-56.2)
	0.01	0.5 µg/cm²	10.1	9.6 (10)	25.5 (23.4-27.6)
	0.1	5 µg/cm²			n/t
Dichlovos	0.001	50 ng/cm²	3.3	10.2 (58)	122.0 (112.4-254.8)
	0.01	0.5 µg/cm²	6.6	9.1 (32)	58.4 (54.3-62.4)
	0.1	5 µg/cm²			n/t

LT_{50} indicates exposure time (minutes) at which 50% of the flies had died.
n/t indicates not tested.

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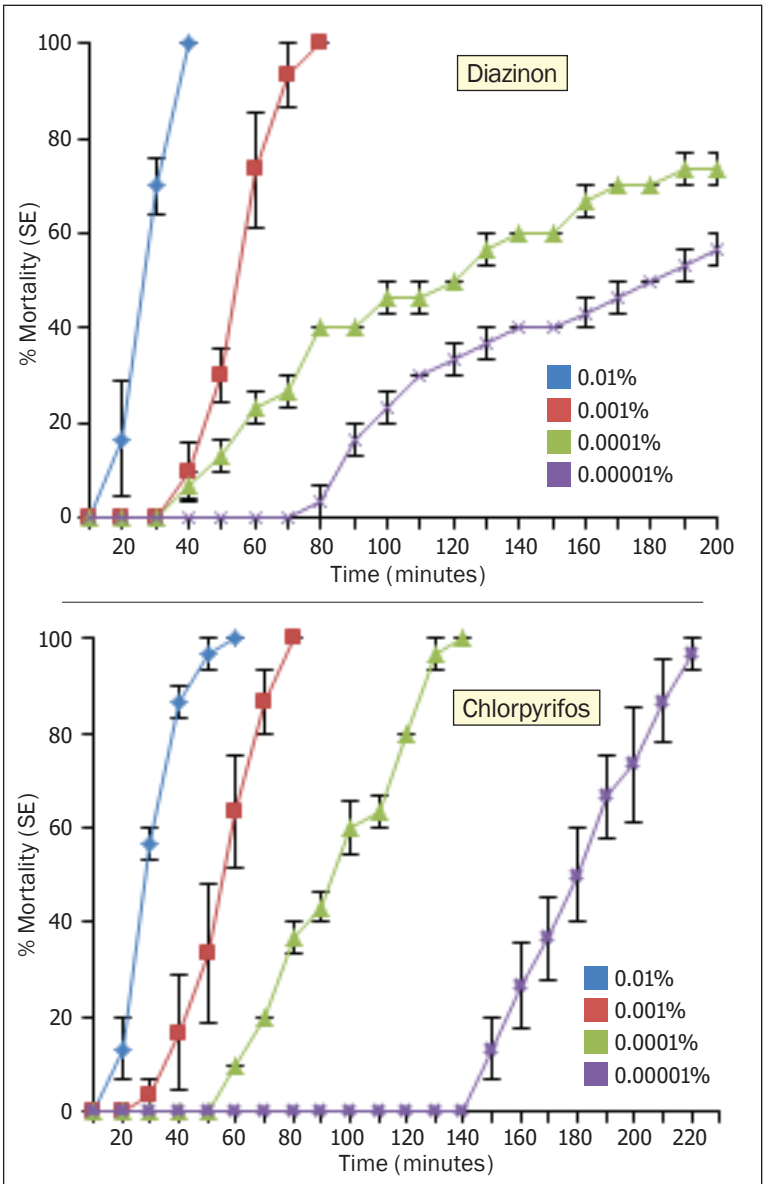

Figure 4. Exposure time-mortality responses of male *P papatasi* to different concentrations of representative organophosphate (diazinon, chlorpyrifos) insecticides.

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Table 2. Results of glass vial bioassays of pyrethroid and organophosphate insecticides against the Israeli strain of *P duboscqi* strain maintained at USAMRU-K field station in Marigat, Kenya.

	Solution Concentration (%)	Surface Concentration	Slope	χ^2 (n)	LT_{50} (95% CI)
Pyrethroids					
Cypermethrin	0.0001	5 ng/cm²	2.8	34.3 (19)	109.2 (74.7-503.2)
	0.001	50 ng/cm²	6.0	8.3 (13)	24.1 (21.0-27.0)
	0.01	0.5 µg/cm²	/	/	<10
Deltamethrin	0.0001	5 ng/cm²	4.0	58.2 (19)	57.1 (43.6-77.8)
	0.001	50 ng/cm²	10.5	6.8 (10)	20.2 (17.8-22.0)
	0.01	0.5 µg/cm²	/	/	<10
Etofenprox	0.0001	5 ng/cm²	5.8	34.6 (13)	25.0 (18.9-30.7)
	0.001	50 ng/cm²	3.4	36.8 (22)	32.5 (23.5-40.8)
	0.01	0.5 µg/cm²	5.6	5.0 (6)	17.9 (14.3-21.2)
λ-cyhalothrin	0.0001	5 ng/cm²	2.0	8.1 (22)	65.3 (52.9-91.5)
	0.001	50 ng/cm²	/	/	<20
	0.01	0.5 µg/cm²	/	/	<5
Permethrin	0.0001	5 ng/cm²	3.1	11.2 (25)	80.8 (69.8-101.4)
	0.001	50 ng/cm²	4.9	19.4 (23)	33.1 (29.4-36.5)
	0.01	0.5 µg/cm²	/	/	<20
Prallethrin	0.0001	5 ng/cm²	2.4	10.5 (13)	22.4 (17.8-27.0)
	0.001	50 ng/cm²	/	/	<10
	0.01	0.5 µg/cm²	/	/	<5
Organophosphates					
Carbaryl	0.001	50 ng/cm²	8.1	141.1 (19)	32.2 (21.8-40.3)
	0.01	0.5 µg/cm²	7.5	270.4 (13)	/
	0.1	5 µg/cm²	11.4	3.9 (10)	26.8 (24.8-28.8)
Chlopyrifos	0.001	50 ng/cm²	6.2	33.1 (13)	21.4 (16.6-25.5)
	0.01	0.5 µg/cm²	/	/	<10
	0.1	5 µg/cm²	/	/	<10
Malathion	0.001	50 ng/cm²			n/t
	0.01	0.5 µg/cm²	6.3	8.6 (7)	13.1 (10.6-15.5)
	0.1	5 µg/cm²			n/t

LT_{50} indicates exposure time (minutes) at which 50% of the flies had died.
n/t indicates not tested.

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Efficacy of Permethrin Treated Bed Nets Against *Leishmania major* Infected Sand Flies

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Abstract

Insecticide treated nets (ITNs) are a potential tool to help control sand flies and prevent Leishmaniasis. However, little is currently known about the response of Leishmania infected sand flies to ITNs. In this study, Phlebotomus duboscqi sand flies were infected with the parasite Leishmania major. Infected and noninfected sand flies were then evaluated against permethrin treated and untreated bed nets in a laboratory assay that required sand flies to pass through suspended netting material to feed on a mouse serving as an attractive host. The number of sand flies passing through the nets and blood feeding was recorded. There was not a significant difference in the ability of infected or noninfected sand flies to move through treated or untreated nets. Fewer sand flies entered the permethrin treated nets compared to the untreated nets, indicating that permethrin creates an effective barrier. The results show that in addition to reducing the nuisance bites of noninfected sand flies, ITNs also protect against Leishmania infected sand flies and therefore can play in key role in reducing the rates of Leishmaniasis. This study is important to the Department of Defense as it continues to develop and field new bed nets to protect service members.

Leishmaniasis is caused by parasitic protozoa in the genus *Leishmania* that are vectored by Phlebotomine sand flies. The disease manifests in cutaneous, mucocutaneous, or visceral forms. There are an estimated 12 million people in tropical and subtropical regions currently infected with some form of this disease.[1] The cutaneous form of the disease was frequently observed among US service members deployed in Iraq and Afghanistan with 1,670 confirmed cases reported from 2003 to 2014.[2] However, the actual number of cases was probably much larger. Initial efforts by the US military to control sand flies relied on insecticides and proved largely unsuccessful due to harsh environmental conditions and the cryptic nature of sand flies.[3] Current guidelines for managing sand flies endorse an integrated approach and stress the importance of personal protective measures that include using topical repellents on the skin, treating uniforms with permethrin, and sleeping under insecticide treated nets (ITNs).[4]

Insecticide treated nets are recognized by the global health community as an important tool to help control sand flies and prevent Leishmaniasis.[5,6] They have been most frequently used and are most well known for their use in malaria control programs where there is a current emphasis to switch to long lasting insecticide nets that do not require periodic retreatment with an insecticide.[7] Sand flies cannot be adequately controlled with standard mosquito nets because most species are small enough to pass through the mesh, and decreasing the mesh size restricts air flow and makes the nets uncomfortable to use in hot environments. Therefore, protection is only observed after the addition of an insecticide.[8] Insecticide treated nets have been compared to baited traps where sand flies are attracted to host odors emanating from inside the net and then contact the insecticide treated fabric while trying to enter.[5] Even if sand flies are able to pass through ITNs, the nets are capable of killing sand flies or changing their feeding behavior.[9,10]

One important question that has not been investigated is whether ITNs provide the same level of protection against *Leishmania* infected sand flies as they do for noninfected sand flies. It is well documented that *Leishmania* infection causes many changes to sand fly feeding behavior and host seeking.[11-13] During normal feeding, noninfected sand flies will land on a host, search for a suitable location, begin probing, and then usually obtain a full blood meal within 2 to 3 minutes. In contrast, infected sand flies will often probe multiple times and for several minutes and never successfully obtain a blood meal. They are also more persistent and are more

likely to return and feed if interrupted.[13] This behavior likely enhances transmission of *Leishmania* parasites, but it is not known if it makes sand flies more likely to enter ITNs and seek a blood meal.

In this laboratory study, the ability of *Leishmania major* infected *Phlebotomus duboscqi* to pass through a permethrin treated net and take a blood meal was compared to noninfected flies. Permethrin was selected because it is the insecticide used by the military to treat bed nets and belongs to the pyrethroid class of insecticides which is preferred by most global health organizations.[14] The sand fly *P duboscqi* is a major vector of cutaneous leishmaniasis in Africa. The parasite *L major* is found in Africa and the Middle East and was the leading cause of cutaneous Leishmaniasis among service members in Iraq.[4]

Materials and Methods

Sand Flies

Phlebotomus duboscqi from Mali were obtained from the National Institute of Health and reared in the insectary at the Walter Reed Army Institute of Research by the methods described in Modi and Rowton.[15] The sand flies were maintained at 26°C and 80% relative humidity.

Infection with *Leishmania*

Leishmania major RYN strain parasites were used for infections. A membrane feeding apparatus and water bath circulator were used to infect sand flies.[16] Defibrinated rabbit blood was spiked with *L major* promastigotes and placed in the feeding apparatus. The feeding apparatus had a chicken skin membrane attached to the lower opening and was placed on a screened carton holding sand flies. The sand flies were allowed to blood feed to repletion. Dissections on day 13 postinfection and immediately after experimental manipulation revealed 90% to 100% infection rates. Noninfected control sand flies were blood fed in the same way, but *L major* promastigotes were not added to the blood meal.

Insecticide Treated Nets

Untreated white, 196-mesh polyester netting material was obtained from Vestergaad Frandsen, Inc (Lausanne, Switzerland). This material was selected because it was known that sand flies could pass through. The fabric was cut into 232.4 cm^2 squares before treatment. A wooden stand was used to hold an aerosol can of Repel Permanon (0.5% permethrin) (United Industries Corp, Middleton, WI) with the nozzle 20 cm from the center of the material. The fabric was then sprayed for 5 seconds on each side. This method of permethrin application was selected because it simulates how bed nets are currently treated by the US military.[14] The treated material was allowed to dry for 48 hours in a chemical fume hood and then stored individually in plastic bags at room temperature until use. Untreated netting material was cut in similar squares and hung in a separate chemical hood for 48 hours.

Assays

Assays were conducted using a Grieco module.[17] The module consists of 2 Plexiglass cylinders each 15.9 cm long and 10.2 cm in diameter (Figure 1). A Teflon linking section (4.4 cm thick, 10.2 cm diameter) connects the 2 cylinders and contains a butterfly valve (5.5 cm diameter) that allows movement between the 2 modules. Netting material was stretched tightly between the 2 clear chambers with the butterfly door closed. With the door open, the only way for sand flies to move between the cylinders is through the netting material.

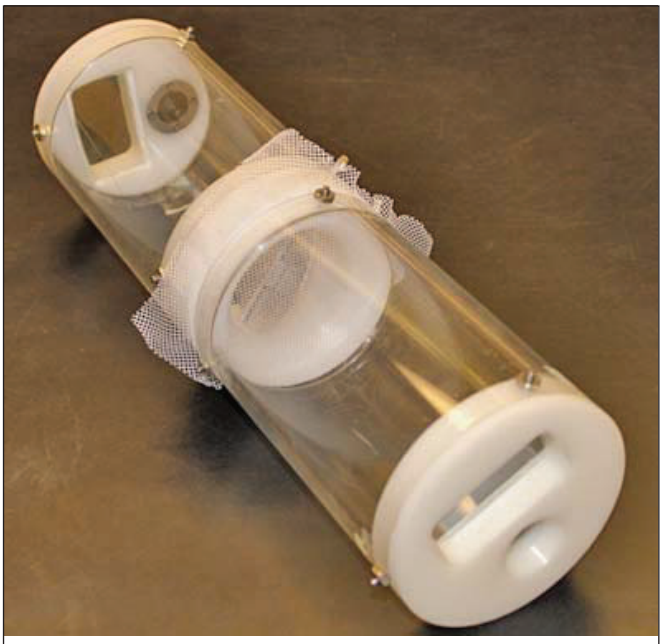

Figure 1. Grieco module used to conduct laboratory assays with insecticide treated nets.

The assays were performed in a glove box with the temperature maintained between 20°C and 24°C and 70% to 80% relative humidity. The sand flies used in the assays were approximately 15 to 17 days postemergence and had been water and sugar starved for 12 hours prior to use. A single ICR (Institute for Cancer Research) mouse (Charles River Laboratory, Frederick, MD) was anesthetized and placed in one chamber to serve as a host for sand flies. Twenty female *P duboscqi* were placed in the opposite chamber and were given 3 minutes to acclimate. The lights were turned off making the room completely dark and the butterfly door was then opened allowing the sand flies to move about undisturbed. After 20 minutes, the sand flies from each side of the module

were counted. The sand flies were then dissected to verify blood feeding with any trace of blood considered a blood fed sand fly. The midguts were also checked for metacyclic promastigotes to confirm infection.

Study Design

There were 4 experimental groups based on the combination of netting material (permethrin treated, untreated) and sand flies (infected, noninfected). Each combination was replicated 6 times. Data was collected on the number of sand flies passing through the nets and blood feeding for all combinations. The data were analyzed by Fisher exact tests, which were conducted using a 2-sided alpha of 0.05.

Results

There was not a significant difference in the movement of *Leishmania* infected or noninfected sand flies through treated or untreated nets as shown by Figure 2. For the treated netting material, 27 of 73 (37%) of the infected sand flies passed through and 23 of 77 (30%) of the noninfected sand flies passed through (P=.6245, χ^2=0.4267). Results for the untreated material were similarly not significant with 42 of 78 (54%) of infected sand flies passing through and 49 of 81 (60%) of the noninfected sand flies passing through (P=.394, χ^2=0.988).

There was a significant difference when comparing the 2 types of netting material. Fewer sand flies passed through the permethrin treated net compared to the untreated net for both infected (P=.0369, χ^2=4.9784) and noninfected sand flies (P=.0002, χ^2=14.6701). This indicates that the treated netting material was more effective than the untreated net at preventing sand flies from moving through the mesh. It was noted for the permethrin treated net that most of the sand flies that passed through the netting material were knocked down or dead after 20 minutes. All of the sand flies exposed to the untreated net were still active.

There were no blood-fed sand flies in modules containing the permethrin treated net. When the untreated netting material was used, 7% of the infected sand flies took a blood meal and 25% of the noninfected sand flies took a blood meal as shown in the Table.

Blood feeding results for sand flies that were able to pass through the netting material to host side of the module.

	Permethrin Treated Net	Untreated Net
Leishmania infected sand flies	0/27 (0%)	3/42 (7%)
NonInfected sand flies	0/23 (0%)	12/49 (24%)

Comment

This is the first study to assess the ability of *Leishmania* infected sand flies to pass through ITNs. Both infected and noninfected sand flies passed through permethrin treated netting material at similar rates. Infections with very high levels of parasites did not lead to changes in behavior,[11-13] and thus allow infected sand flies to bypass the protection of ITNs. The results suggest that ITNs likely play an important role in reducing the transmission of Leishmaniasis in addition to reducing the nuisance bites of sand flies.

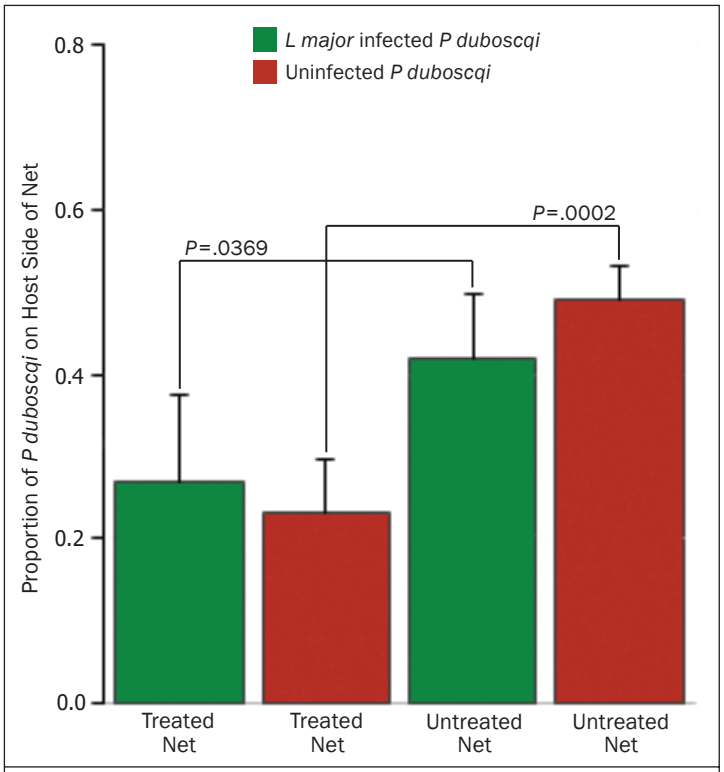

Figure 2. Proportion of Phlebotomus duboscqi that passed through treated or untreated bed net material.

Permethrin treated nets reduced vector host interactions for both infected and noninfected sand flies. This shows that permethrin is effective in lowering the movement of sand flies into bed nets. However, a small percentage of sand flies were still able to pass through treated nets. This may have been an artifact of the testing module since it was small and enclosed, and, without the ability to escape, some sand flies may have found their way through.

It is worth noting that in this study even when sand flies did pass through the permethrin treated net, none of them took a blood meal and most were knocked down. This corresponds to other studies showing that exposure to permethrin alters sand fly feeding behavior even if it does not kill them directly.[9,10] There have been a

few unpublished studies at the Walter Reed Army Institute of Research (WRAIR) where blood feeding was observed after sand flies contacted permethrin treated nets, and contact with permethrin may not always prevent blood feeding. In this study, sand fly probing behavior could not be observed because the assays were conducted in the dark. It is possible that some sand flies moved through the net and probed before being killed or knocked down.

The results showed that infected sand flies were less effective at taking blood meals than the noninfected sand flies. This corresponds to other studies showing that *Leishmania* infection inhibits blood feeding.[11,12] Overall, the blood feeding rates of sand flies in this study were lower than previous studies conducted at WRAIR. Those unpublished studies used noninfected flies that never received a blood meal and feeding rates were 60% to 80%. The lower rates in this study were likely a result of the sand flies receiving a blood meal during the infection process and being gravid at the time of the assay. It was not possible to have nongravid females in this study, since a blood meal was required to become infected and females die in the laboratory immediately after ovipositing their eggs.

Many large scale studies have evaluated the effectiveness of ITNs against sand flies and most have shown that they are beneficial. In a study in Brazil, ITNs were associated with reduced indoor human landing rates and high sand fly mortality.[18] Visceral leishmaniasis rates in Sudan were reduced following the widespread distribution of ITNs.[19] In Syria, the rates of cutaneous leishmaniasis dropped by 85% after the distribution of ITNs.[20] A large World Health Organization effort to eliminate visceral leishmaniasis in India, Bangladesh, and Nepal has shown both positive and negative results. Community-wide distribution of ITNs reduced the indoor density of sand flies by 25% in India and Nepal,[21] and the use of ITNs reduced indoor sand fly densities by 60% to 85% in Bangladesh.[22,23] However, in one large study in India, ITNs did not lead to lowered sand fly densities.[24] In another study in India and Bangladesh, the mass distribution of ITNs only slightly lowered sand fly biting rates based on serological data among communities.[25]

The most important result of this study is that it justifies using noninfected sand flies to evaluate the effectiveness of new ITNs. There have been many unpublished evaluations of ITNs at WRAIR using noninfected sand flies, and other published studies have also used noninfected sand flies.[10] This study indicates that results from noninfected sand flies are most likely similar and applicable for *Leishmania* infected sand flies. It is much easier to use noninfected sand flies in laboratory assays since they can be produced in greater numbers, at

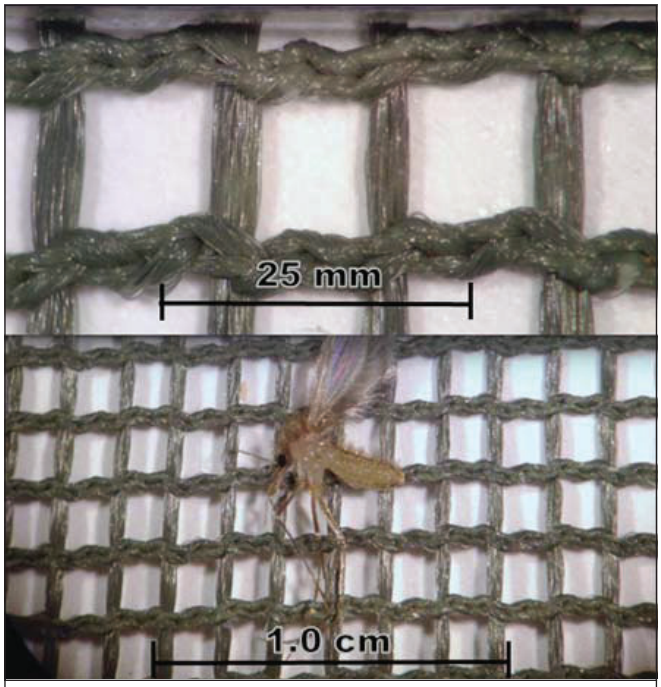

Figure 3. Standard mosquito net (NSN 7210-00-266-9736/9740). This net is not treated with an insecticide and the mesh is too large to physically exclude sand flies. Female sand fly included for size comparison.

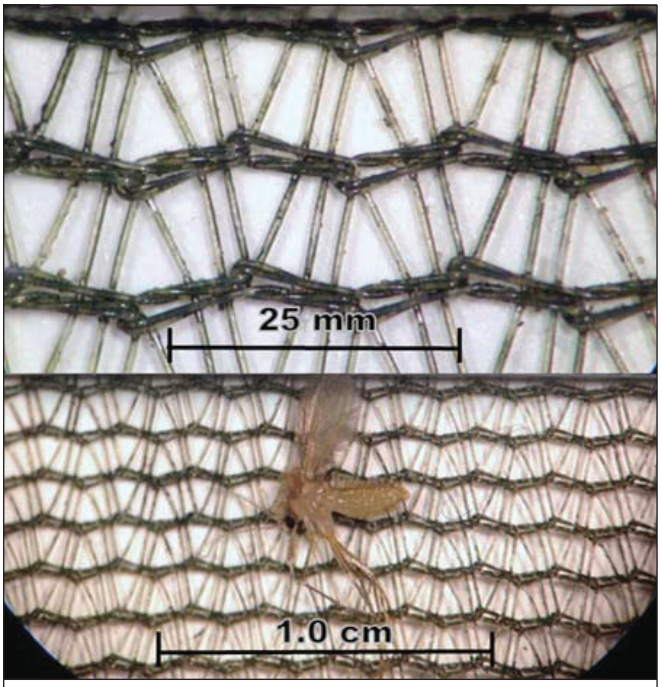

Figure 4. Pop-up style bed net (NSN 3740-01-516-4415). This net is factory treated with permethrin and the mesh size is small enough to physically exclude sand flies. Female sand fly included for size comparison.

cheaper costs and are safer to handle. Research with *Leishmania* infected sand flies must be performed in a Biosafety level 2 laboratory.[26]

The Department of Defense will continue to rely upon ITNs to protect service members from sand fly bites and diseases. There are two bed nets currently available. The standard mosquito net (NSN 7210-00-266-9736/9740), shown in Figure 3, is untreated and its mesh size is too large to physically exclude sand flies. The mesh becomes more permissive to sand flies as the nets become older and receive more wear.[4] It is important to treat these nets with permethrin if they are to provide protection against sand flies. The recommended method is to spray with an aerosol can as described in this study.[14] Pop-up style bed nets (Figure 4) that are now available (NSN 3740-01-516-4415) have a mesh size small enough to exclude sand flies and are issued factory treated with permethrin.[4] These nets are the preferred option for the prevention of sand fly bites. The results from this study will be useful as the Department of Defense continues to develop and evaluate new ITNs and ensure that they provide protection from both infected and noninfected sand flies.

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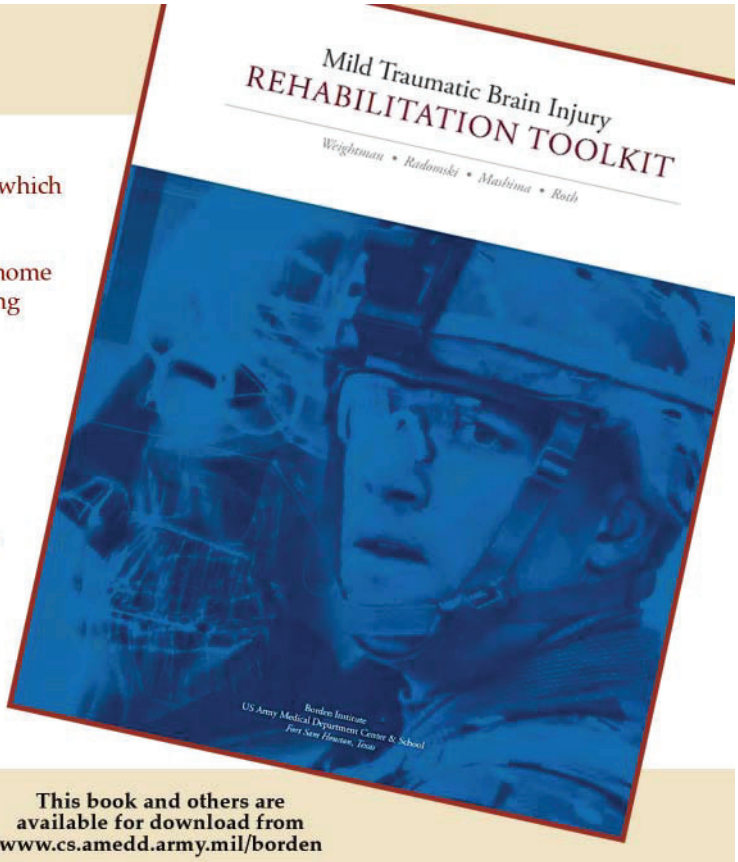

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Controlled Human Malaria Infection at the Walter Reed Army Institute of Research: The Past, Present, and Future From an Entomological Perspective

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Abstract

Thirty years ago, the Entomology Branch at the Walter Reed Army Institute of Research (WRAIR) performed the first controlled human malaria infection, in which lab-reared mosquitoes were infected with lab-cultured malaria parasites and allowed to feed on human volunteers. The development of this model was a turning point for pre-erythrocytic malaria vaccine research and, through decades of refinement, has supported 30 years of efficacy testing of a suite of antimalarial vaccines and drugs. In this article, we present a historical overview of the research that enabled the first challenge to occur and the modifications made to the challenge over time, a summary of the 104 challenges performed by WRAIR from the first into 2015, and a prospective look at what the next generation of challenges might entail.

Malaria remains one of the greatest infectious disease burdens worldwide, with ≈200 million cases and ≈600,000 deaths reported in 2013.[1] Though not endemic to the United States, malaria incidence is reported in 97 countries, indicating this threat to global health is also a threat to travelers and deployed military personnel. Resistance to drugs that kill Plasmodium parasites (the etiological agent of malaria) is common and spreads rapidly upon introduction. There is no currently available vaccine. Therefore, development and testing of antimalarial vaccines and new drugs has been a top priority for infectious disease research within the Department of Defense for decades.

Plasmodium parasites are delivered to humans by the bite of an Anopheles mosquito; as a female mosquito takes blood from a human host, she deposits the sporozoite stage of the parasite into the host's skin along with her saliva. Sporozoites navigate to the liver where they invade hepatic cells, shift to a new form called the merozoite, and multiply, eventually being released into circulation where they can continue an invade-multiply-release cycle, now dependent on erythrocytes. Since the mosquito only deposits about 10 to 100 sporozoites per bite[2-4] and the ensuing life cycle involves exponential multiplication, the pre-erythrocytic sporozoite stage represents a bottleneck in the parasite population that is vulnerable to vaccine and drug activity.

Interventions that specifically target the pre-erythrocytic stage have been in the pipeline since it was first shown that sporozoites elicit an immune response capable of preventing subsequent infection.[5,6] However, as these vaccine and drug candidates were showing efficacy in animal models, it became apparent to medical entomologists that clinical testing of such interventions would require a method of mimicking the natural acquisition of sporozoites by humans via mosquito bite. Previous methods used human gametocyte donors to infect mosquitoes intended to deliver sporozoites to vaccines,[7] but this method was unpredictable and dependent on the availability of people naturally infected with malaria as gametocyte sources. A more controlled, reproducible method was needed; thus, an experimental human malaria infection, later coined as controlled human malaria infection (CHMI),[8] was developed at the Walter Reed Army Institute of Research (WRAIR). The CHMI method encompasses the entirety of a purposeful human malaria infection, from mosquito bite to parasite detection in the blood, to resolution by drug administration. The entomological part of CHMI is considered the "challenge": the transmission of malaria parasites as mosquitoes bite a human volunteer in a safe, reliable, and reproducible way.

Identifying Parasites and Vectors Capable of Infecting Humans

To develop a controlled challenge model, entomologists at WRAIR tested the feasibility of artificially infecting lab-reared Anopheles mosquitoes with lab-cultured Plasmodium. A reliable culture method for growing *P falciparum* in vitro was finally published in 1976.[9] This system became critical for the development of a malaria challenge since it enabled manufacture of parasite

lines with known origin and drug sensitivity, followed a somewhat consistent schedule, and used blood and sera of known type that could be tested for pathogens. The downfall of in vitro parasite growth was (and still is) that most parasite lines adapted to grow well asexually in vitro infect mosquitoes poorly, if at all. WRAIR and the Naval Medical Research Institute (NMRI), predecessor to the Naval Medical Research Center, collaboratively tweaked the Trager-Jensen method to grow the best lines for infecting mosquitoes.[10] Foreseeing the need for a compatible parasite-vector pair on which to base the malaria challenge, WRAIR entomologists exposed various anopheline species to multiple *P falciparum* parasite lines, both lab-adapted and patient-derived, to assay for successful mosquito infection. Although the screening was exhaustive, infection rates were often disappointing, sometimes yielding months of no infectiousness to mosquitoes. By 1983, the 7G8 strain (chloroquine resistant) was cloned from a Brazilian patient sample and, in regular production at WRAIR, showed low numbers of oocysts and sporozoites but with greater consistency than any other strain. In 1985, WRAIR received the African-derived, chloroquine sensitive NF54 *P falciparum* strain from NMRI which had received it from collaborators in Nijmegen, The Netherlands.[11] This quickly became the primary culture in production. In 1987, WRAIR subsequently received 3D7, a strain cloned from NF54 by NIH researchers,[12] from NMRI. Based on the mosquito infectivity studies done at WRAIR, NMRI, and elsewhere, 7G8, NF54, and 3D7 would become the worldwide standards for cultured parasites suitable for infecting mosquitoes and nearly the only strains of *P falciparum* used in malaria challenges as of 2015.

For vector selection, the breadth of available parasite strains were fed to a variety of potential vectors, including *An stephensi*, *An freeborni*, *An balabacensis*, *An albimanus*, *An quadrimaculatus*, and others. The studies showing 7G8, NF54, and 3D7 were infectious to mosquitoes also showed that *An stephensi* was a robust mosquito, amenable to mass rearing with hearty feeding propensity, widely used by other mosquito biologists and displayed excellent susceptibility to both *P falciparum* and *P berghei*, a pre-clinical rodent model for infection. Therefore, it is not surprising that *An stephensi* is the primary colony supported within WRAIR and, to date, 3 challenges used *An freeborni* but the rest have used *An stephensi*.

Initial Development of the WRAIR Challenge Model

During vector-parasite compatibility experiments in 1982, exposure of a laboratory worker to an escaped infectious mosquito resulted in accidental transmission of cultured 7G8 *P falciparum* by lab-reared *An freeborni* to a human.[13] While this study highlighted the acute need for a safety regimen to safeguard workers' health, it also showed for the first time that parasites grown in culture and capable of infecting a mosquito could also retain infectiousness to humans, inadvertently paving the way for CHMI.

The first CHMI was performed in 1985 as a proof-of-concept trial to assess whether 6 volunteers would develop malaria after being bitten by 5 mosquitoes infected with NF54.[14] Collectively, WRAIR, NMRI, and NIH contributed *An freeborni* and *An stephensi* that were given a blood meal containing cultured parasites in donor blood; only blood-fed (and therefore potentially infected) mosquitoes were retained for possible use. At appropriate times, subpopulations were dissected and numbers of oocysts and sporozoites were quantified in midgut and salivary glands, respectively. Mosquitoes determined to likely be infectious were sorted into cups of 5 and allowed access to a volunteer's arm for 5 minutes. Mosquitoes were then checked for the presence of a blood meal (confirmed they fed on the volunteer) and the presence of sporozoites on a 0 to 4 quantification scale (rating of 2 or greater confirmed infectiousness) and, if fewer than all 5 satisfied those criteria, the volunteer was exposed to more mosquitoes until 5 infectious bites were confirmed. This process was performed on a rolling basis—volunteers were called when mosquitoes were ready and not all on the same day. All 6 of the volunteers came down with malaria. This method was independently repeated at the University of Maryland[15] with success (4 of 4 volunteers infected) and the fundamentals of the process are largely how challenges are performed today.

Questions were raised about the validity of using 5 mosquito bites for a challenge. In nature, people are typically infected by the bite of one mosquito; could vaccine-derived immunity be overwhelmed by a 5-bite dosage? And, if so, would a vaccine that would be efficacious against a natural 1 or 2 bite dose be erroneously perceived as ineffective in a 5-bite challenge? Also, how does sporozoite load affect dosage? Compared to natural conditions, laboratory conditions can load mosquito salivary glands with a much heavier burden of sporozoites[16]; however, the number of sporozoites successfully deposited in the skin is orders of magnitudes lower than in the salivary glands and highly variable.[3,17] Direct enumeration of sporozoites put into each volunteer is ethically impossible, so 2 challenges were performed by WRAIR for the Navy to assess the feasibility of a 1- or 2-bite challenge. Three out of 5 volunteers receiving a

single bite became malaria positive, while 2 of 5 receiving 2 bites became positive.[18] A third 2-bite challenge was performed by WRAIR for Johns Hopkins University with only 1 of 3 volunteers becoming malaria positive.[19] Therefore, a 5-bite challenge has been standard since about 1990. Later studies show that 3 bites from aseptically reared *An stephensi* can result in 100% infectivity,[20,21] but the consistency and the theoretical advantages of this model have yet to be demonstrated, so WRAIR continued to provide a 5-bite challenge. In 2012, a series of meetings were held to generate a consensus of all CHMI-capable centers, ultimately agreeing on the WRAIR challenge model of 5 bites from *An stephensi* using a 0 to 4 rating scale as the global standard.[8]

After 24 years, a second parasite species was introduced to the 5-bite challenge. In 2009, the Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok, Thailand, sent 2 challenges using *P vivax* in *An dirus* to WRAIR for infectivity studies. Since *P vivax* cannot be easily cultured in vitro, the lab-reared mosquitoes were infected with blood from a human gametocytemic patient in Thailand, then shipped to WRAIR for challenge administration. All 12 volunteers from these studies became infected, demonstrating that the challenge model has a measure of flexibility.

Variations on the Traditional Challenge

Off-site Challenges

Shipping or hand-carrying infected mosquitoes to perform a challenge overseas was initially tested for feasibility in 2000. A batch of prepared mosquitoes was flown from Washington, DC, to London as a mock challenge test of transport and mosquito viability in anticipation of challenges performed by WRAIR personnel for collaborators from Oxford University. This validated the feasibility of a "traveling" challenge that, with slight variations that defer to site-specific clinical trial centers, is performed similarly to in-house challenges. This includes not just the supply of infectious mosquitoes but of dissectors, entomologists, quality assurance/quality control, standard operating procedures, and challenge day methodology that has produced success in the past. This still requires the receiving facility to have minimal insectary infrastructure for mosquito storage but requires no entomological experience, parasite culture, or mosquito rearing on the part of the receiver.

Mosquito Bites as Vaccines

Soon after the debut of CHMI, a second mosquito-biting-humans method was developed, in which volunteers were exposed to hundreds or even thousands of bites from mosquitoes infected with attenuated parasites. At first, this was radiation-attenuated sporozoites as a natural progression from the animal studies and few human studies that already demonstrated this produced a protective immune response.[22] These studies, performed on a rolling basis over several years, would collectively use 23,279 mosquitoes. Later, sporozoites would also be genetically attenuated,[23] but the role of entomology remained the same, differing from traditional challenges in that many more mosquitoes were required and real-time dissections were not necessary. These trials culminated with a traditional challenge to test the efficacy of the mosquito-delivered "vaccine" and/or investigate the immune response generated. Eventually, production of mosquitoes that functioned as a vaccine would be considered by the Federal Drug Administration (FDA) to be a manufacturing process (reviewed later in this article), instituting a sum of regulatory requirements that would impose the greatest modification of the challenge process since inception.

Challenge in a Bottle

Not surprisingly, challenges can be expensive, time-consuming, and require specialized facilities and entomological expertise. Innovations in sporozoite cryopreservation by Sanaria, Inc (Rockville, MD)[24] initiated an effort to overcome these limitations by vialing aseptic, cryopreserved sporozoites into an FDA-regulated product called PfSPZ Challenge, colloquially referred to as "challenge in a bottle." This mosquito-free challenge delivers sporozoites by needle inoculation and is capable of reasonable infectivity rates at a dose of 3,500 sporozoites per vial. This type of challenge is most useful in field settings or locations where facilities cannot support insect maintenance; however, in bypassing the skin, it does not fully mimic the natural route of sporozoite inoculation by mosquito.[25] This means it also bypasses immune responses elicited by skin-deposited parasites in the dermis and draining lymph nodes, and may affect the degree of protection observed.[26,27]

Meeting the Increasing Needs of the Challenge

By 1989, demand for infected mosquitoes, stemming from both clinical and preclinical vaccine research, shifted Entomology into a production role. Every aspect of producing infected mosquitoes, from obtaining enough blood and serum to rearing enough mosquitoes to having the tools and infrastructure to safely handle so many infectious mosquitoes, was reexamined and retooled to meet the needs of CHMI. General rearing rooms were outfitted with specialized equipment to improve insect production and increase efficiency, while smaller equipment such as aspirators to transfer mosquitoes, water

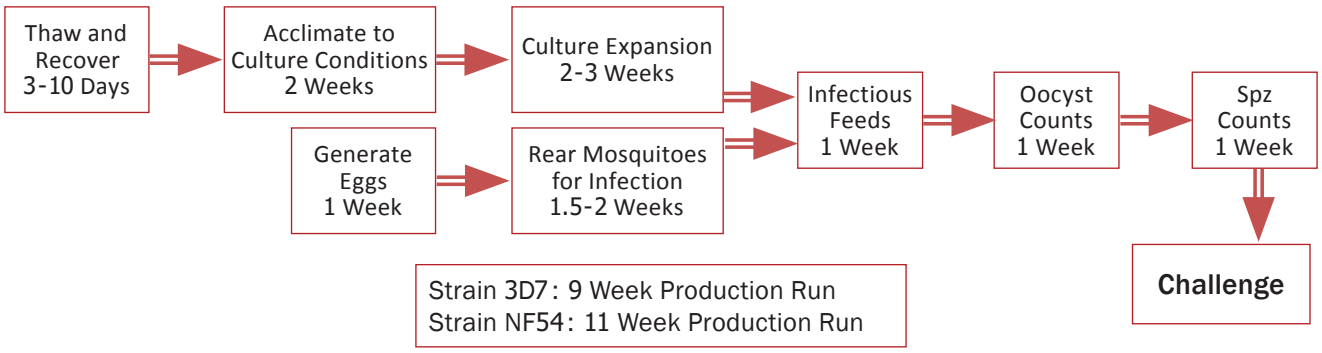

Figure 1. Workflow diagram describing the regimen for producing infected mosquitoes for CMHI.

jacketed membrane feeders, and mosquito containment devices underwent multiple rounds of innovation to comply with increased demand and increased safety precautions. Mosquito rearing conditions and parasite culture methods were optimized and Entomology personnel began to routinely record data on prevalence and intensity of mosquito infection, no longer as basic research but as indicators of mosquito quality for use in CHMI.

The biggest physical innovation in mosquito production occurred in the late 1990s as WRAIR moved from downtown Washington, DC, to the Forest Glen Annex in Silver Spring, MD. The insectary facilities in that building were specifically designed to meet the needs of the challenge. The challenge suite exists separate from general insect rearing and consists of (1) an empty vestibule to discourage accidental mosquito release as doors are opened, (2) a main room where volunteers and noninsectary personnel are stationed on challenge day, (3) an adjacent room that houses both walk-in and reach-in incubators for infected mosquitoes, and (4) a separate adjacent room for real-time dissection of mosquito salivary glands. Doors with screens allow personnel to communicate with one another but also contain any escaped mosquitoes in work areas away from the main challenge room where visitors are permitted. Incubator set-up facilitates scale production depending on the sizes and numbers of clinical trials in progress and enable segregation of mosquitoes infected with different parasite lines. The dissection room is designed for the comfort, safety, and efficiency of up to 5 dissectors. A person must pass through 5 doors and a downward air current to get from infected mosquito housing to the main corridor, ensuring the safety of all who work in the building. Two distinct labs specific for parasite culture exist separately from the insectary and other lab space, isolating challenge-specific cultures from general lab work while simultaneously enabling segregation of different *P falciparum* strains destined for challenges.

The 1990s also ushered in an extensive suite of methodological innovations, transitioning the orientation of challenge preparation from academic to production. Extensive screens for fail-proof stocks of NF54, 3D7, and 7G8 were undertaken, mass sporozoite harvesting methods were adopted, and individualized fine-tuning of each round of parasite culture/mosquito infection was abandoned in favor of a scheduled, standardized culture/rearing/infection regimen used for every round of production (Figure 1). This was also highly influenced by the advent of new regulatory requirements as discussed in the next section.

Regulatory Influence on the Challenge

Until 1993, challenges were performed with mosquitoes infected as they would be for routine laboratory experiments. At that time, the FDA became interested in the challenge as a systemized and monitored part of a clinical trial and introduced a wave of new regulatory requirements, exponentially increasing the labor and planning required to carry out each successful trial. A batch master file was created in the fall of 1994 and, within one year, entire cell banks comprised of 140, 110, and 75 vials of NF54, 3D7, and 7G8, respectively, were manufactured under good manufacturing practices (GMP) conditions at the Pilot Bioproduction Facility also located on the Forest Glen Annex. These cell banks, derived from blood collected from clinical trial volunteers, have provided the seed parasites for every WRAIR challenge through 2015, though a new bank was created for 3D7 in 2014 as the original lot dwindled. Every cell bank creation was preceded by months of methodical selection of line isolates that gave robust infection in *An stephensi* mosquitoes.

The use of infected mosquito bites as a vaccine (reviewed in previous section) precipitated the need to treat infected mosquitoes as an investigational product and to treat anything related to culture, husbandry, and feeding

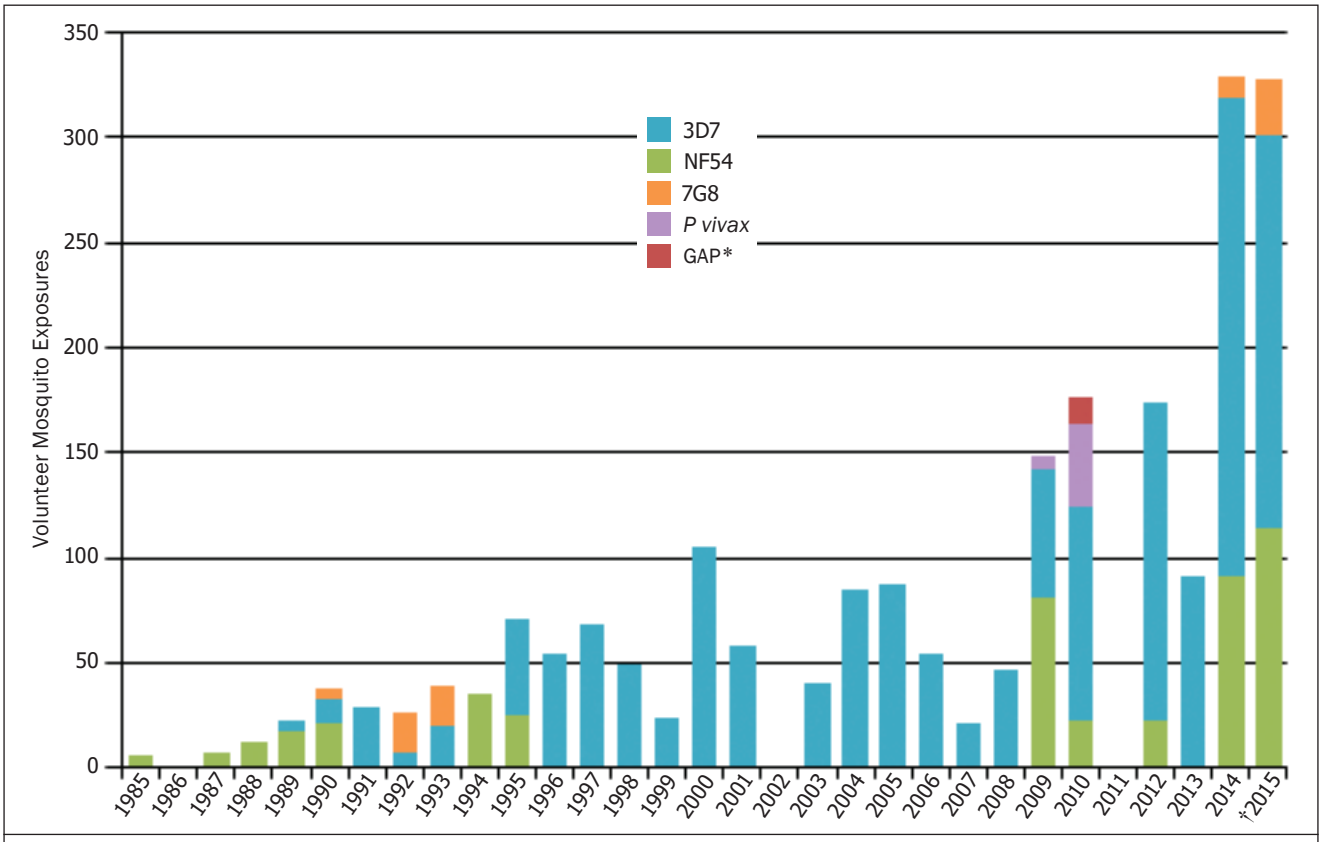

Figure 2. Volunteer mosquito exposures involved in malaria challenges performed by the WRAIR Entomology Department from 1985 through 2015† and distribution of strains of parasites used for those challenges.
*Genetically attenuated parasites.
†Actual and projected volunteer mosquito exposures shown for 2015.

as a manufacturing process. By 2009, production of infected mosquitoes was performed as close to GMP standards as possible for a population of live insects: a library of standard operating procedures were written; the batch master file for parasite production was improved; raw materials and equipment were tracked and certified; forms were added; and each step of the process was documented, reviewed by quality assurance/quality control (QA/QC) personnel, and filed. These methods were extended to traditional challenges and have become standard.

Summary of Challenges Performed

One hundred and four challenges or immunizations by mosquito bite have been performed or are planned through the end of 2015, resulting in over 2200 volunteer mosquito exposures (VME) (some volunteers are counted more than once due to rechallenges or cumulative immunizations on the same person). About half of all challenges have been with the 3D7 strain of *P falciparum* and another 20.5% were with NF54. 7G8, *P vivax*, and genetically attenuated parasites with an NF54 background comprise the remainder (Figure 2). All data is summarized from recordkeeping within the Mosquito Biology/Vector and Parasite Biology department within the Entomology Branch at WRAIR.

While the number of challenges performed by year did not remarkably increase until about 2009, the number of VME per year displays a growth trend throughout the 30 year time line. The average number of VME per year for the 1980s is 9.8, for the 1990s is 43.6, for the 2000s is 64.8, and for 2010 to 2015 is 183, with particularly active years in 2014 and 2015 (recorded and projected) (Figure 2). Increase in demand for challenges reflects, first, advancement of vaccine and drug interventions to clinical trials and, second, tentative success of several vaccine candidates leading to follow-up trials to refine dosage, schedule, and durability. This escalation in activity parallels the advent of many organizations with the mission of controlling malaria, such as the Roll Back Malaria Partnership in 1998, the PATH-Malaria Vaccine Initiative in 1999, The Global Fund in 2002, and the President's Malaria Initiative in 2005. Funding from

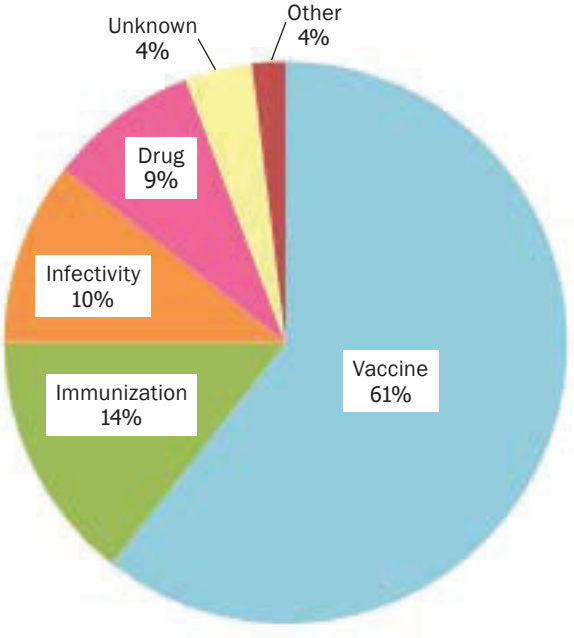

Figure 3. Proportions of challenges performed for indicated purposes.

PATH-MVI in particular has directly influenced the increased demand for the WRAIR challenge model to test their sponsored vaccines.

Vaccines as a malaria control intervention has been by far the most common use for the WRAIR malaria challenge model with 61% of all challenges administered for that reason. Another 14% have used the immunization-by-mosquito-bite, primarily by repeated exposure to radiation attenuated parasites. Verification that the model (or modification) is infectious comprised 10% of all challenges, a prudent step before new parasites or changes are made to the model for testing interventions or immunity. As shown in Figure 3, 9% of challenges have been used for research into experimental therapeutics, and several challenges either served a purpose unknown or unique (eg, test of transport and viability overseas).

To date, WRAIR has performed 28 off-site challenges (27%), both domestically and overseas. The remaining 73% were performed in the WRAIR insectary suite as described in previous sections. Exclusive of challenges performed for Oxford University, nearly 65,000 mosquitoes have been used in the CHMIs summarized here (through February 2015). Over 23,000 were used for the irradiated sporozoite vaccinations in the 1990s and over 27,000 were used in irradiated sporozoite vaccination studies in 2014. These numbers denote the numbers of mosquitoes actually exposed to human volunteers. Exponentially greater numbers of mosquitoes are prepared for QA/QC and to ensure mosquito availability is not a limiting factor for CHMI success. Furthermore, up to 10,000 mosquitoes are produced weekly by the WRAIR insectary to support clinical and preclinical malaria research.

Current and Future Directions of the WRAIR Challenge Model

Although the core of the challenge model has not changed much since the 1980s, the model has improved significantly with new scientific knowledge, applications, technology, and varying needs of users. The next generation of WRAIR challenges anticipates the following variations:

Heterologous Challenge

As vaccine candidates display efficacy against homologous parasites that meets or exceeds the levels called for by the target product profile, demand for heterologous challenges is increasing. NF54 and its derivative, 3D7 are of African origin and serve as the template typically used when designing vaccines. 7G8, a Brazilian isolate, displays a high degree of polymorphism compared to NF54 and 3D7[28] and is, therefore, an excellent heterologous parasite. However, as observed by labs from multiple institutions, 7G8 is unreliably infectious to mosquitoes. An intradepartmental effort at WRAIR to develop new heterologous strains has evaluated a plethora of field-isolated parasite strains for in vitro cultivation and mosquito infection and, to date, has found none to be dually suitable. Ideally, Entomology would possess a library of heterologous strains from around the world such that parasites with different genetic backgrounds could be tested against vaccines, and those with different drug susceptibility profiles could be tested against candidate therapeutics.

Additional species

Concurrently, the need for challenges using non-falciparum Plasmodium species is rising. Entomology anticipates at least one challenge using *P vivax* within the next 2 years and more to follow. This encompasses not only late-stage testing of the breadth of protection offered by *P falciparum* vaccines, but also *P vivax*-specific vaccines currently in research and development. Despite extensive efforts, *P vivax* in vitro culture is nearly impossible and existing workarounds (such as constant addition of purified reticulocytes[29]) are incompatible with the challenge model. *P vivax*-infected mosquitoes can be sourced from AFRIMS, but, as this process uses gametocyte donors, it is not nearly as flexible as what exists for *P falciparum*. Nonhuman primate challenges

(NHP) have also been provided by WRAIR using *P knowlesi*-infected mosquitoes sourced from partners.[30] In the future, full in-house NHP challenges with *P cynomolgi* cycled from NHP to mosquito and back are expected at WRAIR. No plans for *P ovale* or *P malariae* challenges are in place. Each new tweak to a challenge model requires an investigation into which mosquito species (and strain) is the best to vector the target parasite and how well sporozoites can be recovered, both in terms of prevalence and intensity of infection.

Transmission Blocking Interventions

Vaccines and drugs with transmission-blocking potential should be investigated for efficacy at the clinical level, inciting a need for an inverse challenge: a controlled human-to-mosquito malaria transmission that elevates the standard membrane feeding assay to natural transmission dynamics. Arms of volunteers who received a transmission blocking intervention (TBI) or placebo and who acquire malaria would be offered to mosquitoes for feeding and the efficacy of transmission blocking assessed by Plasmodium prevalence and intensity in those mosquitoes. Currently, most TBIs are still in development, but at least one is moving on to Phase I trials employing this type of methodology.

Dengue Human Infection Model (and Others)

Just as entomologists in the 1980s foresaw the need for a way to test candidate malaria vaccines against natural routes of transmission, it is now obvious that virologists will soon need such a way to test candidate dengue vaccines. CHMI has the distinct advantage of using a pathogen that is susceptible to available drugs and can be completely cured by a simple dosing regimen. This is not a characteristic of other vector-borne diseases that need a CHMI-like challenge to properly test vaccine candidates.

A controlled challenge for dengue is the most pressing need, but it would present ethical considerations (ie, if the vaccine is not protective, you can only provide supportive care, not cure, to a volunteer). From the entomological point of view, CHMI presents an excellent template in which to substitute other mosquito-borne pathogens but with careful consideration of where the processes differ biologically. Dengue human infection model (DHIM) requires a different mosquito species, *Aedes aegypti*, which displays high variability in vector competence across strains that is often dependent on the specific virus strain used.[31,32] A suitable *Ae aegypti* strain would have to be validated for every viral strain desired in challenges. Dengue virus prevalence and intensity cannot be determined in real-time similar to the confirmation of malaria sporozoites via light microscopy, so DHIM would rely on either pre- or postscreening of mosquitoes for positive infection. Additionally, the number of bites optimal for guaranteeing dengue transmission while avoiding overwhelming the immune response would require investigation. The feasibility of such a challenge and some theoretical design elements were reviewed by Mores et al.[33]

Summary

Controlled human malaria infection is a powerful tool in antimalarial testing that requires or benefits from mimicking the natural route of infection. All of the leading pre-erythrocytic vaccines have been tested using this model, and even after 30 years its utility is still increasing. Preparing infected mosquitoes for a challenge is a task that forces a complex and tenuous biological interaction into a manufacturing-style operation of precision and predictability. The challenge portion of CHMI as it exists at WRAIR today is the result of decades of research and refinement. Such cumulative effort is reflected not only in how well the challenge has performed historically but also in the ways that it can adapt to answer new questions about malaria and vector-borne disease.

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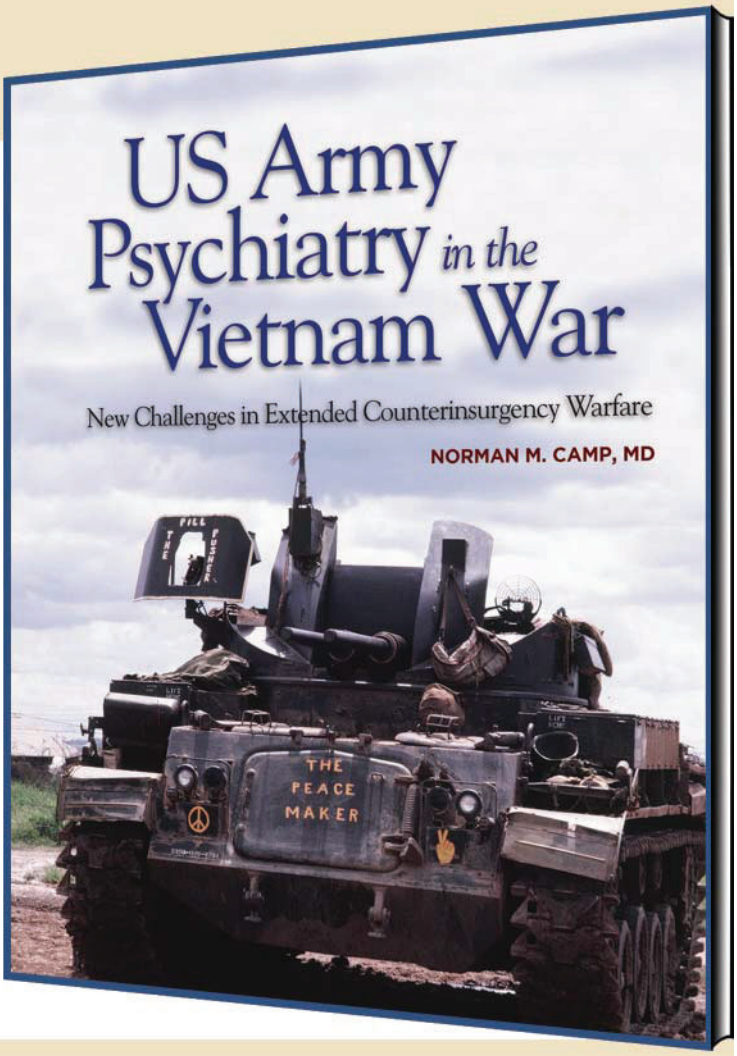
RECENTLY RELEASED

During the Vietnam War (1965–1973), the US Army suffered a severe breakdown in soldier morale and discipline in Vietnam —matters that not only are at the heart of military leadership but also ones that can overlap with the mission of Army psychiatry. The psychosocial strain on deployed solders and their leaders in Vietnam, especially during the second half of the war, produced a wide array of individual and group symptoms that thoroughly tested Army psychiatrists and their mental health colleagues there. In the aftermath of the Vietnam War, the Army Medical Department apparently intended to sponsor a history of Army psychiatry along with other medical specialties, but that project was never begun. This book seeks to consolidate a history of the military psychiatric experience in Vietnam through assembling and synthesizing extant information from a wide variety of sources, documenting the successes and failures of Army psychiatry in responding to the psychiatric and behavioral problems that changed and expanded as the war became protracted and bitterly controversial.



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Mosquito Fauna of Lao People's Democratic Republic, With Special Emphasis on the Adult and Larval Surveillance at Nakai District, Khammuane Province

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Abstract

This article includes the distribution records and updated checklist of mosquitoes (Culicidae, Diptera) from the Lao People's Democratic Republic (PDR), based on the literature, specimens deposited at the US National Museum of Natural History mosquito collections, and our recent field collections from the Nakai District, Khammuane Province. Ten of 101 species in the updated checklist of mosquitoes are new records for the Lao PDR.

Laos People's Democratic Republic (Lao PDR) (18° 00′ N; 105° 00′ E; area 236,800 km^2) is a landlocked Southeastern Asian country, surrounded by 5 countries: Burma, Cambodia, China, Thailand, and Vietnam.[1] These 5 countries, together with the Lao PDR, formed the Greater Mekong Subregion (GMS), which have a combined population of 92 million. Vector-borne diseases have a significant effect on morbidity in these countries, and of these diseases, malaria causes more deaths in remote and border areas.[2,3] In addition to malaria and high heterogeneity in *Plasmodium falciparum* (Welch) risk,[4] dengue, scrub typhus, Japanese encephalitis,[5] and filariasis[6] are common insect-borne diseases in the GMS. However, their effects on human populations are poorly characterized and the taxonomic identities of most vectors should be studied and clarified.

The mosquito fauna of the Lao PDR are not well known, except for several scattered reports.[7-14] In this study, we updated the records and checklist of mosquito species from the Lao PDR based on the literature, specimens deposited at the US National Mosquito Collections (USNMC), National Museum of Natural History (NMNH), Smithsonian Institution, Washington, DC, and our latest specimen collections from Khammuane Province, particularly at the Phou Hin Poun National Biodiversity Conservation Area (PHP NBCA). This area, which has a human population of approximately 30,000, is located in a limestone tower karst region of the Annamite Range in Khammuane Province. It is composed mainly of rugged caves, porous karst terrain, and dry evergreen forest and scrubland. It is also the home to a number of rare or newly discovered species of animals.[15-17] We are in the process of confirming the identification of several species of mosquitoes and sand flies, and possibly describing new species from our recent collections in the area.

Materials and Methods

Mosquito Field Collection, Museum Specimens and Identification

Specimen collections were conducted from May 1 to May 31, 2012, and from February 21 to March 10, 2014, from various areas in the PHP NBCA (17.99524° N, 104.82108° E), Ban Natan, Nakai District, Khammuane Province (Figure 1). Adults were collected using modified Centers for Disease Control and Prevention traps (Figure 2A, B) with light attractants, and were suspended about 1.3 m above ground level on selected sites and inside the caves. Larvae were collected using a standard larval dipper (350 ml, 13 cm diameter: BioQuip, Rancho Dominguez, CA) (Figure 2C, D) from various habitats including water pockets along edges of rivers, rock holes, temporary pools in between rocks, caves, etc (Figures 2, 3, and 4). They were individually link-reared to adult stage, as morphological voucher specimens for this work. Emergent adults were pinned on paper points, each given a unique collection number, properly labeled, and identified using diagnostic morphological characters.[18-23] Voucher specimens were deposited at the USNMC NMNH, Smithsonian Institution, Washington, DC, USA, and at the Entomology Laboratory, Institut Pasteur du Laos, Vientiane, Lao PDR. In addition, old mosquito specimens at the NMNH repository were examined, and their collection data were recorded.

Figure 1. Map of Lao PDR showing 16 provinces and the national capital Vientiane.

Key to Provinces:

AU=Attapu	OU=Oudomxai
BO=Bokeo	PH=Phongsali
BL=Borikhamxay	SA=Saravane
CH=Champasak	SV=Savannakhet
HO=Houaphan	VI=Viangchan
KH=Khammuane	XA=Xayaboury
LO=Louangnamtha	XE=Sekong
LU=Luangprabang	XI=Xiangkhouang

VT indicates national capital city area of Vientiane.

P indicates Phou Hin Poun National Biodiversity Conservation Area, Nakai District where recent collections were conducted.

Provinces or localities where mosquitoes were collected or reported in the literature.

Results

The summary of mosquito collections from the PHP NBCA, Khammuane Province is presented in Table 1. Figure 1 shows the map of the Lao PDR, with 10 of 16 provinces, Vientiane (capital city) and PHP NBCA (all with asterisks as shown in the map) where adult and larval mosquitoes were collected or reported in the literature. In the PHP NBCA, mosquito habitats included water pockets along edges of rivers, rock holes, temporary pools along the edges of rivers, in between rocks, and in caves (Figures 2-4). A total of 43 mosquito taxa were collected from PHP NBCA in 9 genera (*Aedes*, *Anopheles*, *Culex*, *Heizmannia*, *Mansonia*, *Orthopodomyia*, *Topomyia*, *Toxorhynchites*, *Tripteroides*). Among the 3 genera examined, *Aedes* (19 species) had the greatest number of species, followed by *Culex* (8 species) and *Anopheles* (7 species). Only 18 species out of 43 (42%) were morphologically identified, while the rest (25 species; 58%) need further analyses (including molecular techniques) to clarify their taxonomic identities. Known or potential vectors of human infectious diseases were also collected from PHP NBCA, including *Aedes vexans* (Meigan), *Ae albopictus* (Skuse), and several unconfirmed species of *Anopheles (Anopheles)*, *An (Cellia*), *Culex (Culex)*, and *Mansonia*.

An updated checklist of mosquitoes in the Lao PDR (Table 2) includes a total of 101 species. They are in 16 genera, namely *Aedes* (22 species), *Anopheles* (33), *Armigeres* (14), *Coquillettidia* (2), *Culex* (12), *Ficalbia* (1), *Heizmannia* (1), *Hodgesia* (1), *Mansonia* (4), *Mimomyia* (2), *Orthopodomyia* (1), *Topomyia* (1), *Toxorhynchites* (2), *Tripteroides* (2), *Uronotanea* (2), *Verrallina* (1). About 80 of 101 species were reported in the Walter Reed Biosystematics Unit (WRBU) catalog,[23] 2 species found from the Smithsonian/NMNH collections, 17 species from current PHP NBCA collections, and the remaining species from the literature. About 10 species of mosquitoes are new records for the Lao PDR. They include 9 species under 7 subgenera of the genus *Aedes* and one species in the genus *Orthopodomyia* (Table 2).

Comment

The Lao PDR, like other countries comprising the GMS, has a high biodiversity of vector species, a great number of mosquito species complexes, enormous spatial heterogeneity in distribution patterns, and extensive behavioral plasticity both between and within species[2].

In 1934[7] and 1938,[8] *Anopheles* mosquitoes were reported in the Laos PDR (Table 2). In December 1999, malaria vector surveys were carried out by Vythilingam et al[11] in 7 provinces, namely Borikhamxay, Champasak, Luangprabang, Saravane, Savannakhet, Xayaboury, and Sekong, and in the capital city of Vientiane in the Lao PDR. Using bare leg collections from indoors and outdoors from 6 PM to 5 PM, a total of 438 *Anopheles* mosquitoes belonging to 19 species were obtained. Of these, only 3 species were found infected with oocysts, namely *An maculatus* Theobald, *An dirus* Peyton and Harrison, and *An minimus* Theobald. *Anopheles aconitus* Doenitz was the predominant species in the 1999 collection, but its vectorial status was unknown. The prevalence of *Anopheles* and epidemiology of malaria were also reported in the provinces of Xekong[12] and Attapeu.[13,14] In 2014, Hii and Rueda[2] listed 3 species in *Anopheles (Anopheles)* and 20 species in *Anopheles (Cellia)* in the Lao PDR, including known and potential

Table 1. Summary of collected mosquito adults and larvae in Phou Hin Poun NBCA, Ban Natan, Nakai District, Khammuane Province, Lao PDR (17.99524° N, 104.82108° E), from May 1 thru May 31, 2012, and February 21 thru March 10, 2014.

Species	Sex*	Collection no.
Aedes (Aedimorphus) alboscutellatus (Theobald)	3F	LN-048, 050, 060
Aedes (Aedimorphus) sp	1F	LN-012
Aedes (Aedimorphus) vexans (Meigen)	1F	LN-047
Aedes (Bothealla) eldridgei Reinert	3F†	LN-002, 041, 068
Aedes (Bothealla) sp	3F, 1M	LN-018, 022, 023, 069
Aedes (Collessius) sp	1F	LN-013
Aedes (Downsiomyia) ganapathi Colless	1F	LN-024
Aedes (Downsiomyia) harinasutai Knight	1F	LN-001
Aedes (Downsiomyia) sp	1M	LN-008
Aedes (Fredwardsius) vittatus (Bigot)	2F, 1M	LN-015, 065, 066
Aedes (Hulecoeteomyia) chrysolineatus (Theobald)	1F	LN-035
Aedes (Hulecoeteomyia) formosensis Yamada	1F, 1M	LN-036, 037
Aedes (Hulecoeteomyia) sp (near *reinerti* or *formosensis*)	1F, 1M	LN-031, 046
Aedes (Kenknightia) dissimilis (Leicester)	1F	LN-063
Aedes (Kenknightia) sp	1F	LN-044
Aedes (Stegomyia) albopictus (Skuse)	1F	LN-043
Aedes *(Stegomyia) pseudoscutellaris* (Theobald)	1F	LN-039
Aedes (Tewarius) pseudonummatus Reinert	2F	LN-003
Aedes sp	4F, 2M	LN-011, 013, 019, 020, 026, 042
Anopheles (Anopheles) sp (Barbirostris Group)	1F	LN-011
Anopheles (Anopheles) sp (Asiaticus Group)	1F	LN-049
Anopheles (Anopheles) sp	1F	LN-046
Anopheles (Anopheles) sp (Culiciformis Group)	1F	LN-062
Anopheles (Cellia) pseudowillmori Theobald	1F	LN-045
Anopheles (Cellia) sp (Leucosphyrus Group)	1F	LN-005
Anopheles (Cellia) sp	2F	LN-009, 010
Coquillettidia (Coquillettidia) ochracea (Theobald)	1F	LN-004
Culex (Culex) sp (Vishnui Complex)	1F	LN-052
Culex (Culex) sp (Sitiens Group)	1F	LN-054
Culex (Culex) sp	3F	LN-028, 055, 071
Culex (Culex) tritaeniorhynchus Giles	1F	LN-053
Culex (Culiciomyia) nigropunctatus Edwards	2F, 1M	LN-064, 074,075
Culex (Culiciomyia) sp	1F, 1M	LN-067, 070
Culex (Eumelanomyia) sp (Temipalpus Complex)	1F, 2M	LN-017, 072, 073
Culex (Lophoceraomyia) sp	1F, 1M	LN-007, 016
Heizmannia sp	3F	LN-025, 038, 059
Mansonia (Mansonioides) uniformes (Theobald)	1F	LN-057
Mansonia sp	1F	LN-051
Orthopodomyia albipes Leicester	1M	LN-033
Orthopodomyia sp	1F	LN-032
Topomyia sp	1F	LN-030
Toxorhynchites sp	3M†	LN-076, 077, 078
Tripteroides sp	2F, 1M	LN-021, 040, 058

*F indicates female adult, M indicates male adult.
†Larvae collected using plastic larval dipper.

malaria vectors in countries of the Mekong Subregion. While there are numerous examples of *An dirus* mostly feeding outdoors and much earlier in the evening,[24,25] Vythilingam et al[13] reported an unusual stereotypical nocturnal indoor and late feeding behavior in Attapeu province, Laos PDR. In 2002, Tsuda et al[10] conducted an ecological survey of *Aedes* dengue vectors in the central part of the Lao PDR. A new hydroelectric project, Nam Theun 2, created ideal conditions for *Aedes aegypti* (Linnaeus) breeding in water storage jars and tires, and *Ae albopictus* was abundant.[26]

The present study indicates the species diversity of mosquitoes in the Lao PDR. The difficulty in doing morphological comparisons among species warrants further molecular analysis to ascertain taxonomic identities and to clarify hierarchic classifications. With the diversity of the habitats, particularly the caves and surrounding areas, we expect that more unknown species will be collected and described in the near future. Deforestation, water resources and management,[27,28] conventional agricultural practices, and unregulated destruction of many habitats are major human activities that may adversely affect the floral and animal fauna of the Lao PDR, including the creation or elimination of suitable breeding sites of mosquitoes and other arthropods. While habitats in some government protected areas are not hugely damaged yet, continuous inventories of arthropod fauna, particularly those groups (mosquitoes, sand flies, ticks, mites, etc) with known disease vectors, should be conducted to accumulate much needed data for developing strategies to manage and control infectious human diseases. Proper vector surveillance, including ecological surveys, should be performed in areas where human diseases (malaria, dengue, tick-borne viruses, filariasis, etc) are common and severely affect the local human populations. The updated checklist of mosquitoes in this article (including several vector species) may help health personnel in mapping out some risk areas for infectious diseases in the Lao PDR.

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Table 2A. Updated checklist of mosquito species from Lao PDR.

Species	Reference[a]
Aedes (Aedimorphus) alboscutellatus (Theobald)	14, 23, X
Aedes (Aedimorphus) pipersalatus (Giles)	14, 23
Aedes (Aedimorphus) vexans (Meigen)	14, 23, X
Aedes (Bothaella) eldridgei Reinert[b]	X
Aedes (Collessius) macfarlanei (Edwards)	23
Aedes (Diceromyia) iyengari Edwards	14, 23
Aedes (Downsiomyia) ganapathi Colless[b]	X
Aedes (Downsiomyia) harinasutai Knight[b]	X
Aedes (Downsiomyia) niveus (Ludlow)	23
Aedes (Fredwardsius) vittatus (Bigot)	10, X
Aedes (Hulecoeteomyia) chrysolineatus (Theobald)	14, 23, X
Aedes (Hulecoeteomyia) formosensis Yamada[b]	X
Aedes (Hulecoeteomyia) reinerti Rattanarithikul and Harrison[b]	X
Aedes (Kenknightia) dissimilis (Leicester)[b]	X
Aedes (Neomelaniconion) lineatopennis (Ludlow)	19
Aedes (Paraedes) ostentatio (Leicester)	19
Aedes (Phagomyia) prominens (Barraud)[b]	X
Aedes (Stegomyia) albopictus (Skuse)	14, X
Aedes (Stegomyia) aegypti (Linnaeus)	10, 23, M
Aedes (Stegomyia) pseudalbopictus Borel	14, 23
Aedes (Stegomyia) pseudoscutellaris (Theobald)[b]	X
Aedes (Tewarius) pseudonummatus Reinert[b]	X
Anopheles (Anopheles) albotaeniatus (Theobald)	11
Anopheles (Anopheles) argyropus (Swellengrebel)	2
Anopheles (Anopheles) baileyi Edwards	23
Anopheles (Anopheles) barbirostris Van der Wulp	2, 8, 11, 14, 23
Anopheles (Anopheles) donaldi Reid	2, 14, 23
Anopheles (Anopheles) sinensis Wiedemann	3, 8
Anopheles (Anopheles) umbrosus (Theobald)	12
Anopheles (Cellia) aconitus Doenitz	2, 7, 11, 14, 23
Anopheles (Cellia) annularis Van der Wulp	2
Anopheles (Cellia) culicifacies Giles	2, 7, 23
Anopheles (Cellia) dirus Peyton and Harrison	2, 10, 11, 12, 23
Anopheles (Cellia) dravidicus Christophers	2, 14, 23
Anopheles (Cellia) harrisoni Harbach and Manguin	23
Anopheles (Cellia) indefinitus (Ludlow)	2, 23
Anopheles (Cellia) jamesii Theobald	2, 23
Anopheles (Cellia) jeyporiensis James	2, 7, 8, 12, 23
Anopheles (Cellia) karwari (James)	2, 11, 12, 23
Anopheles (Cellia) kochi Donitz	2, 8, 11, 14, 23
Anopheles (Cellia) maculatus Theobald	2, 7, 8, 11, 12, 14, 23
Anopheles (Cellia) minimus Theobald	2, 8, 11, 12, 14, 23
Anopheles (Cellia) nivipes (Theobald)	11, 12, 14, 23
Anopheles (Cellia) notanandai Rattanarithikul and Green	2, 14, 23
Anopheles (Cellia) pallidus Theobald	11, 12

[a] X indicates observed, field collection; M indicates observed, Smithsonian/National Museum of Natural History museum collection.
[b] New record for Lao PDR.

Table 2B. Updated checklist of mosquito species from Lao PDR (continued).

Species	Reference[a]
Anopheles (Cellia) pampanai Buttiker and Beales	11, 14, 23
Anopheles (Cellia) philippinensis Ludlow	2, 8, 11, 12, 14, 23
Anopheles (Cellia) pseudowillmori Theobald	2, 14, 23, X
Anopheles (Cellia) rampae Harbach and Somboon	29
Anopheles (Cellia) sawadwongporni Rattanarithikul and Green	14, 23
Anopheles (Cellia) splendidus Koidzumi	11, 12, 14, 23
Anopheles (Cellia) subpictus Grassi	2, 11
Anopheles (Cellia) sundaicus (Rodenwaldt)	2
Anopheles (Cellia) tessellatus Theobald	2, 11, 14, 23
Anopheles (Cellia) vagus Donitz	2, 7, 8, 11, 12, 14
Anopheles (Cellia) varuna Iyengar	2, 11, 12, 14, 23
Armigeres (Armigeres) aureolineatus (Leicester)	23
Armigeres (Armigeres) durhami (Edwards)	23
Armigeres (Armigeres) kuchingensis Edwards	23
Armigeres (Armigeres) laoensis Toma and Miyagi[c]	23, M
Armigeres (Armigeres) moultoni Edwards	23
Armigeres (Armigeres) setifer Delfinado	14, 23
Armigeres (Armigeres) subalbatus (Coquillett)	14, 23
Armigeres (Armigeres) theobaldi Barraud	14, 23
Armigeres (Leicesteria) annulitarsis (Leicester)	23
Armigeres (Leicesteria) dolichocephalus (Leicester)	23
Armigeres (Leicesteria) flavus (Leicester)	23, M
Armigeres (Leicesteria) longipalpis (Leicester)	23
Armigeres (Leicesteria) magnus (Theobald)	23
Armigeres (Leicesteria) pectinatus (Edwards)	23
Coquillettidia (Coquillettidia) crassipes (Van der Wulp)	14, 23
Coquillettidia (Coquillettidia) ochracea (Theobald)	23, X
Culex (Culex) fuscocephala Theobald	14, 23
Culex (Culex) gelidus Theobald	23
Culex (Culex) hutchinsoni Barraud	14, 23
Culex (Culex) pseudovishnui Colless	14, 23
Culex (Culex) quinquefasciatus Say	14, 23, M
Culex (Culex) tritaeniorhynchus Giles	14, 23, X
Culex (Culex) vishnui Theobald	14, 23
Culex (Culex) whitmorei (Giles)	14, 23
Culex (Culiciomyia) nigropunctatus Edwards	14, 23, X
Culex (Oculeomyia) bitaeniorhynchus Giles	14, 23
Culex (Oculeomyia) pseudosinensis Colless	14, 23
Culex (Oculeomyia) sinensis Theobald	14, 23
Ficalbia minima (Theobald)	23
Heizmannia (Heizmannia) complex (Theobald)	23
Hodgesia malayi Leicester	23

[a] X indicates observed, field collection; M indicates observed, Smithsonian/National Museum of Natural History museum collection.
[c] Holotype male, 1 paratype female, 4 females, 3 whole larvae, and 3 larval exuviae, deposited in the Smithsonian/National Museum of Natural History museum collection.

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Table 2C. Updated checklist of mosquito species from Lao PDR (continued).

Species	Reference[a]	Species	Reference[a]
Mansonia (Mansonioides) annulifera (Theobald)	14, 23	*Toxorhynchites (Toxorhynchites) albipes* (Edwards)	23
Mansonia (Mansonioides) dives (Schiner)	14	*Toxorhynchites (Toxorhynchites) kempi* (Edwards)	23
Mansonia (Mansonioides) indiana Edwards	14, 23	*Tripteroides (Rachionotomyia) aranoides* (Theobald)	23
Mansonia (Mansonioides) uniformes (Theobald)	14, 23, X	*Tripteroides (Rachionotomyia) ponmeki* Miyagi and Toma	9, 23
Mimomyia (Mimomyia) chamberlaini Ludlow	23	*Uranotaenia (Pseudoficalbia) nivipleura* Leicester	23, M
Mimomyia (Mimomyia) hybrida (Leicester)	23	*Uranotaenia (Pseudoficalbia) novobscura* Barraud	23, M
Orthopodomyia albipes Leicester[b]	X	*Verrallina (Verrallina) dux* (Dyar and Shannon)	23
Topomyia (Topomyia) gracilis Leicester	23		

[a] X indicates observed, field collection; M indicates observed, Smithsonian/National Museum of Natural History museum collection.
[b] New record for Lao PDR.

Figure 2. Nakai District cave showing mosquito adult wall resting areas (A, D) and larval habitats inside the cave (B, C). A modified light trap hung from the cave wall (D at arrow). Samples were obtained from the cave water pocket (C at arrow) using a larval dipper (C inset).

Figure 3. Nakai District river showing mosquito larval habitats along river edge (A), and in rock holes (B, C, D).

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Figure 4. Nakai District river and tributaries showing typical mosquito larval habitats including river edge with floating grasses (A, B), water pocket (C inset), and temporary water pool (D).

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Erratum

In the article "A Heart Gripping Case: Carcinoid Heart Disease" published on pages 93-96 of the January-March 2015 issue of the *AMEDD Journal*, the byline entry "Capt John P. Magulik" is incorrect. The correct byline entry is "Capt John P. Magulick."

The article "Performance Differences Between Male and Female Marines on Standardized Physical Fitness Tests and Combat Proxy Tasks: Identifying the Gap" appearing on pages 12-21 in the print edition of the April-June 2015 issue of the *AMEDD Journal* has been retracted by the authors. The article does not appear in the online digital version of that issue, nor does data describing the article appear in the PubMed MEDLINE record database.

Records and Distribution of New World Phlebotomine Sand Flies (Psychodidae, Diptera), With Special Emphasis on Primary Types and Species Diversity

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Abstract

This article includes the records and distribution of Phlebotomine sand flies (Psychodidae, Diptera) in the New World based on the specimen collections housed in 2 repositories, the US National Museum of Natural History and the Museum of Entomology, Florida State Collection of Arthropods. Approximately 128 species have primary types housed in the 2 repositories, including holotypes (47 species, 3 subspecies), "types" (7 species), allotypes (52 species, 6 subspecies), lectotypes (4 species), paratypes (93 species, 10 subspecies), and neoallotype (1 species), mounted on slides, with a total of 1,107 type slides. For species diversity, collection data from 24 countries in the sand fly database were analyzed according to the number of species present, specimen records, decade of collections, and countries where collections were conducted.

Phlebotomine sand flies (Subfamily Phlebotominae, Family Psychodidae, Order Diptera) are of major health importance because they are capable of transmitting pathogens, including protozoans (*Leishmania*), bacteria (*Bartonella*), and viruses (Phleboviruses, sand fly fever).[1] Like mosquitoes, only female sand flies, particularly species of *Phlebotomus* and *Lutzomyia*, suck blood, including humans. Species of *Sergentomyia* species primarily feed on reptiles, and rarely bite man.[2] Of approximately 900 sand fly species, only about 70 species are capable of transmitting protozoan *Leishmania* parasites that cause visceral leishmaniasis (kala-azar) and various forms of cutaneous leishmaniasis (oriental sore, espundia, etc.) in man.[3,4] A few sand fly species have been associated with Phlebovirus and other viruses,[3-6] and only one, *Lutzomyia verrucarum* (Townsend) *sensu lato*, can transmit the bacterium *Bartonella bacilliformis* (Strong, Tyzzer, Brues, Sellards and Gastiaburu) causing bartonellosis (Oroya fever, Carrion's disease) in the Andean Region of South America.[7,8] Ready[9] reviewed the biology of Phlebotomine sand flies as vectors of disease agents, including the transmission cycles of human leishmaniasis both in the Old and New Worlds, mostly in rural communities. Additional Phlebotomine reviews also focused on sand fly biology,[10] and emphasis on leishmaniasis control.[11]

Leishmaniasis has a great impact on military operations, particularly those of the United States.[12] Since World War II, more than 1,000 US service personnel were infected with cutaneous leishmaniasis.[13] In Afghanistan (Operation Enduring Freedom, OEF) and Iraq (Operation Iraqi Freedom, OIF), more US soldiers have been exposed to significant leishmaniasis risk than any time since World War II. During the disease surveillance period from 2001-2006, there were 1,287 incident diagnoses/reports of leishmaniasis, both cutaneous (1,283 cases) and visceral (4 cases) forms, among OEF/OIF deployers.[13] Furthermore, in an effort to establish the Leishmaniasis Control Program (LCP) during OIF, US military entomologists conducted comprehensive phlebotomine sand fly surveillance at Tallil Air Base (TAB), Iraq from April 2003-November 2004. They determined the biology and temporal distribution of sand flies at TAB, and noted the impact of sand fly vectors on military operations, including the leishmanial threat to deployed troops in Iraq.[14-16]

The phlebotomine sand flies are found between 50° N and 40° S, with the majority distributed in the tropics and subtropics, and none reported on Pacific Islands or in New Zealand. In the Old World, the anthropophilic *Phlebotomus* sand flies (and principally Leishmaniasis transmission) are confined in the subtropics (particularly in dry, semiarid areas), with a few human biting species in Africa south of the Sahara and none in Southeast Asia (although *Phlebotomus* species are found). In the New World (Nearctic and Neotropical Regions), the

Figure 1. Phlebotomine sand fly collection sites on the New World, based on specimens deposited in the USNMNH and MEFSCA.

transmission of leishmaniasis is mainly in the tropics (particularly in the forests and savanna areas) of South America.[2]

In this article, we examine the types and related specimens of New World Phlebotomine sand flies housed in the US National Museum of Natural History (USNMNH), and those borrowed from the Museum of Entomology, Florida State Collection of Arthropods (MEFSCA). We record the collection data of sand flies, including their geographical distribution, past and present taxonomic arrangement and related information. The species occurrence and diversity of these sand flies, according to the number of collections for each country over certain periods, were analyzed and reported. Other collection or occurrence data of sand fly specimens (including non-types, from the Nearctic and Neotropical Regions) from the 2 repositories (USNMNH and MEFSCA) were also examined and recorded, and will be posted later to the Walter Reed Biosystematics Unit (WRBU)/VectorMap

website (www.vectormap.org).[17] They may be helpful in developing world sand fly taxonomic catalogs, and in creating sand fly vector risk maps and prediction distribution models for WRBU/VectorMap. In addition to increasing the knowledge of sand fly distribution, the collection holdings in these repositories, particularly the primary types, will assist future phlebotomine researchers in their taxonomic and related studies.

Materials and Methods

Species Types and Related Specimens

New World Phlebotomine sand fly specimens used in this study are either housed in the USNMNH repository in Suitland, MD, or were borrowed from the MEFSCA in Gainesville, FL. The slide mounted specimens (about 10,000 slides in more than 300 slide boxes) were examined and their collection data were recorded. All collection data from both repositories were entered into the USNMNH/MEFSCA database. They were processed and used for analyses in this article. The primary types (holotypes, allotypes, neoallotype, paratypes, metatypes) of sand flies were examined for collection records and related information.

Other sand fly slides (more than 3,000 slides, mainly from Afrotropical and Palearctic Regions) housed in 5 other repositories were also examined and their collection or occurrence data were also recorded in separate databases. Those sand fly repositories included Institut de Reserche pour le Developpement, Montpellier, France; Institut Pasteur, Paris, France; Museum National d'Historie Naturelle, Paris, France; and Royal Museum for Central Africa, Tervuren, Belgium. However, data from the above 5 repositories were not included in this article, but may be processed for another report.

Species Diversity

The number of sand fly species according to each country in the New World was compiled in MS Excel and maps were constructed in ArcMap 10.1 (ESRI, Redlands, CA). Georeferences for individual specimens were determined and uncertainty calculated using the point-radius method.[18-20] Label data from each specimen was recorded verbatim and entered into an Excel spreadsheet. These text descriptions were then assigned coordinates using a web-based gazetteer.[21] For named places, the geographic center of the locality was used as the latitude and longitude anchor. Once the coordinates were established, a measurement of uncertainty was calculated for each point. This measurement is defined as the radius of a circle surrounding the coordinate anchor, indicating that the collection site is within this circle. The uncertainty measurement takes into consideration errors involving the extent of the named place, the geographic datum, map scale, and imprecision of collectors' location descriptions. All information including the verbatim locality description, gazetteer results and geo-referencing calculations were recorded and will be available for user review via VectorMap.[17] The sand fly data from our database (USNMNH and MEFSCA) were sorted and ranked according to: (*a*) number of species per country, (*b*) number of collection records per species, and (*c*) number of records by decade of collections.

Results

Species Types

The list of New World Phlebotomine species with type specimens housed in the USNMNH and MEFSCA is shown in Table 1, using the new taxonomic arrangements.[22,23] The number of slides for each type and species and the country of type origin are also included in Table 1 at the end of this article. A comparison of the new[22,23] and old[4] generic and subgeneric classifications of types at both repositories is shown in Table 2 at the end of this article. About 139 species have primary types housed in those 2 repositories, including holotypes (49 species, 3 subspecies), "types" (8 species) , allotypes (51

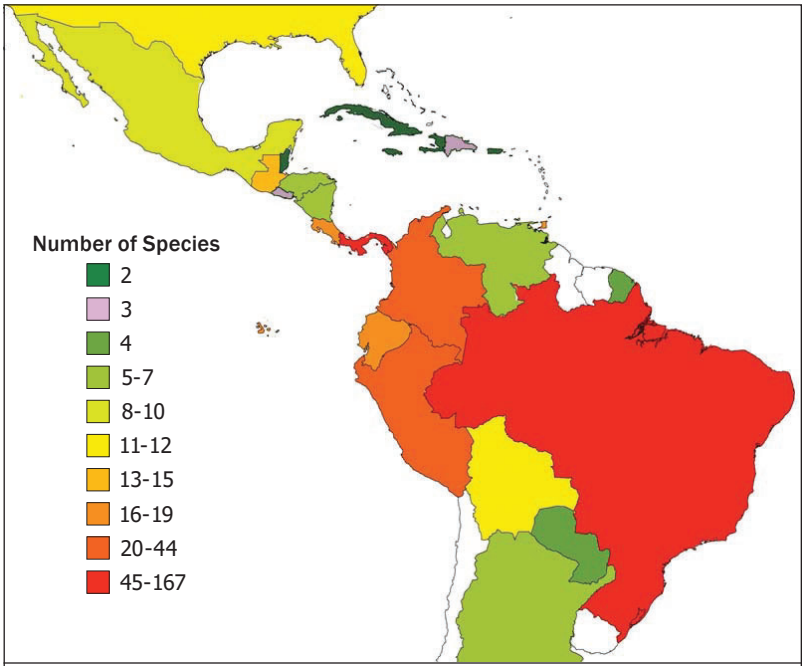

Figure 2. Map of the New Word showing the number of New World Phlebotomine sand fly species in the USNMNH and MEFSCA sand fly database according to country of collection.

species, 6 subspecies), paratypes (93 species, 10 subspecies), lectotypes (4 species), neoallotype (1 species), and metatype (1 species), mounted on slides, with a total of 1,113 type slides. The number of paratypes (103) ranged from 1 to 53/species or subspecies, with a total of 917. Those specimens on slides labeled "Type" could be considered as holotypes, but proper designations should be done later. Attempts to check for any additional primary types housed in the MEFSCA are still ongoing, and they will be listed in separate reports, if any types are found later. Species, without type specimens, will be posted later on the VectorMap website.[17]

Species Diversity

The total number of specimen slides considered in this database numbered 2,743. Depending on the amount of location detail, georeference uncertainty ranged from highest (country only information recorded) through to lowest (country, province and village information recorded), with an average of 234,678 m (n=2,573). Twenty-four countries of the New World were represented in our database of species collection record. The maps showing the collections sites in the New World countries, based on the specimens housed in the USNMNH and MEFSCA, are presented in Figures 1 and 2. They include (from smallest to largest number of species occurring in the collection database): Belize=Cuba=Haiti=Jamaica=Puerto Rico (2 species)<Dominican Republic=El Salvador (3)<French Guyana=Paraguay(4)<Nicaragua=Argentina=Honduras=Venezuela (5)<Mexico (10)<Bolivia=United States (12)<Guatemala (15)<Ecuador=Trinidad and Tobago (17)<Costa Rica (19)<Peru (22)<Colombia (44)<Panama (77)<Brazil (167). These New World countries, with the top 4 countries (Brazil, Panama, Colombia, Peru) in the ranks according to the number of sand fly species present in the USNMNH/MEFSCA database, are shown in Figure 3. When the sand fly collection data were further analyzed according to the number of records present in the database, *Psychodopygus geniculatus* (Mangabeira) was the dominant species, followed by *Nyssomyia ylephiletor* (Fairchild and Hertig) and *Lutzomyia panamensis* (Shannon) (Figure 4). The number of New World sand fly records in the database according to the decade of collections is shown in Figure 5. Most collections were done during the 1950s followed by the 1970s then the 1940s. The earliest collection was done during 1906 and no collections in the database occurred beyond 1986. Based on the number of sand fly species in the database according to the country of collections, Brazil has the greatest number of species (167), followed by Panama (77), Colombia (44), and Peru (22) (Figure 3). Mapping species numbers by countries reveals that the greatest diversity appears to occur around equatorial regions (Figures 1 and 2). When the primary types were considered, according to the country of occurrence, Panama has the greatest number of primary types (21 species with holotypes), followed by Brazil (19 spp) and Colombia (7 spp) (Table 1).

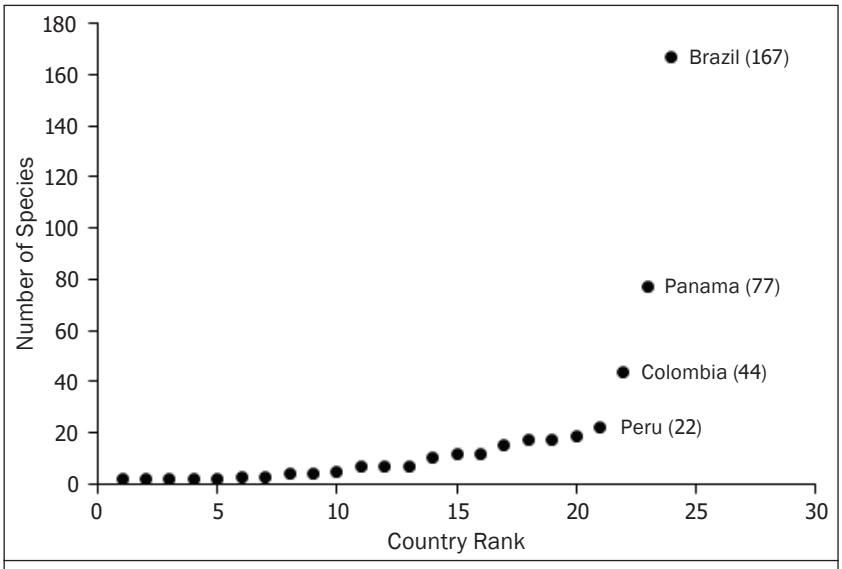

Figure 3. New World countries ranked according to the number of Phlebotomine sand fly species present in the USNMNH and MEFSCA sand fly database. The top 4 countries (and number of species) in the database are identified.

Comment

The Phlebotomine sand fly collections, including primary types and voucher specimens, are essential for vector identifications, surveillance, and control efforts. The USNMNH and MEFSCA have voucher specimens of 7 (of 8) major species incriminated as vectors[9] of various *Leishmania* species involved in the transmission of human leishmaniasis in the New World. Twenty of 27 suspected vector species[9] of *Leishmania* in the New World have types and/or voucher specimens deposited in the 2 repositories. For example, the holotypes or "types" of the suspected vectors, namely *Nyssomyia anduzei* (Rozeboom), *Psychodopygus panamensis* (Shannon), and *Lutzomyia diabolica* (Hall), are found in the USNMNH. Considering the distribution records (mostly from 1940 to 1970) at our database (USNMNH/MEFSCA), there is an urgent need for additional collections of New World sand flies to obtain fresh voucher specimens for both molecular and morphological studies, and for safe deposits in the USNMNH, MEFSCA, or local Neotropical country repositories.

Specimen collections from USNMNH and MEFSCA were used in developing

the LUCID[24] interactive keys for the New World Neotropical Phlebotomine sand flies, particularly Neotropical Region (South and Central America, Southern Command, SOUTHCOM). Twenty-four morphological keys for males and females of the Neotropical Region (South and Central America), were created by L. M. Rueda with assistance from the WRBU staff, particularly J. Stoffer for the Automontage images which are now posted at the WRBU website,[25] namely:

- Phlebotomine Sand Fly Genera, Neotropical (SOUTHCOM): Females, Males
- Phlebotomine Sand Flies, Subgenera, Neotropical (SOUTHCOM): Females, Males
- Subgenus *Dampfomyia* Sand Flies, Adults, Neotropical (SOUTHCOM): Females, Males
- Subgenus *Evandromyia* Sand Flies, Adults, Neotropical (SOUTHCOM): Females, Males
- Subgenus *Helcocyrtomyia* Sand Flies, Adults, Neotropical (SOUTHCOM): Females, Males
- Subgenus *Lutzomyia* Sand Flies, Adults, Neotropical (SOUTHCOM): Females, Males
- Subgenus *Nyssomyia* Sand Flies, Adults, Neotropical (SOUTHCOM): Females, Males
- Subgenus *Pintomyia* Sand Flies, Adults, Neotropical (SOUTHCOM): Females, Males
- Subgenus *Psathyromyia* Sand Flies, Adults, Neotropical (SOUTHCOM): Males
- Subgenus *Psychodopygus* Sand Flies, Adults, Neotropical (SOUTHCOM): Females, Males
- Subgenus *Sciopemyia* Sand Flies, Adults, Neotropical (SOUTHCOM): Females, Males
- Species Grp. Verrucarum Sand Flies, Adults, Neotropical (SOUTHCOM): Females, Males
- Subgenus *Trichophoromyia* Sand Flies, Adults, Neotropical (SOUTHCOM): Males

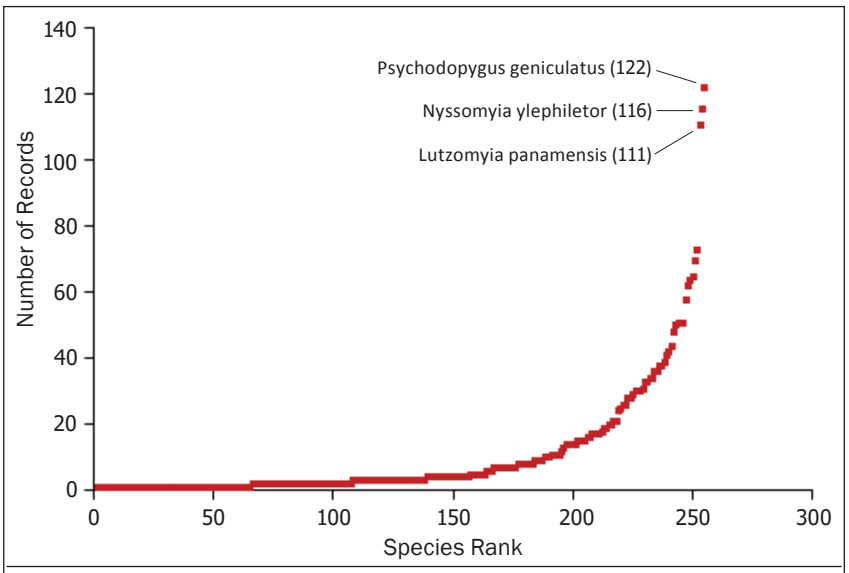

Figure 4. New World Phlebotomine sand fly species ranked according to the number of records present in the USNMNH and MEFSCA sand fly database. The top 3 most common species (and number of records) in the database are identified.

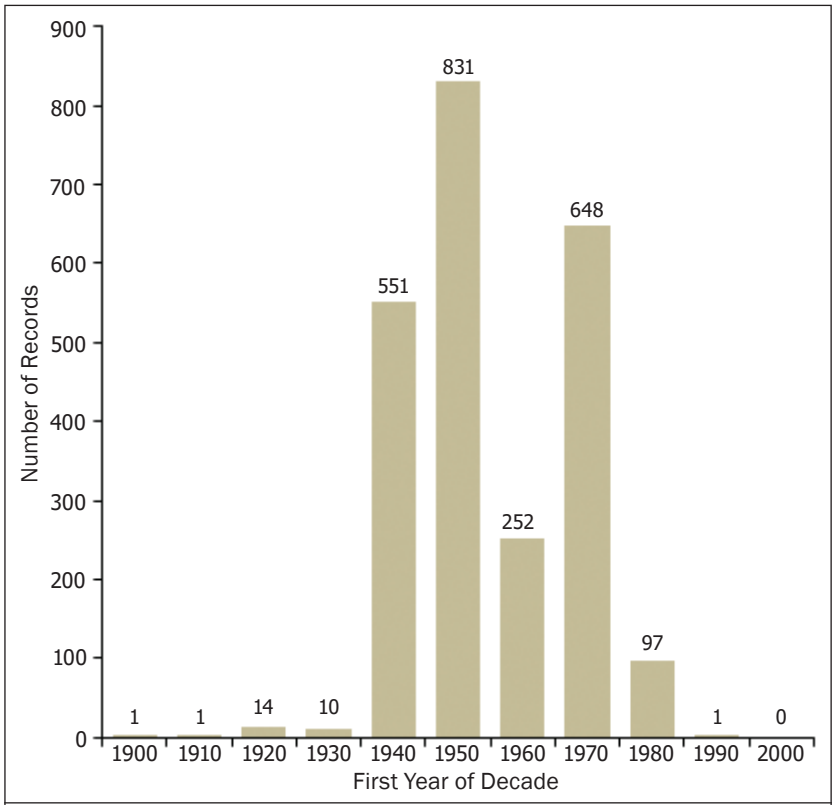

Figure 5. Number of New World Phlebotomine sand fly specimen records in the USNMNH and MEFSCA sand fly database according to decade of collection.

The old arrangement of sand fly taxa by Young and Duncan[4] was followed in the above keys. Other interactive keys for Africa and Central, Eastern, and Southwest Asia are still in preparation by the WRBU staff.

Concerning species diversity, a latitudinal biodiversity gradient was observed for mosquitoes, with species richness increasing toward the equator.[26] For mosquitoes, the total number of species increases with geographic area, according to a linear log-log relationship, and island

countries are more species-rich and have a higher number of endemic species than do mainland countries. With 17 sand fly taxa in the USNMNH/MEFSCA sand fly database, Trinidad-Tobago is the most species-rich. Foley et al[26] also found that this country is species-rich for mosquitoes, even in comparison with other island nations. As in mosquitoes, there appears to be little relationship (ie, no shared species) between the sand fly fauna in the database for Trinidad-Tobago and Venezuela (the closest continental area), despite these countries having been joined during the Pleistocene.[27] Separating the effects of sampling effort, taxonomic output and species richness may be difficult, as a species-rich or endemic area will initially result in higher numbers of new species per sampling effort, and may attract the greatest sampling effort. According to Foley et al,[26] Brazil, Panama, French Guiana, and Costa Rica had the highest number of mosquito species, including endemic species. With the exception of French Guiana, this pattern is also seen for sand flies from the USNMNH/MEFSCA database. The list of New World countries with above average species-level mosquito taxonomic output (type locations, taxonomic publications) included El Salvador, Venezuela, Brazil, Ecuador, Guatemala, Costa Rica, Panama, French Guiana, Belize, and Trinidad-Tobago, while Haiti and Uruguay were below average.[26] The numbers of sand fly species in the USNMNH/MEFSCA database from Haiti and Uruguay were also low, possibly reflecting a similar lack of taxonomic output. A number of assumptions and limitations are inherent in the present study. For example, Hijmans et al[28] identified 4 types of bias that could apply in the present case, namely species bias (eg, oversampling species of sand flies due to greater abundance); species-area bias (eg, oversampling island endemics compared with mainland species); hotspot bias (eg, oversampling areas where previous studies indicated a high species richness); and infrastructure bias (eg, oversampling near roads and towns).

Recently, about 12,000 slides of Phlebotomine sand flies were donated by retired COL Philip Lawyer to the USNMNH for safekeeping. These slides were temporarily mounted using Hoyer's medium, and should be remounted permanently. Their locality data from slide labels and collection sheets will be retrieved and recorded. Additional collection data from other regions of the world (including Old World countries) will be loaded into VectorMap to enable further analysis of species diversity, and to create sand fly vector distribution models that will be useful for leishmaniasis risk assessments.

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Table 1A. Types of New World sand flies (Phlebotominae, Psychodidae) deposited in the USNMNH and MEFSCA, including country of type origin (continued through 1B, 1C, 1D).

Species	Repository Type* (No. of Slides)	Country of Type Origin†
Bichromomyia olmeca olmeca (Vargas and Najera 1959)	F: P(1)	Brazil
Bichromomyia olmeca bicolor (Fairchild and Theodor 1971)	F: H, A, P(44)	Panama
Bichromomyia olmeca nociva (Young and Arias 1982)	F: A, P(14); U: P(10)	Brazil
Brumptomyia galindoi (Fairchild and Hertig 1947)	F: H	Panama
Brumptomyia hamata (Fairchild and Hertig 1947)	F: H, A, P(1)	Panama
Brumptomyia leopoldoi (Rodriguez 1953)	F: P(3)	Panama
Dampfomyia (Coromyia) steatopyga (Fairchild and Hertig 1958)	F: P(2); U: P(1)	Mexico
Dampfomyia (Coromyia) vesicifera (Fairchild and Hertig 1947)	F: H, A, P(8); U: P(2)	Panama
Dampfomyia (Coromyia) vespertilionis (Fairchild and Hertig 1947)	F: H, A, P(24)	Panama
Dampfomyia (Coromyia) viriosa (Fairchild and Hertig 1958)	F: P(1); U: P(1)	Costa Rica
Dampfomyia (Coromyia) zeledoni Young and Murillo 1984	F: H, P(1)	Costa Rica
Dampfomyia (Dampfomyia) anthophora (Addis 1945)	U: P(1)	United States
Dampfomyia (Dampfomyia) rosabali (Fairchild and Hertig 1956)	F: P(2)	Panama
Dampfomyia (Incertae sedis) caminoi (Young and Duncan 1994)	F: H,A,P(1)	Mexico
Evandromyia (Aldamyia) sericea (Floch and Abonnenc 1944)	U: H	Brazil
Evandromyia (Aldamyia) williamsi (Damasceno, Causey and Arouck 1945)	U: H	Brazil
Evandromyia (Evandromyia) begonae (Ortiz and Torres 1975)	U: P(1)	Brazil
Evandromyia (Evandromyia) inpai (Young and Arias 1977)	F: P(11)	Brazil
Evandromyia (Evandromyia) wilsoni (Damasceno and Causey 1945)	U: H	Brazil
Hertigia hertigi Fairchild 1949	F: A	Costa Rica
Lutzomyia (Helcocyrtomyia) botella (Fairchild and Hertig 1961)	F: H, P(6)	Panama
Lutzomyia (Helcocyrtomyia) cirrita Young and Porter 1974	F: A, P(5)	Colombia
Lutzomyia (Helcocyrtomyia) hartmanni (Fairchild and Hertig 1957)	F: H, P(10)	Panama
Lutzomyia (Helcocyrtomyia) imperatrix (Alexander 1944)	U: H	Peru
Lutzomyia (Helcocyrtomyia) noguchii (Shannon 1929)	U: T	Peru
Lutzomyia (Helcocyrtomyia) peruensis (Shannon 1929)	U: T	Peru
Lutzomyia (Helcocyrtomyia) pescei (Hertig 1943)	U: L	Peru
Lutzomyia (Helcocyrtomyia) strictivilla Young 1979	F: A, P(6)	Colombia
Lutzomyia (Helcocyrtomyia) tortura Young and Rogers 1984	F: A	Ecuador
Lutzomyia (Incertae sedis) tanyopsis Young and Perkins 1984	F: P(1)	United States
Lutzomyia (Lutzomyia) battistinii (Hertig 1978)	U: L, P(2)	Peru
Lutzomyia (Lutzomyia) lichyi (Floch and Abonnenc 1950)	F: A, P(6)	Panama
Lutzomyia (Tricholateralis) carvalhoi (Damasceno, Causey and Arouck 1945)	U: H	Brazil
Lutzomyia (Tricholateralis) cruciata (Coquillett 1907)	U: T	Guatemala
Lutzomyia (Tricholateralis) diabolica (Hall 1936)	U: T	United States
Lutzomyia (Tricholateralis) falcata Young, Morales and Ferro 1994	F: P(7)	Brazil
Lutzomyia (Tricholateralis) marinkellei Young 1979	F: A, P(3)	Colombia
Martinsmyia gasparviannai Martins, Godoy and Silva 1962	F: P(1)	Brazil
Martinsmyia waltoni Arias, Freitas and Barrett 1984	F: P(2); U: P(2)	Brazil
Micropygomyia (Coquillettimyia) apache (Young and Perkins 1984)	F: A, P(2)	United States
Micropygomyia (Coquillettimyia) stewarti (Mangabeira and Galindo 1944)	F: H; U: P(1)	United States
Micropygomyia (Coquillettimyia) vexator (Coquillett 1907)	U: H	United States
Micropygomyia (Micropygomyia) cayennensis cayennensis (Floch and Abonnenc 1941)	F: P(3)	Guatemala
Micropygomyia (Micropygomyia) cayennensis hispaniolae (Fairchild and Trapido 1950)	U: P(2)	Dominican Republic
Micropygomyia (Micropygomyia) cayennensis jamaicensis (Fairchild and Trapido 1950)	F: H, A, P(1)	Jamaica
Micropygomyia (Micropygomyia) cayennensis maciasi (Fairchild and Hertig 1948)	F: (P1); U: (P1)	Mexico

*Key to Repository Type:
H=holotype (1 specimen) A=allotype (1 specimen)
F=MEFSCA P=paratype (1 or more specimens)
N=neoallotype (1 specimen) T=Type (1 specimen)
U=USNMNH

†Based on types and repositories, as listed in adjacent column.

Table 1B. Types of New World sand flies (Phlebotominae, Psychodidae) deposited in the USNMNH and MEFSCA, including country of type origin (continued).

Species	Repository Type* (No. of Slides)	Country of Type Origin†
Micropygomyia (Micropygomyia) cayennensis puertoriciensis (Fairchild and Hertig 1948)	F: A, P(5); U: P(1)	Puerto Rico
Micropygomyia (Micropygomyia) cayennensis viequesensis (Fairchild and Hertig 1948)	F: H, A, P(4); U: P(2)	Puerto Rico: H, A, P(4); Panama: P(2)
Micropygomyia (Micropygomyia) cubensis (Fairchild and Trapido 1950)	F: H, A, P(5); U: P(1)	Cuba
Micropygomyia (Micropygomyia) duppyorum (Fairchild and Trapido 1950)	F: A, P(6); U: P(2)	Jamaica
Micropygomyia (Micropygomyia) hispaniolae (Fairchild and Trapido 1950)	F: A, P(8)	Dominican Republic: A, P(5); Haiti: P(3)
Micropygomyia (Micropygomyia) pilosa (Damasceno and Causey 1944)	U: H	Brazil
Micropygomyia (Micropygomyia) xerophila (Young, Brener, and Wargo 1983)	F: A, P(10); U: P(1)	United States
Micropygomyia (Sauromyia) atroclavata (Knab 1913)	U: P(1)	Trinidad and Tobago
Micropygomyia (Sauromyia) ferreirana (Barretto, Martins, and Pellegrino 1956)	U: H	Brazil
Micropygomyia (Sauromyia) quechua (Martins, Llanos, and Silva 1975)	F: A, P(1)	Peru
Micropygomyia (Sauromyia) quinquefer (Dyar 1929)	U: T, A	Argentina
Migonemyia (Blancasmyia) cerqueirai (Causey and Damasceno 1945)	U: H	Brazil
Migonemyia (Blancasmyia) gorbitzi (Blancas 1959)	F: A, P(50)	Panama
Nyssomyia anduzei (Rozeboom 1942)	U: H	Venezuela
Nyssomyia trapidoi (Fairchild and Hertig 1952)	F: H, A, P(29); U: P(2)	Panama
Nyssomyia ylephiletor (Fairchild and Hertig 1952)	F: H, P(36)	Panama
Nyssomyia yuilli Young & Porter 1972	U: H, A; F: P(20)	Colombia
Oligodontomyia oligodonta (Young, Pérez, and Romero 1985)	F: A, P(9)	Peru
Pintomyia (Pifanomyia) andina Osorno, Osorno-Mesa, and Morales 1972	U: P(1)	Colombia
Pintomyia (Pifanomyia) boliviana (Velasco and Trapido 1974)	U: H	Bolivia
Pintomyia (Pintomyia) christenseni Young and Duncan 1994	F: H, A, P(34)	Panama: H, A , P(20); Colombia: P(12); Brazil (P2)
Pintomyia (Pifanomyia) christophei (Fairchild and Trapido 1950)	F: H, A, P(3)	Dominican Republic
Pintomyia (Pifanomyia) gruta Ryan 1986	F: P(1)	Brazil
Pintomyia (Pifanomyia) moralesi Young 1979	F: P(4)	Colombia
Pintomyia (Pifanomyia) odax (Fairchild and Hertig 1961)	F: A, P(17)	Panama
Pintomyia (Pifanomyia) oresbia (Fairchild and Hertig 1961)	F: A, P(2)	Panama
Pintomyia (Pifanomyia) orestes (Fairchild and Trapido 1950)	F: H, P(1)	Cuba
Pintomyia (Pifanomyia) pia (Fairchild and Hertig 1961)	F: H, A, P(10)	Panama
Pintomyia (Pifanomyia) torvida Young, Morales, and Ferro 1994	F: A, P(1)	Colombia
Pintomyia (Pifanomyia) youngi Feliciangeli and Murillo 1985	F: P(2)	Venezuela
Pressatia camposi (Rodriguez 1952)	F: A, P(31)	Panama
Pressatia dysponeta (Fairchild and Hertig 1952)	F: A, P(53)	Panama
Pressatia trispinosa (Mangabeira 1942)	F: H, A, P(7)	Colombia
Psathyromyia (Incertae sedis) ignacioi (Young 1972)	F: P(1)	Venezuela
Psathyromyia (Forattiniella) barrettoi barrettoi (Mangabeira 1942)	U: P(1)	Panama
Psathyromyia (Forattiniella) barrettoi majuscula (Young 1979)	F: A, P(15); U: P(2)	Panama: A, P(11); Colombia: P(3); Costa Rica: P(1); Ecuador (P=1); Nicaragua: P(1)
Psathyromyia (Forattiniella) carpenteri (Fairchild and Hertig 1953)	F: H, A, P(36)	Panama
Psathyromyia (Forattiniella) runoides (Fairchild and Hertig 1953)	F: H, A, P(28)	Panama
Psathyromyia (Forattiniella) texana (Dampf 1938)	U: T	United States

*Key to Repository Type:
H=holotype (1 specimen) A=allotype (1 specimen)
F=MEFSCA P=paratype (1 or more specimens)
N=neoallotype (1 specimen) T=Type (1 specimen)
U=USNMNH

†Based on types and repositories, as listed in adjacent column.

Table 1C. Types of New World sand flies (Phlebotominae, Psychodidae) deposited in the USNMNH and MEFSCA, including country of type origin (continued).

Species	Repository Type* (No. of Slides)	Country of Type Origin†
Psathyromyia (Psathyromyia) campbelli (Damasceno, Causey, and Arouck 1945)	U: H	Brazil
Psathyromyia (Psathyromyia) cratifer (Fairchild and Hertig 1961)	F: H, P(1)	Mexico
Psathyromyia (Psathyromyia) dasymera (Fairchild and Hertig 1961)	F: H, A, P(45)	Panama: H, A, P(42); Mexico: P(1); Nicaragua: P(2)
Psathyromyia (Psathyromyia) guatemalensis Porter and Young 1986	F: A, P(1)	Guatemala
Psathyromyia (Psathyromyia) shannoni (Dyar 1929)	U: L, P(1); F: P(2)	Argentina: H; Panama: L, P(2); Peru (P1)
Psathyromyia (Psathyromyia) soccula (Fairchild and Hertig 1961)	F: P(2)	Panama
Psathyromyia (Psathyromyia) souzacastroi (Damasceno and Causey 1944)	U: H	Brazil
Psathyromyia (Psathyromyia) undulata (Fairchild and Hertig 1950)	U: P(1)	Guatemala
Psathyromyia (Psathyromyia) volcanensis (Fairchild and Hertig 1950)	F: N, P(3)	Panama
Psathyromyia (Xiphomyia) aclydifera (Fairchild and Hertig 1952)	F: A	Panama
Psychodopygus amazonensis (Root 1934)	U: (L); F: P(1)	Peru: L; French Guyana: P(1)
Psychodopygus ayrozai (Barretto and Coutinho 1940)	F: P(2)	Panama
Psychodopygus bispinosus (Fairchild and Hertig 1951)	F: H, A, P(4)	Panama
Psychodopygus carrerai carrerai (Barretto 1946)	F: P(1)	Panama
Psychodopygus carrerai thula (Young 1979)	F: A, P(27)	Panama: A, P(17); Colombia: P(10)
Psychodopygus davisi (Root 1934)	U: P(1)	Brazil
Psychodopygus fairchildi Barretto 1966	F: H,A, P(4)	Colombia
Psychodopygus fairtigi (Martins 1970)	F: H, P(1)	Colombia
Psychodopygus nocticolus (Young 1973)	F: A, P(7)	Colombia
Psychodopygus panamensis (Shannon 1926)	U: T	Panama
Psychodopygus recurvus (Young 1973)	F: A, P(12)	Colombia
Sciopemyia nematoducta Young and Arias 1984	F: A, P(23); U: P(8)	Brazil
Sciopemyia pennyi Arias and Freitas 1981	F: P(2); U: P(1)	Brazil
Sciopemyia preclara Young and Arias 1984	F: P(1)	Peru
Sciopemyia servulolimai (Damasceno and Causey 1945)	U: H	Brazil
Sciopemyia sordellii (Shannon and Del Ponte 1927)	U: L	Argentina
Trichophoromyia castanheirai (Damasceno, Causey, and Arouck 1945)	U: H	Brazil
Trichophoromyia dunhami (Causey and Damasceno 1945)	U: H	Brazil
Trichophoromyia gibba Young and Arias 1994	F: P(1)	Brazil
Trichophoromyia lopesi (Damasceno, Causey, and Arouck 1945)	U: H	Brazil
Trichophoromyia loretonensis (Llanos 1964)	F: P(1)	Peru
Trichophoromyia meirai (Causey and Damasceno 1945)	U: H	Brazil
Trichophoromyia melloi (Causey and Damasceno 1945)	U: H	Brazil
Trichophoromyia napoensis Young and Rodgers 1984	F: A, P(12)	Ecuador
Trichophoromyia pabloi (Barreto, Burbano, and Young 2002)	F: P(1)	Colombia
Trichophoromyia reburra (Fairchild and Hertig 1961)	F: H, A, P(2)	Panama
Trichophoromyia ruii Arias and Young 1982	F: P(31)	Brazil
Trichophoromyia sinuosa Young and Duncan 1994	F: H, P(1)	Peru
Trichopygomyia elegans Martins, Falcao and Silva 1976	U: P(1)	Peru
Trichopygomyia ferroae (Young and Morales 1987)	F: H, A, P(1)	Colombia
Trichopygomyia martinezi Young and Morales 1987	F: H, A, P(1)	Colombia
Trichopygomyia ratcliffei Arias, Ready, and Freitas 1983	U: P(5)	Brazil
Trichopygomyia triramula (Fairchild and Hertig 1952)	F: H, A, P(28)	Panama
Trichopygomyia wagleyi (Causey and Damasceno 1945)	U: H	Brazil

*Key to Repository Type:
H=holotype (1 specimen) A=allotype (1 specimen)
F=MEFSCA P=paratype (1 or more specimens)
N=neoallotype (1 specimen) T=Type (1 specimen)
U=USNMNH

†Based on types and repositories, as listed in adjacent column.

Table 1D. Types of New World sand flies (Phlebotominae, Psychodidae) deposited in the USNMNH and MEFSCA, including country of type origin (continued).

Species	Repository Type* (No. of Slides)	Country of Type Origin†
Trichopygomyia wilkersoni Young and Rodgers 1984	F: A, P(1)	Ecuador
Trichopygomyia witoto Young and Morales 1987	F: H, P(1)	Colombia
Viannamyia fariasi (Damasceno, Causey, and Arouck 1945)	U: H	Brazil
Warileya nigrosaccula Fairchild and Hertig 1951	F: H	Panama
Warileya phlebotomanica Hertig 1948	F: H	Peru
Warileya rotundipennis Fairchild and Hertig 1951	F: H, A, P(6)	Panama
Warileya yungasi Velasco and Trapido 1974	F: P(1); U: H, P(1)	Bolivia

*Key to Repository Type:
H=holotype (1 specimen) A=allotype (1 specimen)
F=MEFSCA P=paratype (1 or more specimens)
N=neoallotype (1 specimen) T=Type (1 specimen)
U=USNMNH

†Based on types and repositories, as listed in adjacent column.

Table 2A. Types of New World sand flies (Phlebotominae, Psychodidae), deposited in the USNMNH and MEFSCA, with old and new generic and subgeneric classifications (continued through 2B, 2C, 2D).

New Arrangement	Old Arrangement†
Bichromomyia olmeca olmeca (Vargas and Najera 1959)	*Lutzomyia (Nyssomyia) olmeca olmeca*
Bichromomyia olmeca bicolor (Fairchild and Theodor 1971)	*Lutzomyia (Nyssomyia) olmeca bicolor*
Bichromomyia olmeca nociva (Young and Arias 1982)	*Lutzomyia (Nyssomyia) olmeca nociva*
Brumptomyia galindoi (Fairchild and Hertig 1947)	*Brumptomyia galindoi*
Brumptomyia hamata (Fairchild and Hertig 1947)	*Brumptomyia hamata*
Brumptomyia leopoldoi (Rodriguez 1953)	*Brumptomyia leopoldoi*
Dampfomyia (Coromyia) steatopyga (Fairchild and Hertig 1958)	*Lutzomyia (Coromyia) steatopyga*
Dampfomyia (Coromyia) vesicifera (Fairchild and Hertig 1947)	*Lutzomyia (Coromyia) vesicifera*
Dampfomyia (Coromyia) vespertilionis (Fairchild and Hertig 1947)	*Lutzomyia (Coromyia) vespertilionis*
Dampfomyia (Coromyia) viriosa (Fairchild and Hertig 1958)	*Lutzomyia (Coromyia) viriosa*
Dampfomyia (Coromyia) zeledoni Young and Murillo 1984	*Lutzomyia (Coromyia) zeledoni*
Dampfomyia (Dampfomyia) anthophora (Addis 1945)	*Lutzomyia (Dampfomyia) anthophora*
Dampfomyia (Dampfomyia) rosabali (Fairchild and Hertig 1956)	*Dampfomyia (Dampfomyia) rosabali*
Dampfomyia (Incertae sedis) caminoi (Young and Duncan 1994)	*Lutzomyia caminoi*
Evandromyia (Aldamyia) sericea (Floch and Abonnenc 1944)	*Lutzomyia sericea*
Evandromyia (Aldamyia) williamsi (Damasceno, Causey, and Arouck 1945)	*Lutzomyia williamsi*
Evandromyia (Evandromyia) begonae (Ortiz and Torres 1975)	*Lutzomyia (Evandromyia) begonae*
Evandromyia (Evandromyia) inpai (Young and Arias 1977)	*Lutzomyia inpai*
Evandromyia (Evandromyia) wilsoni (Damasceno and Causey 1945)	*Lutzomyia wilsoni*
Hertigia hertigi Fairchild 1949	*Hertigia hertigi*
Lutzomyia (Helcocyrtomyia) botella (Fairchild and Hertig 1961)	*Lutzomyia (Helcocyrtomyia) botella*
Lutzomyia (Helcocyrtomyia) cirrita Young and Porter 1974	*Lutzomyia (Helcocyrtomyia) cirrita*
Lutzomyia (Helcocyrtomyia) hartmanni (Fairchild and Hertig 1957)	*Lutzomyia (Helcocyrtomyia) hartmanni*
Lutzomyia (Helcocyrtomyia) imperatrix (Alexander 1944)	*Lutzomyia (Helcocyrtomyia) imperatrix*
Lutzomyia (Helcocyrtomyia) noguchii (Shannon 1929)	*Lutzomyia (Helcocyrtomyia) noguchii*
Lutzomyia (Helcocyrtomyia) peruensis (Shannon 1929)	*Lutzomyia (Helcocyrtomyia) peruensis*
Lutzomyia (Helcocyrtomyia) pescei (Hertig 1943)	*Lutzomyia (Helcocyrtomyia) pescei*
Lutzomyia (Helcocyrtomyia) strictivilla Young 1979	*Lutzomyia (Helcocyrtomyia) strictivilla*
Lutzomyia (Helcocyrtomyia) tortura Young and Rogers 1984	*Lutzomyia (Helcocyrtomyia) tortura*
Lutzomyia (Incertae sedis) tanyopsis Young and Perkins 1984	*Lutzomyia tanyopsis*
Lutzomyia (Lutzomyia) battistinii (Hertig 1978)	*Lutzomyia (Lutzomyia) battistinii*
Lutzomyia (Lutzomyia) lichyi (Floch and Abonnenc 1950)	*Lutzomyia (Lutzomyia) lichyi*
Lutzomyia (Tricholateralis) carvalhoi (Damasceno, Causey, and Arouck 1945)	*Lutzomyia (Lutzomyia) carvalhoi*

*Based on WRBU[22] and Galati.[23]

†Based on Young and Duncan[4] and various references.

Table 2B. Types of New World sand flies (Phlebotominae, Psychodidae), deposited in the USNMNH and MEFSCA, with old and new generic and subgeneric classifications (continued).

New Arrangement	Old Arrangement†
Lutzomyia (Tricholateralis) cruciata (Coquillett 1907)	*Lutzomyia (Lutzomyia) cruciata*
Lutzomyia (Tricholateralis) diabolica (Hall 1936)	*Lutzomyia (Lutzomyia) diabolica*
Lutzomyia (Tricholateralis) falcata Young, Morales and Ferro 1994	*Lutzomyia (Lutzomyia) falcata*
Lutzomyia (Tricholateralis) marinkellei Young 1979	*Lutzomyia (Lutzomyia) marinkellei*
Martinsmyia gasparviannai Martins, Godoy and Silva 1962	*Lutzomyia (Lutzomyia) gasparviannai*
Martinsmyia waltoni Arias, Freitas and Barrett 1984	*Lutzomyia (Nyssomyia) waltoni*
Micropygomyia (Coquillettimyia) apache (Young and Perkins 1984)	*Lutzomyia apache*
Micropygomyia (Coquillettimyia) stewarti (Mangabeira and Galindo 1944)	*Lutzomyia (Helcocyrtomyia) stewarti*
Micropygomyia (Coquillettimyia) vexator (Coquillett 1907)	*Lutzomyia (Helcocyrtomyia) vexator*
Micropygomyia (Micropygomyia) cayennensis cayennensis (Floch and Abonnenc 1941)	*Lutzomyia (Micropygomyia) cayennensis cayennensis*
Micropygomyia (Micropygomyia) cayennensis hispaniolae (Fairchild and Trapido 1950)	*Lutzomyia (Micropygomyia) cayennensis hispaniolae*
Micropygomyia (Micropygomyia) cayennensis jamaicensis (Fairchild and Trapido 1950)	*Lutzomyia (Micropygomyia) cayennensis jamaicensis*
Micropygomyia (Micropygomyia) cayennensis maciasi (Fairchild and Hertig 1948)	*Lutzomyia (Micropygomyia) cayennensis maciasi*
Micropygomyia (Micropygomyia) cayennensis puertoriciensis (Fairchild and Hertig 1948)	*Lutzomyia (Micropygomyia) cayennensis puertoriciensis*
Micropygomyia (Micropygomyia) cayennensis viequesensis (Fairchild and Hertig 1948)	*Lutzomyia (Micropygomyia) cayennensis viequesensis*
Micropygomyia (Micropygomyia) cubensis (Fairchild and Trapido 1950)	*Lutzomyia (Micropygomyia) cubensis*
Micropygomyia (Micropygomyia) duppyorum (Fairchild and Trapido 1950)	*Lutzomyia (Micropygomyia) duppyorum*
Micropygomyia (Micropygomyia) hispaniolae (Fairchild and Trapido 1950)	*Micropygomyia (Micropygomyia) hispaniolae*
Micropygomyia (Micropygomyia) pilosa (Damasceno and Causey 1944)	*Lutzomyia pilosa*
Micropygomyia (Micropygomyia) xerophila (Young, Brener and Wargo 1983)	*Lutzomyia xerophila*
Micropygomyia (Sauromyia) atroclavata (Knab 1913)	*Lutzomyia (Micropygomyia) atroclavata*
Micropygomyia (Sauromyia) ferreirana (Barretto, Martins and Pellegrino 1956)	*Lutzomyia ferreirana*
Micropygomyia (Sauromyia) quechua (Martins, Llanos and Silva 1975)	*Lutzomyia quechua*
Micropygomyia (Sauromyia) quinquefer (Dyar 1929)	*Lutzomyia quinquefer*
Migonemyia (Blancasmyia) cerqueirai (Causey and Damasceno 1945)	*Lutzomyia (Evandromyia) cerqueirai*
Migonemyia (Blancasmyia) gorbitzi (Blancas 1959)	*Lutzomyia gorbitzi*
Nyssomyia anduzei (Rozeboom 1942)	*Lutzomyia (Nyssomyia) anduzei*
Nyssomyia trapidoi (Fairchild and Hertig 1952)	*Lutzomyia (Nyssomyia) trapidoi*
Nyssomyia ylephiletor (Fairchild and Hertig 1952)	*Lutzomyia (Nyssomyia) ylephiletor*
Nyssomyia yuilli Young & Porter 1972	*Lutzomyia (Nyssomyia) yuilli yuilli*
Oligodontomyia oligodonta (Young, Pérez and Romero 1985)	*Lutzomyia oligodonta*
Pintomyia (Pifanomyia) andina Osorno, Osorno-Mesa and Morales 1972	*Lutzomyia andina*
Pintomyia (Pifanomyia) boliviana (Velasco and Trapido 1974)	*Lutzomyia boliviana*
Pintomyia (Pintomyia) christenseni Young and Duncan 1994	*Lutzumyia (Pintomyia) christenseni*
Pintomyia (Pifanomyia) christophei (Fairchild and Trapido 1950)	*Lutzomyia christophei*
Pintomyia (Pifanomyia) gruta Ryan 1986	*Lutzomyia gruta*
Pintomyia (Pifanomyia) moralesi Young 1979	*Lutzomyia moralesi*
Pintomyia (Pifanomyia) odax (Fairchild and Hertig 1961)	*Lutzomyia odax*
Pintomyia (Pifanomyia) oresbia (Fairchild and Hertig 1961)	*Lutzomyia oresbia*
Pintomyia (Pifanomyia) orestes (Fairchild and Trapido 1950)	*Lutzomyia orestes*
Pintomyia (Pifanomyia) pia (Fairchild and Hertig 1961)	*Lutzomyia pia*
Pintomyia (Pifanomyia) torvida Young, Morales and Ferro 1994	*Lutzomyia torvida*
Pintomyia (Pifanomyia) youngi Feliciangeli and Murillo 1985	*Lutzomyia youngi*
Pressatia camposi (Rodriguez 1952)	*Lutzomyia (Pressatia) camposi*
Pressatia dysponeta (Fairchild and Hertig 1952)	*Lutzomyia (Pressatia) dysponeta*
Pressatia trispinosa (Mangabeira 1942)	*Lutzomyia (Pressatia) trispinosa*

*Based on WRBU[22] and Galati.[23]

†Based on Young and Duncan[4] and various references.

Table 2C. Types of New World sand flies (Phlebotominae, Psychodidae), deposited in the USNMNH and MEFSCA, with old and new generic and subgeneric classifications. (continued).

New Arrangement	Old Arrangement†
Psathyromyia (Incertae sedis) ignacioi (Young 1972)	*Lutzomyia ignacioi*
Psathyromyia (Forattiniella) barrettoi barrettoi (Mangabeira 1942)	*Lutzomyia barrettoi barrettoi*
Psathyromyia (Forattiniella) barrettoi majuscula (Young 1979)	*Lutzomyia barrettoi majuscula*
Psathyromyia (Forattiniella) carpenteri (Fairchild and Hertig 1953)	*Lutzomyia carpenteri*
Psathyromyia (Forattiniella) runoides (Fairchild and Hertig 1953)	*Lutzomyia runoides*
Psathyromyia (Forattiniella) texana (Dampf 1938)	*Lutzomyia texana*
Psathyromyia (Psathyromyia) campbelli (Damasceno, Causey and Arouck 1945)	*Lutzomyia (Psathyromyia) campbelli*
Psathyromyia (Psathyromyia) cratifer (Fairchild and Hertig 1961)	*Lutzomyia (Psathyromyia) cratifer*
Psathyromyia (Psathyromyia) dasymera (Fairchild and Hertig 1961)	*Lutzomyia (Psathyromyia) dasymera*
Psathyromyia (Psathyromyia) guatemalensis Porter and Young 1986	*Lutzomyia (Psathyromyia) guatemalensis*
Psathyromyia (Psathyromyia) shannoni (Dyar 1929)	*Lutzomyia (Psathyromyia) shannoni*
Psathyromyia (Psathyromyia) soccula (Fairchild and Hertig 1961)	*Lutzomyia (Psathyromyia) soccula*
Psathyromyia (Psathyromyia) souzacastroi (Damasceno and Causey 1944)	*Lutzomyia (Psathyromyia) souzacastroi*
Psathyromyia (Psathyromyia) undulata (Fairchild and Hertig 1950)	*Lutzomyia (Psathyromyia) undulata*
Psathyromyia (Psathyromyia) volcanensis (Fairchild and Hertig 1950)	*Lutzomyia (Psathyromyia) volcanensis*
Psathyromyia (Xiphomyia) aclydifera (Fairchild and Hertig 1952)	*Lutzomyia aclydifera*
Psychodopygus amazonensis (Root 1934)	*Lutzomyia (Psychodopygus) amazonensis*
Psychodopygus ayrozai (Barretto and Coutinho 1940)	*Lutzomyia (Psychodopygus) ayrozai*
Psychodopygus bispinosus (Fairchild and Hertig 1951)	*Lutzomyia (Psychodopygus) bispinosus*
Psychodopygus carrerai carrerai (Barretto 1946)	*Lutzomyia (Psychodopygus) carrerai carrerai*
Psychodopygus carrerai thula (Young 1979)	*Lutzomyia (Psychodopygus) carrerai thula*
Psychodopygus davisi (Root 1934)	*Lutzomyia (Psychodopygus) davisi*
Psychodopygus fairchildi Barretto 1966	*Lutzomyia (Psychodopygus) fairchildi*
Psychodopygus fairtigi (Martins 1970)	*Lutzomyia (Psychodopygus) fairtigi*
Psychodopygus nocticolus (Young 1973)	*Lutzomyia (Psychodopygus) nocticola*
Psychodopygus panamensis (Shannon 1926)	*Lutzomyia (Psychodopygus) panamensis*
Psychodopygus recurvus (Young 1973)	*Lutzomyia (Psychodopygus) recurva*
Sciopemyia nematoducta Young and Arias 1984	*Lutzomyia (Sciopemyia) nematoducta*
Sciopemyia pennyi Arias and Freitas 1981	*Lutzomyia (Sciopemyia) pennyi*
Sciopemyia preclara Young and Arias 1984	*Lutzomyia (Sciopemyia) preclara*
Sciopemyia servulolimai (Damasceno & Causey 1945)	*Lutzomyia (Sciopemyia) servulolimai*
Sciopemyia sordellii (Shannon and Del Ponte 1927)	*Lutzomyia (Sciopemyia) sordellii*
Trichophoromyia castanheirai (Damasceno, Causey and Arouck 1945)	*Lutzomyia (Trichophoromyia) castanheirai*
Trichophoromyia dunhami (Causey and Damasceno 1945)	*Lutzomyia (Trichophoromyia) dunhami*
Trichophoromyia gibba Young and Arias 1994	*Lutzomyia (Trichophoromyia) gibba*
Trichophoromyia lopesi (Damasceno, Causey and Arouck 1945)	*Lutzomyia (Trichophoromyia) lopesi*
Trichophoromyia loretonensis (Llanos 1964)	*Lutzomyia (Trichophoromyia) loretonensis*
Trichophoromyia meirai (Causey and Damasceno 1945)	*Lutzomyia (Trichophoromyia) meirai*
Trichophoromyia melloi (Causey and Damasceno 1945)	*Lutzomyia (Trichophoromyia) melloi*
Trichophoromyia napoensis Young and Rodgers 1984	*Lutzomyia (Trichophoromyia) napoensis*
Trichophoromyia pabloi (Barreto, Burbano and Young 2002)	*Lutzomyia (Trichophoromyia) pabloi*
Trichophoromyia reburra (Fairchild and Hertig 1961)	*Lutzomyia (Trichophoromyia) reburra*
Trichophoromyia ruii Arias and Young 1982	*Lutzomyia (Trichophoromyia) ruii*
Trichophoromyia sinuosa Young and Duncan 1994	*Lutzomyia (Trichophoromyia) sinuosa*
Trichopygomyia elegans Martins, Falcao and Silva 1976	*Lutzomyia (Trichopygomyia) elegans*
Trichopygomyia ferroae (Young and Morales 1987)	*Lutzomyia (Trichopygomyia) ferroae*
Trichopygomyia martinezi Young and Morales 1987	*Lutzomyia (Trichopygomyia) martinezi*
Trichopygomyia ratcliffei Arias, Ready and Freitas 1983	*Lutzomyia (Trichopygomyia) ratcliffei*
Trichopygomyia triramula (Fairchild and Hertig 1952)	*Lutzomyia (Trichopygomyia) triramula*
Trichopygomyia wagleyi (Causey and Damasceno 1945)	*Lutzomyia (Trichopygomyia) wagleyi*

*Based on WRBU[22] and Galati.[23]

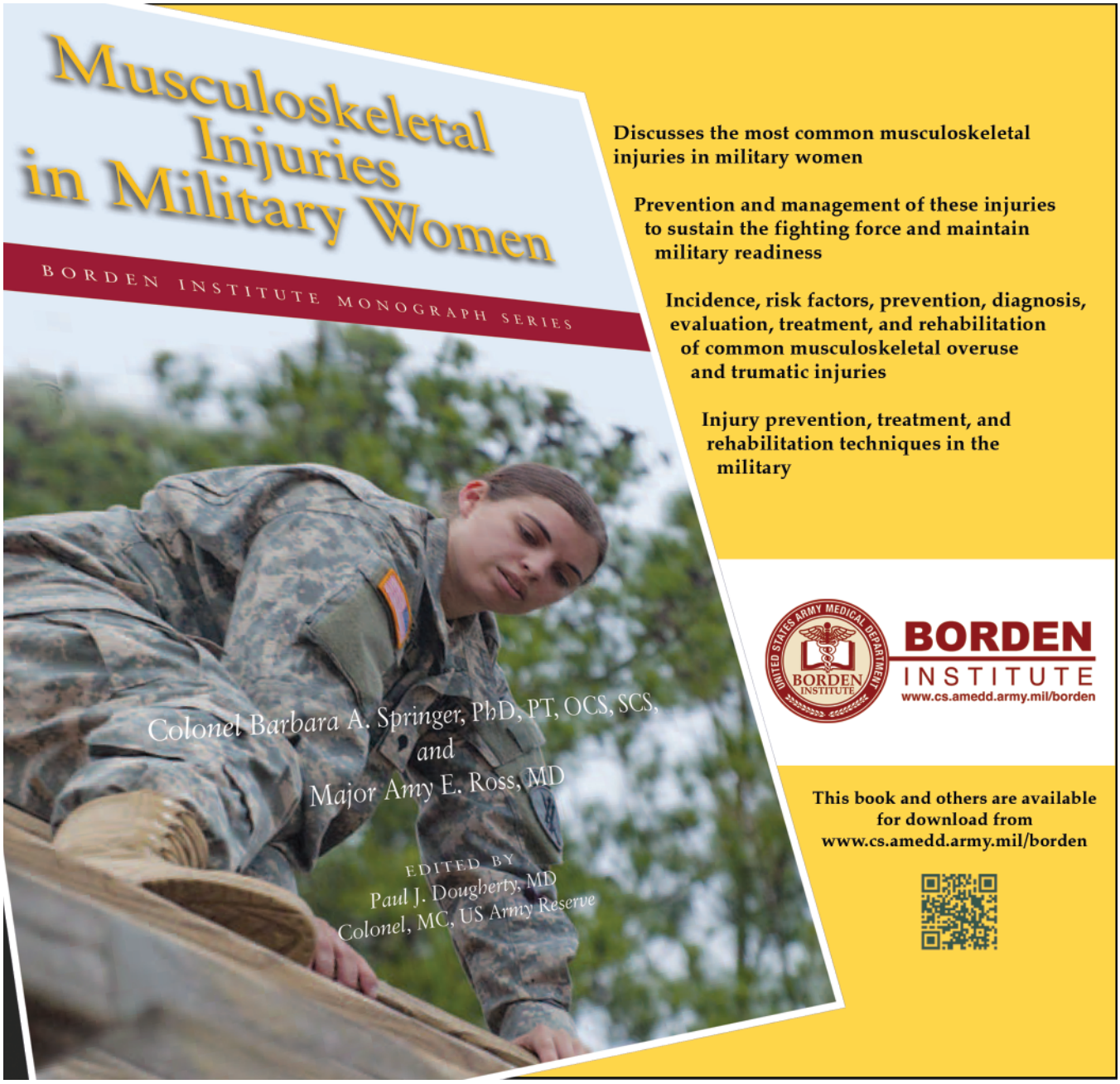
†Based on Young and Duncan[4] and various references.

Table 2D. Types of New World sand flies (Phlebotominae, Psychodidae), deposited in the USNMNH and MEFSCA, with old and new generic and subgeneric classifications (continued).

New Arrangement	Old Arrangement†
Trichopygomyia wilkersoni Young and Rodgers 1984	*Lutzomyia (Trichophoromyia) wilkersoni*
Trichopygomyia witoto Young and Morales 1987	*Lutzomyia (Trichopygomyia) witoto*
Viannamyia fariasi (Damasceno, Causey and Arouck 1945)	*Lutzomyia (Viannamyia) fariasi*
Warileya nigrosaccula Fairchild and Hertig 1951	*Warileya nigrosaccula*
Warileya phlebotomanica Hertig 1948	*Warileya phlebotomanica*
Warileya rotundipennis Fairchild and Hertig 1951	*Warileya rotundipennis*
Warileya yungasi Velasco and Trapido 1974	*Warileya yungasi*

*Based on WRBU[22] and Galati.[23]

†Based on Young and Duncan[4] and various references.



Development of Air Force Aerial Spray Night Operations: High Altitude Swath Characterization

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Abstract

Multiple trials were conducted from 2006 to 2014 in an attempt to validate aerial spray efficacy at altitudes conducive to night spray operations using night vision goggles (NVGs). Higher altitude application of pesticide (more than 400 ft (121.9 m) above ground level (AGL)) suggested that effective vector control might be possible under ideal meteorological conditions. A series of lower altitude daytime applications (300 ft (91.4 m) AGL) demonstrated effective and repeatable mortality of target sentinel insects more than 5,000 ft (1,524 m) downwind, and control of natural vector populations. From these results we believe further pursuit of aerial night applications of pesticide using NVGs at 300 ft (91.4 m) AGL by this group is warranted.

Since World War II, the US military has used aerial spray to mitigate vector-borne disease in combat situations as well as domestic postemergency scenarios. The benefit of aerial application is that large areas which may be inaccessible from the ground can be covered rapidly, thus quickly breaking the cycle of infection.[1] Currently, USAF aerial spray operations for mosquito and other insect-borne disease control are conducted during daylight hours. The application platform for USAF aerial sprays is a C-130 H2 aircraft with a modular aerial spray system (MASS). Current operational parameters use a flight profile spraying at 150 ft (45.7 m) above ground level (AGL) at 200 knots (370.4 km/hr) groundspeed. These parameters were derived from efficacy tests coupled with the need to maintain relatively high airspeeds and low altitudes to mitigate the potential effect of ground-fire hazards.[2-4] Prior to the common use of night vision goggles (NVGs) to offset the unacceptable hazards of low-flying nighttime applications, daytime aerial application of pesticides was the only viable option.

Benefits of night-spray capability are multiple. Application of pesticides concurrent to night feeding mosquito (or other) vector activity will potentially maximize vector mortality and reduce human pathogen transmission. It is generally considered that the optimal timing for application of a fast-acting pesticide is when the target insect is active and in search of a blood meal or other specialized flight periods.[5] Based on this concept, the need for a nighttime aerial spray application capability becomes self-evident. The need for this capability was reinforced when West Nile virus (WNV) quickly spread through the Americas beginning in 1999. Though most of the endemic encephalitides were well documented to be vectored by mosquitos of the genus *Culex*, the introduction of WNV galvanized the public health field with respect to the importance of controlling populations of these vectors.[6] The *Culex* and some *Aedes* species responsible for transmitting viruses like WNV to humans are reportedly far more active in the crepuscular and night hours rather than the daytime hours.[7] Because of this, a majority of aerial adulticiding conducted by civilian mosquito control agencies in the United States is conducted in the evening and nighttime hours.

Benefits of night spraying also include minimizing exposure of diurnal beneficial insects to pesticides. The potential for pollinator and other beneficial insect mortality is a significant concern with aerial spray pesticide application. In the case of honeybees, standard operating procedure for daytime aerial spray application of pesticides includes notifying local beekeepers so that they can cover and isolate their hives prior to any application. Nighttime application does not remove the responsibility from the applicators to inform the beekeepers, but it reduces potentially negative effects of pesticide application (bee kill) as honeybees are normally naturally ensconced in a protected hive at time of application. The same level of protection may not be as well known for other diurnal beneficial insects, and nocturnally active beneficial insects might not be so protected from night pesticide applications.

Night spraying also leverages favorable meteorological conditions that may reduce effects of unwanted pesticide drift. Convective atmospheric activity during daytime aerial pesticide applications has always plagued uniform pesticide application. Differential heating may cause convective surface currents to blow away sections of pesticide application plumes at altitude, thereby potentially leaving significant application gaps. Lowered convective activity during nighttime applications may reduce this effect. At night, surface wind currents generally become more laminar in nature and pesticide drift may become more predictable and effective (conversation with H. Thistle, January 2009).

Despite these benefits of nighttime aerial spray application, the obvious significant danger to night application is the lack of ability to easily see and identify objects that present collision hazards at low altitude. In addition to natural obstructions, man-made obstructions such as buildings, towers, or antennae present formidable obstacles to low-level aircraft navigation. In the contiguous United States, 68% of all surface obstructions are less than 300 ft (91.4 m) AGL, 75% of all surface obstructions are less than 350 ft (106.7 m) AGL, and 5% of all surface obstructions are greater than 500 ft (152.4 m) AGL (conversation with Lt Col John Kochansky, September 2014). Thus, flying at higher altitudes (300+ ft AGL) presents significantly less risk than flying a 150 ft (45.7 m) ground profile at night. Although NVGs have mitigated some hazards of night flying, they have limitations regarding which objects are visible at night.

In an attempt to increase the military spray capability to include nighttime application, the USAF aerial spray flight investigated the feasibility of pesticide application flying on NVGs. This investigation was especially challenging as the flight profile was outside the parameters of the standard low-level NVG routines which are most exclusively employed by special operations C-130 missions using a modified-contour scenario flying at 300 ft (91.4 m) AGL on NVGs. Aerial spray mission requirements for low-level NVG operations required a new, unique set of operating conditions that had not been previously considered.

Proposals including a point paper to convince USAF leadership of the benefits of aerial spray nighttime operation were initiated in 2004. Following a series of inquiries and safety reviews, and with endorsement from agencies including the US Centers for Disease Control and Prevention, the Armed Forces Pest Management Board, the Office of the Surgeon General of the Air Force, and the support of several US mosquito and vector control agencies, permission was granted to the USAF aerial spray flight to begin low-level NVG training activities specific to night-time aerial spray operations in 2014.

The potential *operational* capability by the USAF aerial spray flight to conduct night spray missions must be supported by evidence of *functional* capability. This would include demonstrations that night pesticide applications were effective against target medically important insect vectors of significant disease while operating within an acceptable USAF operational scenario. Herein, we report the results of multiple daytime aerial pesticide application trials conducted to determine pesticide droplet size and effective swath width, including field mosquito sentinel mortality, at a variety of altitudes and with a variety of spray nozzle sizes to support USAF NVG operations for night-spray capability at 300 ft (91.4 m) AGL.

Methods and Materials

Each of the trials detailed below, from earliest to most recent, was designed as a standalone evaluation, generally without replication. The aerial spray platform for all trials was a USAF C-130 H2 aircraft equipped with a MASS, with a variety of nozzle sizes and configurations on 2 fuselage booms as detailed below. Meteorological conditions were measured using a Swath Kit weather monitoring station (Droplet Technologies, College Station, PA) (trial sets 1-3) or a Kestrel NV4500 logging portable weather station (Nielsen-Kellerman, Boothwyn, PA) (trial sets 4-6). Trial sets 7 and 8 used 4 Kestrel NV4500 logging portable weather stations placed at various intervals along each sampling line, and meteorological data were averaged between data derived from each station over the course of each test.

Trial Set 1

The trials were conducted January 13-18, 2006, at Avon Park Training Range, Avon Park, Florida (Figure 1).

On January 14, 10 aerosol impingers (spinners) (John Hock Company, Gainesville, FL) were placed in an east-west orientation on Kissimmee Road. Impingers were positioned in a linear array with each sampling station separated by 2500 ft (762 m). Each impinger was equipped with 2 Teflon-coated 25mm by 75mm microscope slides to collect droplets from the aerosol cloud released from the aircraft as the cloud drifted downwind past each sampling station.

The fuselage booms were fitted with a total of 6 flat-fan TeeJet 8008 Nozzles (Spraying Systems Company, Wheaton, IL). BVA-13 oil was used to simulate pesticide, applied at 4.5 gallons of material per minute (17.03 L/min) at an operating system pressure of 83 psi. Three north-south overlapped (crosswind) passes of the aircraft

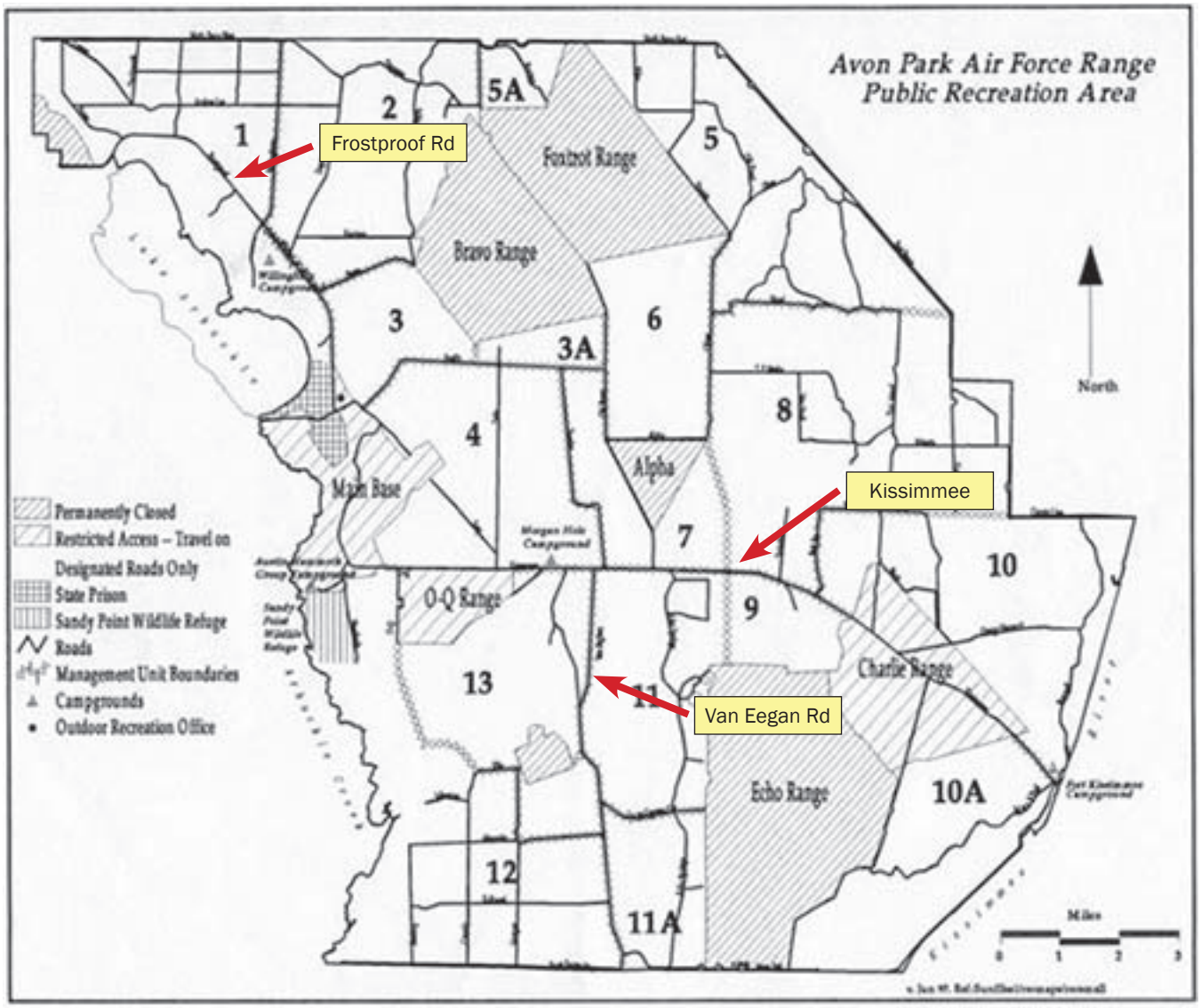

Figure 1. Map of Avon Park Air Force Range (Florida) with testing locations highlighted.

were made to increase the droplet concentrations at the sampling stations. The spray-on time for each pass was 40 seconds, with spraying beginning 30 seconds prior to the aircraft's point of intersection with Kissimmee Road. The aircraft flew directly over the upwind sampling station at 500 ft (152.4 m) AGL.

On January 15, 10 impingers were aligned on Frostproof Road (Figure 1). Sampling stations were placed 1,700 ft (518 m) apart, with the first sampling station located on the northwest point in the road. Three adjacent southwest-northeast passes at 500 ft (152.4 m) AGL were made over the first sampling station, with each pass commencing and ending spray 30 seconds before and after the aircraft's intersection with Frostproof Road. The MASS sprayed BVA-13 oil at 4.75 gal/min (17.98 L/min) at an operating pressure of 56 psi.

On January 16, 10 impingers were placed in a north-south orientation along Van Eegan Road (Figure 1). Sampling stations were positioned 2,500 ft (762 m) apart with the first sampling station located at the southern point of the road. The BVA-13 oil spray flow rate was 4.7 gal/min. Four overlapped west-east passes were flown at 500 ft (152.4 m) AGL, with each pass commencing and ending spray 30 seconds prior to and after intersection with the road at the first sampling station.

Following each set of sprays on the 3 trial days, Teflon slides were collected 45 minutes after the last pass and viewed under a compound microscope. Up to 50 droplets (if available) were counted per slide at each one of the sampling stations. Droplets were measured for volume median diameter (VMD), calculated using the Yeomans method.[8]

Trial Set 2

These trials were conducted at the Avon Park Training Range 13-17 February 2006. In general, the methods were similar to those in Trial set 1. All trials were conducted with the aircraft spraying at 500 ft (152.4 m) AGL. The spray booms were fitted with 6 flat-fan TeeJet 8008 nozzles, three on each boom and in each trial, the MASS delivered approximately 4.5 gal/min (17.03 L/min) of BVA-13 oil with the system operating at approximately 50 psi. In these tests, an optical whitener (Uvitex OB, Ciba Specialty Chemicals, Tarrytown, NY) was added to the BVA-13 oil in an attempt to differentiate between potential contamination of the Teflon slides with ambient atmospheric aerosols versus the spray coming from the aircraft. A fluorescent compound microscope was used to examine droplet collection slides. The Uvitex in the BVA-13 oil caused droplets collected from the spray mission to fluoresce vigorously, making identification unambiguous. Four overlapping spray passes for each trial in this series were conducted on a course perpendicular to the impingers, directly overflying the first sampling station in the series. Each pass totaled 30 seconds of spray-on time, 15 seconds before and 15 seconds following intersection with the road(s) and the first sampling station. Slides were collected and droplet data were calculated as in Trial Set 1, with the exception that droplet density (droplets/cm^2) was also recorded for each of the sampling stations.

On February 14, a trial was conducted with impingers set up on a northwest-southeast orientation along Frostproof Road (Figure 1) with sampling stations separated by 1,700 ft (518.1 m). The first sampling station was located at the southeast end of the road.

On February 15, a test was conducted on an east-west orientation on Kissimmee Road (Figure 1) with sampling stations separated by 2,500 ft (762 m). The first sampling station was at the east end of the road.

On February 16, a north-south linear array of sampling stations was installed on Van Eegan Road (Figure 1). Sampling stations were separated by 1,742 ft (531 m) with the first station located at the junction of Kissimmee and Van Eegan Roads.

Trial Set 3

Trials were conducted December 4-8, 2006, at the Avon Park Training Range. In these trials, a sentinel mosquito bioassay was used in conjunction with the droplet collection system described above. The fuselage booms were fitted with a total of 20 flat-fan TeeJet 8005 nozzles, (10 on each side), which produced flow rates approaching 7.2 gal/min (27.25 L/min) at an operating system pressure of 50 psi. The pesticide used for these trials was Dibrom (Naled; AMVAC Chemical Corp., Los Angeles, CA) organophosphate adulticide, applied at 500 ft (152.4 m) AGL. In each test, 10 impinger sampling stations were collocated with 10 bioassay cages consisting of cardboard rings (approximately 3.175 cm by 15.25 cm), with open ends covered with fine plastic mesh. In each cage was placed approximately 25 adult female *Culex quinquefasciatus* from the laboratory colony at the US Department of Agriculture Agricultural Research Service Center for Medical, Agricultural, and Veterinary Entomology (USDA-ARS CMAVE), Gainesville, FL. Sampling arrays each consisting of an impinger and a sentinel cage were deployed at 0.5 mile (804.6 m) intervals along Van Eegan Road (Figure 1) on December 6 and 7. The first sampling array locations for both trials were at the intersection of Kissimmee and Van Eegan Roads. Four control sentinel mosquito cages were located approximately 0.5 miles (804.6 m) north (upwind) of the first sampler/cage and exposed for the duration of each trial to ambient conditions. The aircraft conducted 2 overlapping passes for each trial on a course perpendicular to the sampling stations, flying directly over the first sampling station. Spray-on time was 30 seconds before and 30 seconds after overflying this location. The aerosol cloud was allowed to drift and settle for 45 minutes, after which time the Teflon slides and the bioassay cages were collected. Droplets were measured as described for the previous Trial Sets, and VMD, numerical median diameter (NMD), and droplet density were calculated for each sampling station. Mosquitoes were aspirated from the bioassay cages and transferred to clean cages equipped with cotton balls soaked with a 10% sugar water solution. Mosquito mortality was determined at 2, 12, and 24 hours postspray, and corrected with Abbott's formula[9] relative to mortality in the controls.

Trial Set 4

Trials were conducted December 6-10, 2009, at the Avon Park Training Range. Ten impinger sampling stations equipped as before were deployed along Van Eegan Road (Figure 1) on December 8 and 9 at 0.25 mile (402.3 m) intervals for the first mile (1,609.3 m) of the sampling array, followed by 0.5 mile (804.6 m) intervals for the remaining 4.5 mile (7,242 m) sampling line. The spray platform was outfitted with 7 flat fan TeeJet 8005 nozzles on each spray boom, producing a flow rate of approximately 7.2 gal/min (27.25 L/min) at an operating pressure of 50 psi. BVA-13 oil was used to simulate pesticide, and application altitude was 300 ft (91.4 m) AGL. The aircraft conducted 2 overlapping spray passes per trial perpendicular to the sampling stations, with each pass totaling 60 seconds of spray-on time (30 seconds prior to and 30 seconds after intersection with first sampling location).

The first sampling station was located at the intersection of Kissimmee and Van Eegan Roads. Teflon slides were collected as described in the above Trial Sets. One hundred drops (if available) were counted from each slide, and VMD, NMD, and droplet density were recorded.

Trial set 5

This trial series was conducted at the Avon Park Training Range the week of January 23, 2012.

On January 24, 10 impinger sampling stations separated by 0.5 miles (804.6 m) were placed in an east-west orientation on Kissimmee Road (Figure 1). Fuselage spray booms on the aircraft were fitted with 8 TeeJet 8005 nozzles, four on each side, for a flow rate of 4.5 gal/min (17.03 L/min) at an operating pressure of 83 psi. Three north-south overlapped perpendicular passes were made by the aircraft beginning directly over the first sampling station in the array. Spray-on times were 20 seconds prior to and 20 seconds after intersection of aircraft flight path and Kissimmee Road. Altitude of the application was 400 ft (121.9 m) AGL and BVA-13 oil was used to simulate pesticide.

On January 25, the January 24 trial was repeated, however, spray booms were outfitted with 10 TeeJet 8005 nozzles, 5 on each side, for a flow rate of 4.75 gal/min (17.98 L/min) at an operating pressure of 56 psi. The first 4 sampling stations were 0.25 miles (402.3 m) apart, with the remainder positioned at 0.5 mile (804.6 m) intervals.

Trial parameters from January 25 were repeated on January 26. However, the sampler array was aligned in a north-south orientation along Van Eegan Road (Figure 1) and aircraft spray passes were from west-east. Flow rate during this test was 4.6 gal/min (17.4 L/min) with a pressure of 45 psi. Droplet size and density data were collected as described for the January 24 trial set.

Trial Set 6

On October 17, 2012, 2 trials were performed in conjunction with an operational aerial spray at Parris Island Marine Corps Recruit Depot (MCRD), South Carolina. In each of these trials, 9 bioassay cages similar to those described in Trial Set 3, each with approximately 20 adult female *Cx. quinquefasciatus*, were placed in a linear array along Wake Blvd. The bioassay cages were clamped to wooden dowels at approximately 2 ft (0.61 m) AGL and fitted with cotton balls saturated with 10% sugar solution. The first 5 cages were separated from each other by 250 ft (76.2 m), with each of the remaining 4 cages separated by 500 ft (152.4 m). The spray booms were outfitted with 18 TeeJet 8003 flat-fan nozzles, 9 on each boom. In each trial in the set, the aircraft flew a swath perpendicular the bioassay sampling line, dispensing Dibrom (Naled) at 300 ft (91.4 m) AGL. Flow rate and pressure recorded from the MASS were 3.3 gal/min (12.5 L/min) at approximately 60 psi. Spray-on times were 20 seconds before and 20 seconds following intersection of the aircraft at the first bioassay station. An additional 3 bioassay cages were included as controls for each trial and exposed to ambient conditions during the trials, approximately 0.5 miles (804.6 m) upwind of the application spray line. Bioassay cages were collected and initial knockdown was recorded approximately 35 minutes after each trial. Mosquitoes were then transferred into clean holding cups fitted with cotton balls saturated with 10% sugar water solution. Mortality was recorded at 1, 12, and 24 hours postspray, and all mortality was corrected with Abbott's formula as described in Trial Set 3.

Trial Set 7

Two independent bioassay trials were conducted on April 9, 2013, at Parris Island MCRD in conjunction with an operational aerial application of the island, and were the result of a cooperative effort between the USAF aerial spray unit and personnel from USDA-ARS-CMAVE. Two tests were conducted using linear arrays of bioassay cages placed either along Wake Blvd (Figure 2A) or the Causeway (Figure 2B). One dispensing swath was used per test. For each test, fuselage booms were equipped with 14 TeeJet 8003 flat fan nozzles, 7 on each boom.

For the first application, 20 bioassay cages identical to those described in Trial Set 6 were placed along Wake Blvd at approximately 500 foot (152.4 m) intervals. The first bioassay cage was positioned at the southwest terminus of Wake Blvd. The aircraft dispensed Dibrom (Naled) adulticide at 300 ft (91.4 m) AGL perpendicular to the sampling array, with an approximately 300 foot (91.4 m) upwind offset. Spray-on and off times were 20 seconds before and after the aircraft intersection with the first sampling station. The MASS parameters were 2.7 gal/min (10.2 L/min) at 52 psi. Five sentinel (control) mosquito control cages were placed at the golf course on the southernmost point on the island outside of the treatment area and exposed to ambient conditions for the duration of each spray test.

For the second test, 25 bioassay cages identical to those described in Trial Set 6 were deployed along the Causeway at approximately 300 foot (91.4 m) intervals, with the first bioassay cage at the northwestern terminus of the road and an additional 5 sentinel (control) cages placed in the open at the golf course upwind from the spray application. The aircraft dispensed adulticide at 300 ft (91.4 m) AGL on a swath perpendicular to the causeway, directly overflying the last sampling station.

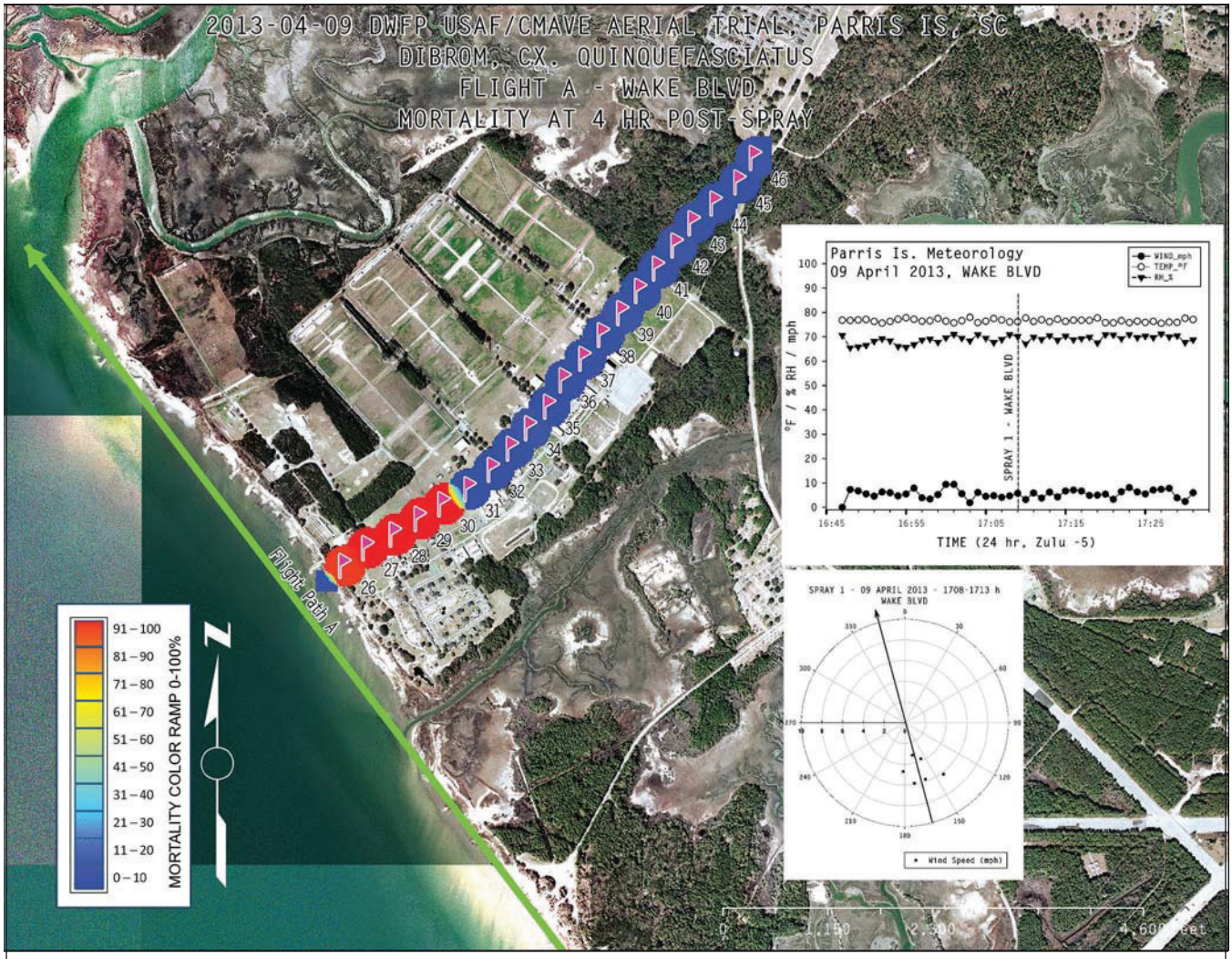

Figure 2A. Caged mosquito mortality at 12 hours after pesticide application and meteorological data for pesticide release April 9, 2013, on sampling array located on Wake Blvd, Parris Island Marine Corps Recruit Depot, SC. Flight path of aircraft is depicted by green arrow.

Initial knockdown from the bioassay cages was recorded after a 40 minute hold following each application, and the sentinel cages were placed in ice chests. Sentinel mosquito mortality was subsequently recorded at 4 and 12 hours postspray, and all mortality was corrected using Abbott's formula as described above.

Trial Set 8

Two bioassay tests were conducted on October 9, 2014, at the Parris Island MCRD, SC, with parameters similar to those in Trial Set 7. In both tests, 30 sentinel mosquito cages were deployed along Wake Blvd, each separated by approximately 250 ft (76.2 m), with an additional set of 10 sentinel cages for each trial located upwind of the spray area on the southern end of the island. The first sampling location in both trials was positioned at the southwest terminus of Wake Blvd. Fuselage booms were equipped with 15 flat-fan TeeJet 8003 nozzles, 7 on the left boom and 8 on the right boom. The MASS parameters during the tests were a flow rate of 2.8 gal/min (10.6 L/min) and an operating system pressure of 40 psi in the first trial and 52 psi in the second trial. Postapplication protocols for tallying mortality data collection were the same as those described in Trial Set 7.

In the first trial, 2 roughly overlapping passes (swaths) were made as the aircraft dispensed adulticide perpendicular to the sampling line over sampling locations 10 and 13 (Figure 3A). Spray-on times were 20 seconds prior to and after intersection of the aircraft with the sampling points on the ground.

In the second trial, 2 swaths were applied with similar spray-on times as in the first test, with the aircraft

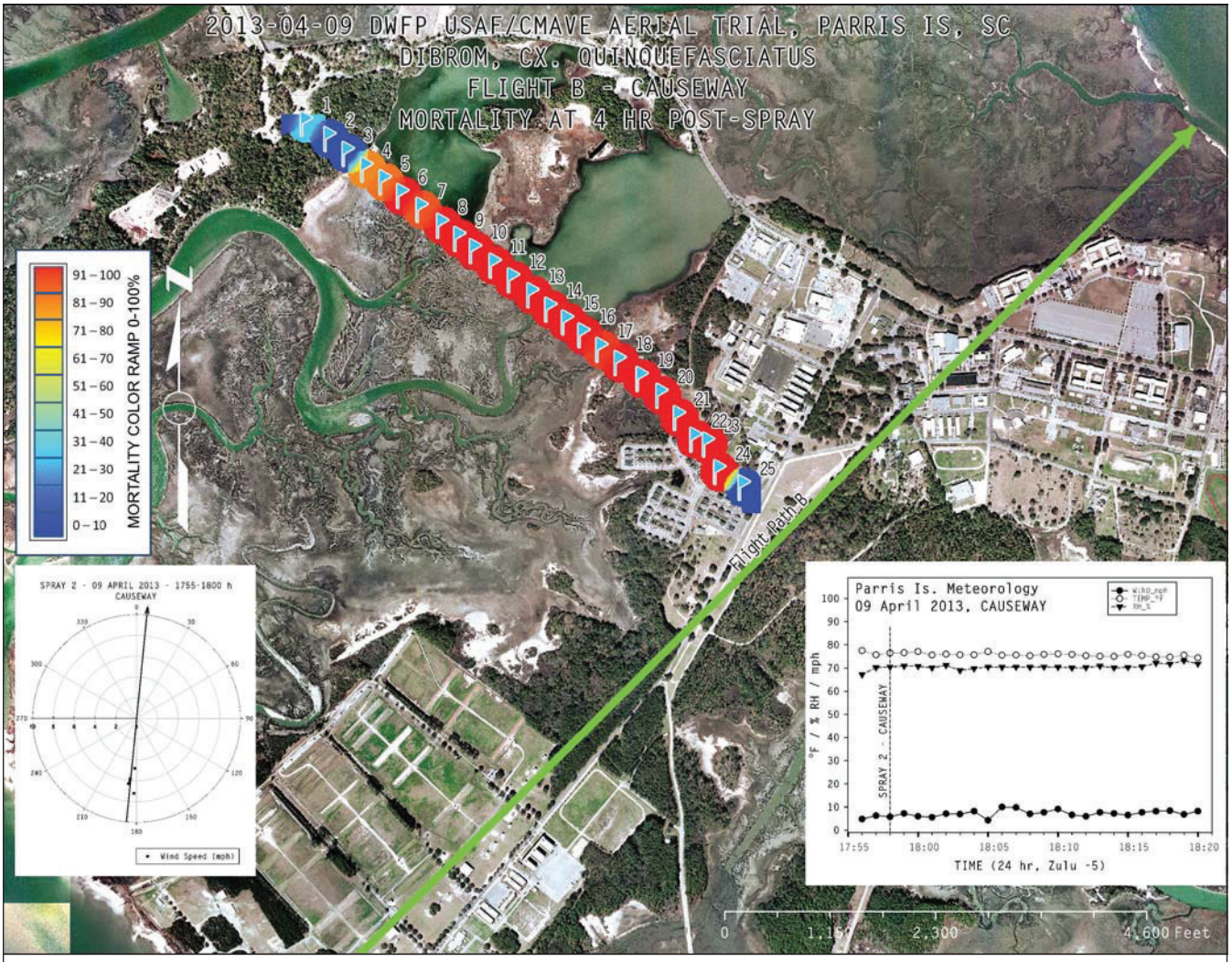

Figure 2B. Caged mosquito mortality at 12 hours after pesticide application and meteorological data for pesticide release April 9, 2013, on sampling array located on Causeway, Parris Island Marine Corps Recruit Depot, SC. Flight path of aircraft is depicted by green arrow.

dispensing adulticide while flying over sampling locations 1 and 7, essentially skipping a 1,000 foot (304.8 m) swath (Figure 3B).

Results and Discussion

Trial Set 1

No data were collected from the first test conducted on January 14, because no droplets were visible on the microscope slides. Wind speed and direction at the time of the test was between 9 and 15 knots from 290 degrees measured at ground level. We speculate that the wind speed was too high, and at the height the simulant was applied combined with the 20 degree crosswind component, the material may have missed the sampling array altogether. Wind speed and direction on the January 15 test was 2-4 knots at 310 degrees. Droplet VMD ranged from approximately 32 μm 1,700 ft (518.2 m) from the aircraft release point, increasing to approximately 50 μm at 3,400 ft (1036 m) and 5,100 ft (1,554.5 m), followed by a roughly linear decrease in VMD at 13,600 ft (4,145.3 m) from release point. Droplets were not detected at sampling stations greater than this distance, or at the sampling location directly beneath the aircraft flight path. Wind speed and direction on the January 16 test was 6-7 knots from 188 degrees. Droplets collected at the sampling location 2,500 ft (762 m) from the release point were approximately 68 μm, with the remainder of droplets collected by the samplers ranging from 30-50 μm up to 22,500 ft (6858 m) from the release point. Droplet density increased from approximately 0.75 drops/cm^2 at the 2,500 foot (762 m) sampling location, to over 2.25 drops/cm^2 at 10,000 ft (3,048 m), and then decreased to between 0.5 and 1.0 drops/cm^2. For both ofthese tests, no droplets were collected directly

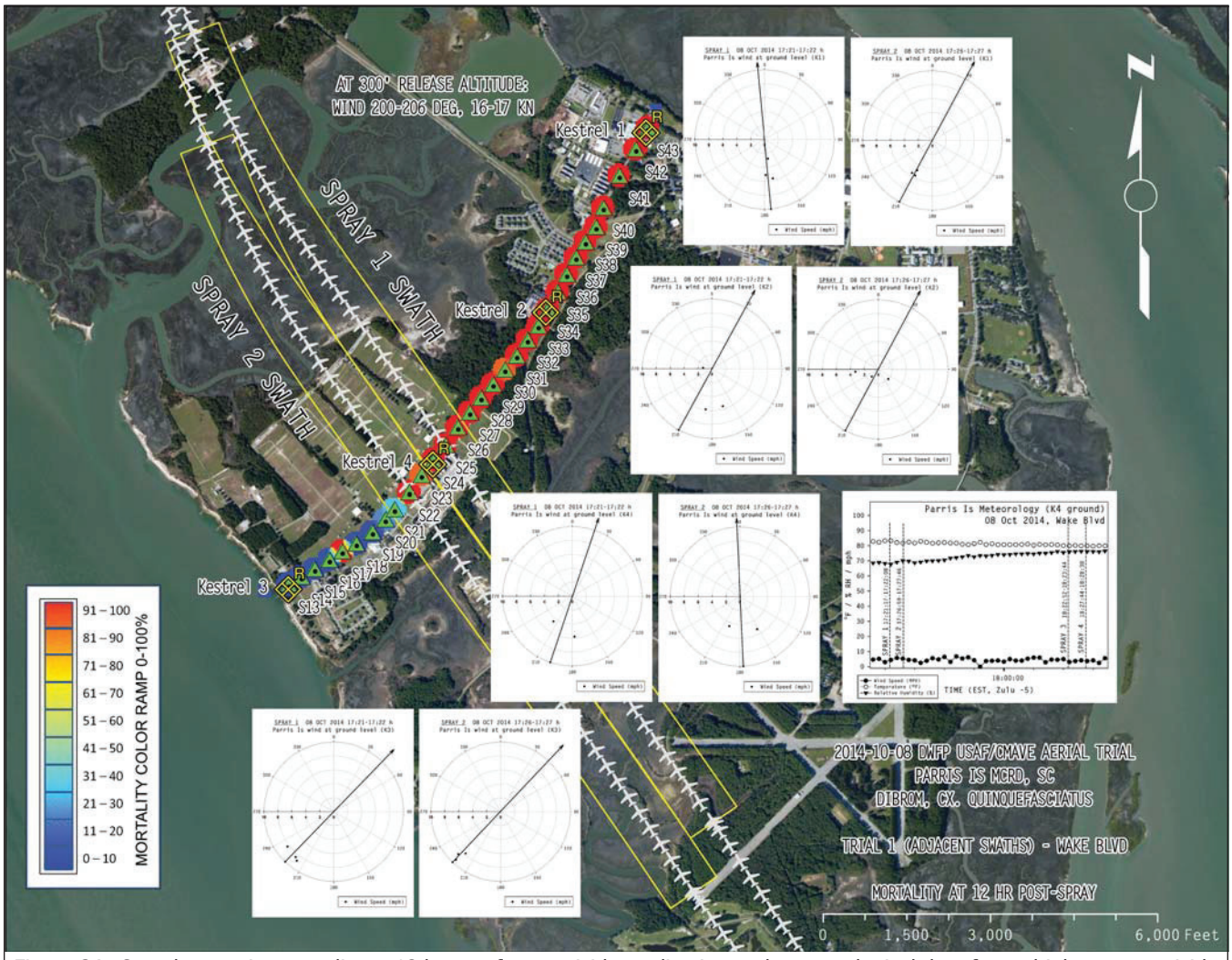

Figure 3A. Caged mosquito mortality at 12 hours after pesticide application and meteorological data for multiple pass pesticide release October 8, 2014, on sampling array located on Wake Blvd, Parris Island Marine Corps Recruit Depot, SC.

beneath the release point. As evidenced by the data from the January 15 trial, a lighter wind condition may have resulted in a tighter and more normal distribution of droplet sizes. No droplets were collected at the far limits of the sampling array. This is in contrast with the January 16 trial, where increased wind speed apparently carried the simulant cloud past the limits of our detection devices. Thus, in light wind conditions it appears that the material drift can be reasonably defined, whereas spraying from 500 ft (152.4 m) AGL may not be optimal in terms of controlling or defining drift when sprayed at higher wind speeds.

Trial Set 2

The first test on February 14 yielded no data, possibly due to light and variable winds. Ground wind speed was less than 2 knots and the wind direction shifted more than 180 degrees several times during the test. Conditions were significantly better on February 15. Surface winds ranged from 1.5 to 3 knots and remained consistent in direction from approximately 070 degrees. The highest droplet densities (10-14 drops/cm^2) were seen at stations 3 and 4 at 4,224 (1,287.5 m) and 6,336 ft (1,931.2 m) downwind from the release point, respectively. Densities dropped dramatically by sampling station 4, and remained low from then on. The VMD of droplets was greatest at sampling station 1, exceeding 50 μm, and ranged from 28-42 μm at the remainder of the stations. Because higher droplet densities are generally correlated with greater insect mortality, it appears the portion of the swath with the greatest potential for effective mosquito control may in this case be an effective swath of approximately 2,000 ft (609.6 m), beginning approximately 3,000 ft (914.4 m) downwind of the

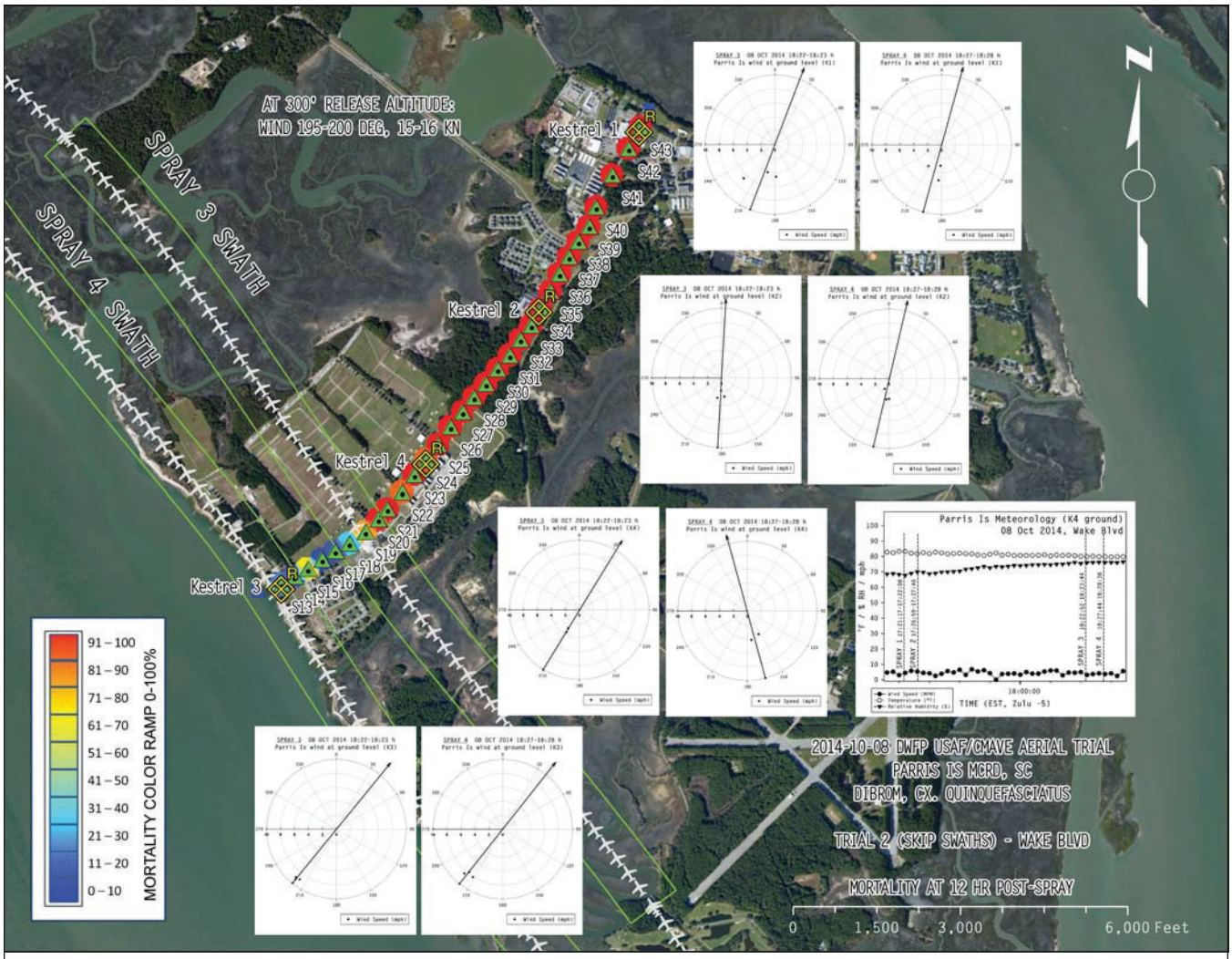

Figure 3B. Caged mosquito mortality at 12 hours after pesticide application and meteorological data for skipped swath test October 8, 2014, on sampling array located on Wake Blvd, Parris Island Marine Corps Recruit Depot, SC.

release point. While this test was encouraging, the lack of a concurrent bioassay data renders this observation unsubstantiated. Ground conditions on the February 16 test remained favorable, with surface winds averaging 1.5 to 4 knots, though wind direction was slightly more variable in nature, ranging from 360 to 030 degrees. Winds at altitude were 11 knots at 060 degrees. Droplets were only recovered downwind as far as station 6 (8,712 ft downrange (2655.4 m)). Droplet sizes were greatest at station 2 (3,485 ft (1,062.2 m)), though droplet density in the aerosol cloud was relatively low (less than 5 drops/cm^2). Droplet densities were greatest at station 1 (1,742 ft (530.9 m) downrange). Discrepancies in the data from the 2 tests might have been attributed to the significant crosswind component at the 500 ft AGL release point, where winds were almost 090 degrees from the theoretical direction that could have led to greater drift and dispersion. In this case it appears only a small leading edge portion of the aerosol cloud was effectively sampled. While both trials effectively indicated that an aerosol cloud generated at 500 ft (152.4 m) AGL can descend to ground level, they also suggest there might be a fairly low tolerance for crosswind components, making spraying from this altitude much more unpredictable.

Trial Set 3

Ground wind conditions for the December 6 test were 3-5 knots at 045-060 degrees for 30 minutes prior to the test, but wind direction subsequently shifted to 080-092 degrees at the time of application. For this test, post-application mosquito mortality was 100% at the second sampling station 2,500 ft (762 m) downrange of release point, and then dropped dramatically to approximately 20% at 5,000 ft (1524 m) downrange. Droplet size and

density remained consistent at both of these sampling locations, with approximate VMDs of 45 and droplet densities of 85-100 drops/cm^2. Data from the December 7 test yielded significantly different results. Surface winds during the test ranged from 1.2 to 3.5 knots at 340-360 degrees, while winds at altitude during the application averaged 2 knots at 324 degrees. As occurred the day before, insect mortality was 100% at the second sampling station 2,500 ft (762 m) downrange, and dropped to approximately 75% and 20% at sampling stations 3 and 4 (1,524 m, and 2,286 m), respectively. Droplet diameters and droplet densities were very different from the previous test: VMDs at sampling stations 2 and 3 were approximately 11 μm, and droplet densities were 20 drops/cm^2 and 10 drops/cm^2 at the sampling stations 2,500 (762 m) and 5,000 ft (1,524 m) downrange. No droplets were collected past the third sampling station. It appears that in the first test the wind shift encountered during the application may have resulted in only a small portion of the aerosol cloud coming in contact with the sampling stations and caged mosquitoes. We speculate that perhaps only the leading edge of the spray with the associated larger and faster depositing droplets encountered the sampling array. Conversely, it would appear that wind shift prior to application in the second trial may have resulted in the heavier fraction of the spray moving perpendicular to the sampling array, with only the finer fractions which dispersed by diffusion or entrainment in the wingtip vortices contacting the remainder of sampling stations. Thus, while it appears that application at 500 ft (152.4 m) AGL can result in insect mortality at ground level, it is apparent that the vagaries of environmental conditions might be greatly amplified while spraying at higher altitudes.

Trial Set 4

Surface wind conditions were ideal during the December 8 trial, with wind speed averaging 3.9 knots from a steady 360 degree direction. Droplet size (VMD) ranged from approximately 43 μm at the sampling station 2500 ft (762 m) downwind, generally decreasing to 27 μm 10,000 ft (3048 m) downrange. Droplet density peaked at the first sampling point (1,250 ft (381 m) past release point) at 25 drops/cm^2. Droplet density decreased at all further sampling stations with the exception of sampling station 3, which was located 3,750 ft (1,143 m) from simulant release point. Trial 2 on December 9 produced generally similar results. Surface winds were again relatively light. Wind speed was 3-5 knots with directions ranging from 340-016 degrees. The highest droplet density was seen at sampling station 2 (2,500 ft (762 m) downrange) with corrected densities of approximately 27 drops/cm^2. Droplet densities fell to roughly 15 drops/cm^2 from station 3 (3,750 ft (1,143 m) downrange), to station 6 (7,500 ft (2,286 m) downrange), after which densities fell dramatically. Droplet size was fairly consistent, in the 40-50 μm range for the first 5,000 ft (1,524 m) downwind, after which it tapered off to approximately 28 μm at the end of the sampling array. Based on relative similarities and consistencies of these 2 tests, the data would suggest under similar circumstances that a nominal swath width of perhaps up to 5,000 ft (1,524 m) may be warranted when spraying at 300 ft (91.4 m) AGL, although that had not been empirically tested with bioassays at this point. Unfortunately, in these trials, there was no sampling station at the release point, and as such, we cannot speculate on any outcomes from the spray release point out to 13,500 ft (4,114.8 m) downwind. Perhaps the most important point from these trials is that it appears detection levels of pesticide (or simulant) when dispensed at a lower altitude (300 ft (91.4 m) AGL), display a much more concentrated effect in terms of droplet densities and VMD than those previously demonstrated in higher altitude tests. Previous trials dispensing at 500 ft (152.4 m) AGL indicated some detection of pesticide/simulant past 18,000 ft (5,486.4 m) downwind. These trials suggest that while material may well indeed drift over 2 miles (3.21 km), the potential for effective mosquito control may be within 5,000 ft (1,524 m) downwind or less of the release point.

Trial Set 5

The surface conditions on January 24 had ground winds of 6-7 knots from 290 degrees. Droplet size ranged from 42 μm to approximately 31 μm in diameter, with most of the VMD size classes around 40 μm out to 4,000 ft (1,219.2 m) downwind of application. Droplet density was greatest at 1,250 ft (381 m) downwind at approximately 5.8 drops/cm^2, and then decreased in a semilinear fashion to where droplet densities were almost zero at 5,000 ft (1,524 m) downwind. Conditions on January 25 were surface winds averaging 4 knots from 280 degrees. As expected, VMD was greatest at the first 2 sampling stations (750 ft (228.6 m) and 1,250 ft (381 m)), and then decreased at 5,000 ft (1,524 m) downrange. Droplet densities were greatest at the first sampling station at 12.7 droplets/cm^2, and decreased to approximately 2.0 drops/cm^2 by sampling station 4 at 2,750 ft (838.2 m) downrange. The January 26 surface conditions were 6-7 knot winds at a direction of 350 degrees. Droplet size (VMD) was greatest at 750 ft (228.6 m) downrange (approximately 60 μm) and then dropped until 2,750 ft (838.2 m) downrange where it peaked again at 61 μm. Droplet density was maximized at 1,250 ft (381 m) downrange at approximately 14.0 drops/cm^2. Droplet density approached zero at 2,750 ft (838.2 m) postrelease point. From these 3 trials at an application altitude of 400 ft (121.9 m) AGL, it appears that the droplets dispensed from the aircraft

did show a fairly predictable swath in terms of width and droplet size. Though the range of detectability appeared acceptable, we consider that the droplet density was not—all measured statistics were a result of 3 passes in these test iterations versus a single pass as would be used in a normal mosquito control operation calibration trial. Again, we speculate that while a certain amount a material drifts to the ground or is pushed to the ground by the dynamic effects of the aircraft, perhaps much of the material, that is, the smaller fractions of the aerosol cloud, may be simply drifting away. However, this hypothesis has not been substantiated by field sentinel bioassay data under these conditions.

Trial Set 6

In this bioassay trial, both tests were conducted on the same day, October 12. Surface conditions on the ground during these field tests was an average wind speed of 1-5 knots from 050 degrees, and remained consistent for both trials. No swath characteristics were recorded. In the first trial, 100% mosquito mortality at 1 hr, 12 hr, and 24 hour was witnessed at the sampling station 500 ft (152.4 m) downwind of release point. This was true also at the station located 1,000 ft (304.8 m) downwind, after which mosquito mortality dropped to approximately 20% at 1,500 ft (457.2 m) downwind, and to almost zero thereafter. The second trial indicated similar, but slightly skewed results. Mosquito mortality of 100% was observed 750 ft (228.6 m) downwind of application, and was continued to 1,500 ft (457.2 m) downrange, at which time mortality dropped to approximately 30% at 2,000 ft (609.6 m) downrange. Interestingly, mortality increased significantly at stations 2,500 ft (762 m) or more. This result was unexpected, and we have no explanations for the apparent anomaly. In these conditions, both of these tests would suggest an effective swath from 300 ft (91.4 m) AGL release height to be at minimum 1,000 ft (304.8 m), with the effective edge at approximately 500 ft (152.4 m) for these particular conditions. Of particular note, however, is that, under these conditions, mosquito mortality was quite low at least after 2,000 ft (609.6 m) downwind of application. The benefit of these bioassay data from a single-pass application is that we might determine a minimum baseline for functionality of aerial application under these conditions when pesticides are applied at 300 ft (91.4 m) AGL.

Trial Set 7

Field sentinel cage insect mortality for a bioassay conducted on April 9, 2013, located on Wake Blvd, Parris Island MCRD is shown in Figure 2A. Wind direction was approximately 160 degrees and wind speed ranged from 2-6 knots. There was zero mortality (Abbott corrected) at all stations when examined at 15 minutes postspray. At 4 hours postspray, the first 5 stations in the sampling array exhibited 100% mortality. The remainder of the stations showed zero to less than 10% corrected mortality. The situation at 12 hours postspray was not significantly different than that the 4 hour observation. It appears that in this test our effective swath was approximately 2,000 ft (609.6 m), which is somewhat expected in that due to a last minute wind shift, the prevailing wind was almost parallel to the flight path of the aircraft, as opposed to the theoretically optimal crosswind application condition to maximize drift. What appears to have happened is that the crosswind component (approximately 10-40 degrees) essentially allowed the applied spray to cut the angle and brought the aerosol cloud in contact with the first few bioassay cages and very little else. The increased altitude may have also caused some significant diffusion of the cloud before it actually reached ground level. Figure 2B shows field sentinel cage mortality data and meteorological data for the sampling array located on Causeway, Parris Island MCRD on April 9, 2013. Winds at spray release were from approximately 190 degrees and ranged from 2-8 knots. When observed at 15 minutes postapplication, no mortality was noted in any of the test cages. When reexamined at 4 hours postapplication, all test cages from station 4 to 24 along the causeway indicated 95% to 100% mortality of caged mosquitos. In this test, no mortality was noted greater than approximately 6,000 ft (1,828.8 m) downwind of aircraft flight path. No mortality was noted at station 25, which was offset from the aircraft flight path by approximately 1,000 ft (304.8 m). No significant differences between 4 hour and 12 hour mortality were observed. Wind direction was much more favorable for this trial than the previous trial conducted on Wake Blvd. Direction was much closer to perpendicular to the flight path of the aircraft, and for this test under these conditions suggests that an effective swath in terms of highest mosquito mortality at or near ground level may be up to 4,000 ft (1,219.2 m). Also, at altitude dispensed with relatively moderate wind speeds, an offset of approximately 1,000 ft (304.8 m) may be anticipated when applying in almost direct crosswind conditions. We were encouraged by these results as they provided an indication that good control over fairly wide areas may be achieved when applying pesticide at 300 ft (91.4 m) AGL.

Trial Set 8

Figures 3A and3B show field sentinel cage mortality data from the trials conducted on October 8, 2014. These trials were slightly different than those in Trial Set 7 in that it was a combined application of 2 adjacent swaths as opposed to a single swath test. Winds at release were from 200-208 degrees (averaged from

4 weather stations), and wind speed varied from 2-8 knots at the time of the test. As before, no mortality was noted in caged mosquitoes 15 minutes after application, however, mortality at 4 hour postspray was substantial among all downwind cages. Figure 3A shows mortality measured 12 hours postapplication, which was not significantly different than mortality noted at 4 hours postapplication. Mortality of 90% to 100% was observed in all cages from station 22 to station 43, an approximate distance of 6,000 ft (1,828.8 m) downwind of the first spray swath flown. Unfortunately, our sampling stations did not extend beyond this point, so it is unknown if the effective swath may be greater than 6,000 ft (1,828.8 m). Interestingly, near 100% mortality was noted at cages which were located directly below the aircraft, which was not expected as the previous single-swath trial under similar conditions suggested a 1,000 foot (304.8 m) offset might be expected. Though we have no definitive explanation for this phenomenon, it is possible that the relative open area associated with the rifle range may have resulted in local unpredictable air movement at the interface of open and wooded areas, or areas dominated by other structures.

Field sentinel cage mortality data for the second trial conducted on October 8, 2014, is shown in Figure 3B. This trial was set up similarly to the previous trial, with a 2-pass application, but the second swath was offset 2,000 ft (609.6 m) from the first. Wind direction averaged from 195-200 degrees, and wind speed ranged from 2-7 knots. Mortality of 90% to 100% was observed at all stations from sampling site 20 to sampling site 43, which encompassed an area approximately 7,000 ft (2,133.6 m) downwind of release of pesticide. Again, it is unknown if the effectiveness of the pesticide may have gone farther downrange, as this was the limit of the sampling array. In this second trial it is interesting to note the apparent lack of efficacy of the second applied swath, as there was very little mortality noted in sampling locations 13 through 18. We speculate that either the interface of the water and land produced some cryptic wind conditions, perhaps an extremely localized offshore breeze, or conditions were such that the pesticide drifted farther downrange before ultimately settling. Regardless, it again appears that pesticide application at this altitude can have significant efficacy against target insects at or near ground level, which is the optimal outcome of aerial sprays of this type.

General Conclusions

This article summarizes outcomes from a series of trials using a variety of aerial spray configurations in an array of meteorological conditions that provide select empirical data to investigate efficacy of higher altitude pesticide applications that may be required for effective nighttime aerial pesticide missions. In general, based on our swath characterizations and bioassays, we believe that while pesticide applications at altitudes of 500 ft (152.4 m) and 400 ft (121.9 m) AGL may be effective under absolutely ideal meteorological conditions, lower altitude application will achieve more consistent results with regard to target insect mortality and allow more reliable prediction of where pesticide may drift. We believe the results of these trials, in particular the 300 ft (91.4 m) AGL mosquito sentinel tests, provide supporting evidence that nighttime applications of pesticides may be very efficacious at lower altitudes. Given that we are developing these innovative, emerging techniques, we acknowledge that these data only provide preliminary quantification of efficacy and drift, which may be different when pesticides are applied in nighttime hours as opposed to daylight. Additional future research and testing will be required to fully validate aerial nighttime pesticide application. However, the research trials described here provide the key baseline data to guide future research.

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Announcing
The 2015 Spurgeon Neel
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Toxicity Testing in the 21st Century: Refining the Army's Toolbox

LTC Erica Eggers Carroll, VC, USA
Mark S. Johnson, PhD, DABT

In 2007, the National Academy of Sciences (NAS) published a comprehensive report, "Toxicity Testing in the 21st Century: A Vision and a Strategy"[1] in response to a US Environmental Protection Agency request to the National Research Council to develop a plan using innovative methods to advance toxicity testing. Toxicity determination in the previous half-century required animal testing of all new chemicals (medicine, food additives, industrial, consumer, and agricultural chemicals) for potential to cause cancer, birth defects, and other adverse health effects. Animal testing is slow, expensive, uses many animals, and often requires assumptions and controversial extrapolations.[1]

The NAS vision discussed harnessing technologies developed in emerging fields such as systems biology (eg, use of computational models fused with in vitro laboratory data) and bioinformatics (computational models to analyze massive data sets), with high throughput screening assays of effects of chemicals on human cells, cellular components, and tissues, at low levels, which are typically more relevant.[1] The report recommended identification not only of changes at the molecular level, but of signal transduction and other pathways that, when perturbed, lead to adverse effects. Study of these influences may lead to improved predictability of health effects in human populations. Mapping such "toxicity pathways" and then discerning actual or predicted perturbations by means of computational models and high throughput screening of chemicals:

> could reduce the backlog of the large number of industrial chemicals that have not yet been evaluated under the current testing system.[1(p30)]

The new approach would most likely reduce animal use as well. However, many of these methods are new; their value remains to be validated in predicting effects or determining safe levels of exposure for humans.

In 1985, the Office of The Surgeon General (OTSG) designated the Army Institute of Public Health (AIPH), now part of the Army Public Health Command, as lead agent of the Health Hazard Assessment Program.[2] In fulfillment of that mission, the AIPH Toxicology Portfolio is charged with evaluating the toxicity of specific military-unique chemicals in materials entering the Army supply system.[3] The primary goals have been (1) to identify health hazards associated with exposure to new substances used in military applications, (2) to provide a technical foundation for approvals (or disapprovals) to eliminate or control hazards associated with manufacturing-related exposures and use and disposal of weapons, equipment, clothing, training devices and other materials.[3] The Toxicity Clearance (TC) is the instrument used for this evaluation. It provides a technical basis to help the acquisition program manager make important life cycle decisions. Solvents, fire extinguishing agents, repellents, fabric finishes, refrigerants, explosives, energetics, propellants, pyrotechnics, hydraulic fluids, metals/alloys, and pest control agents are examples of substances used in systems that have been evaluated. Toxicity Clearances are provided for specific applications and are generally not applicable to systems with different use conditions.[2]

In accordance with the NAS recommendations and Department of Defense mandates to improve efficiency in research, development, and acquisition, the Toxicology Portfolio has implemented a phased approach to toxicity testing and has expanded its toolbox of in silico, in vitro, as well as in vivo methods to better evaluate potential health and environmental threats, ideally, before a new substance is approved for entry into the Army supply chain. Used in a relative manner, these emerging methods can be used in side-by-side comparison to determine which substance is likely to cause health effects from exposure and use and which would present a lower hazard risk.

The phased approach to toxicity testing is intended to identify and characterize occupational and environmental human toxicity concerns as early as possible in the science and technology (S&T) phase of research, prior to transition to an advanced developer. Even before a new material has been synthesized, its properties and performance can be estimated and evaluated using computer modeling techniques that allow toxicity and physical properties to be assessed.[4] Modification, reformulation, or even substitution at the S&T stage of development, generally in budget activity levels (BA) 1 to 3

would most likely be significantly less time-consuming and less costly than comparable changes at BA stages 4 to 8. An example of the phased approach to toxicity testing is underway with the upgrade for the 2.75-inch Hydra rocket:

> The Hydra is one of the most extensively used munitions in the Army, but environmental concerns are associated with it. The training warhead for the rocket contains perchlorate. The propellant contains lead…The phased approach to environmental safety and occupational health (ESOH) was used to replace components of the Hydra with safer formulations. These replacement compounds are now entering the final stages of approval and implementation.[4]

Another success story involved the reformulation of the propellant for the M-115/116/177 (whistle, bang, flash) simulators. The original formulation used ammonium perchlorate as the propellant that resulted in significant contamination of training ranges where used. The new formulation contained a more traditional black powder mix that was just as effective on the ranges, without resulting in toxic environmental residues, loss of performance, or significant addition to cost. Both formulations were evaluated side-by-side and recommendations made using this approach.

Rather than waiting for an upgrade, phased toxicity testing should be a prerequisite to reach a given Technology Readiness Level (TRL) for new products or systems in initial development. The type of toxicity assessment would be selected to be compatible with the stage of development. For example, at the risk of repetitiveness, even before a new material has been synthesized, properties and performance of the substance can be initially evaluated using computer modeling techniques to identify potential toxicity. More sophisticated (and more extensive) tools would be used later in development, but still prior to transition to a weapon system or platform. For example, in silico assays that provided reasonable estimates of confidence regarding toxicity would be followed by in vitro assays, including measures of mutagenesis, genotoxicity, and cytotoxicity assays. These measures provide data that help address targets of toxicity and potential mechanisms for extrapolation to soldiers, civilians, and the environment. Focused animal testing would be reserved for those candidates selected as most likely to be efficacious and safe, in preparation for transition to a program executive officer or advanced developer like the US Army Medical Materiel Development Activity.

Currently, a Programmatic Environmental, Safety, and Occupational Health Evaluation (PESHE) is not required until Milestone B, at which the product must be at TRL 6. New S&T products are often considered for transition about the BA-3 level at roughly TRL 3-4. It is generally accepted that a primary cause of failure to transition includes "lack of technical maturity." The National Environmental Policy Act of 1969 (Pub L No. 91-90, 83 Stat 852 (1969)) mandates full disclosure of possible impacts, alternatives and environmental mitigation measures. Moreover, if not evaluated alongside S&T, the program manager runs the risk of development of a system that may cause injury to the Warfighter or worker, or not lend itself for sustainable use at testing and training ranges. Therefore, it behooves the S&T community to examine the potential for toxicity as part of technology maturity determination, prior to or at least as part of the transition process into an acquisition program of record. Codifying the need for appropriate toxicity assessment in the technology transfer agreement is therefore important. Additionally, since the PESHE is currently the first requirement addressing toxicity and is not required prior to the product advancement to TRL 6 and Milestone B, some products may require reformulation or replacement due to toxicity. This would be fairly late in the acquisition pipeline, by which time the Army might have already committed hundreds of thousands of dollars to a product. This practice is inconsistent with Executive Order 13514 ("Federal Leadership in Environmental, Energy and Economic Performance, 2009) which required:

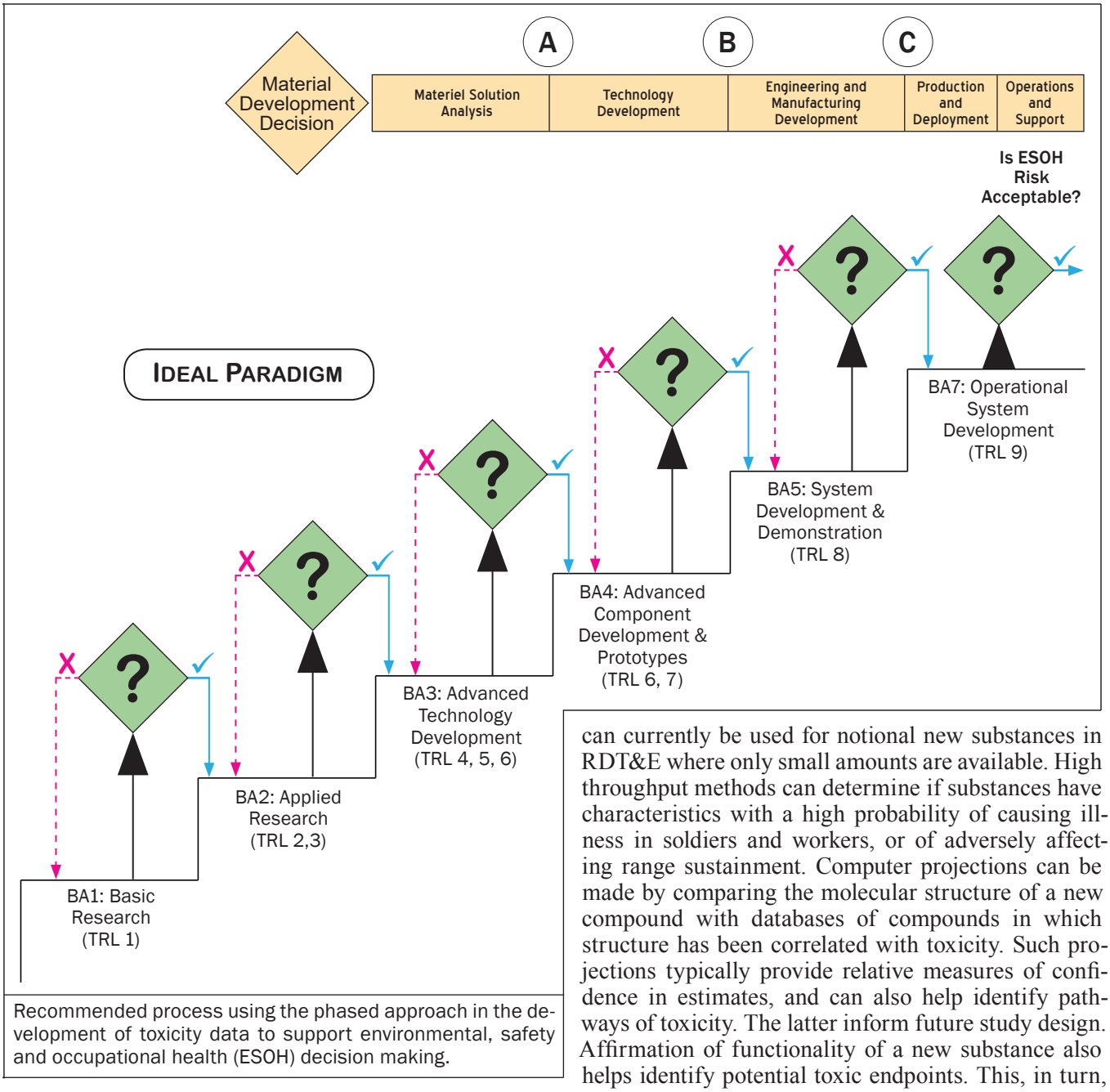
> …minimizing the generation of waste and pollutants… [and] reducing and minimizing the quantity of toxic and hazardous chemicals and materials acquired...[5(p3)]

There is no guidance on what data are needed to help make environmental safety and occupational health (ESOH) decisions for the PESHE or elsewhere regarding toxicity testing, therefore, program managers accept risks based solely on available ESOH data.[6] The proposed phased approach:

> …seeks to make an ESOH evaluation compatible with each stage of the development process by applying appropriate assessment tools…[and] adds a data requirement to each stage for which managers can plan and program,…[6(p53)]

A proposed requirement for appropriate toxicity data at each BA level is illustrated in the Figure.

In addition to a phased approach to early toxicity testing, the Toxicology Portfolio has implemented a number of targeted assays to help research, development, test and evaluation (RDT&E) scientists and managers make funding decisions based upon ESOH risks. Moreover, individuals in the Toxicology Portfolio have been working with the Technical Cooperative Program, Key Technical



Recommended process using the phased approach in the development of toxicity data to support environmental, safety and occupational health (ESOH) decision making.

Area 4-42 to develop internationally harmonized methods for use in the development of new weapon systems or platforms to ascertain ESOH hazards.[7]

Although currently a "hot topic" in discussions of toxicity testing and foreseen in the NAS vision as a valuable in silico method, most high throughput methods are still in the research stage. Much work remains to validate their ability to determine maximum safe exposure levels. Use of high throughput methods will require refined dose metrics for calculation of a safe level of exposure using in vitro results. However, these methods can currently be used for notional new substances in RDT&E where only small amounts are available. High throughput methods can determine if substances have characteristics with a high probability of causing illness in soldiers and workers, or of adversely affecting range sustainment. Computer projections can be made by comparing the molecular structure of a new compound with databases of compounds in which structure has been correlated with toxicity. Such projections typically provide relative measures of confidence in estimates, and can also help identify pathways of toxicity. The latter inform future study design. Affirmation of functionality of a new substance also helps identify potential toxic endpoints. This, in turn, helps industrial hygienists and occupational health physicians conduct meaningful surveillance in the workforce.

The AIPH Toxicology Portfolio attempts to assess high priority military-related substances using the above philosophy wherever possible. In Fiscal Year 14 alone, it completed 39 Toxicity Clearances, 21 technical reports (of which 10 are Toxicity Assessments), and 17 peer-reviewed manuscripts. Doctoral level subject matter experts (SMEs) in endocrine disruption, ecotoxicity, developmental toxicity, genotoxicity,

immunotoxicity, and quantitative structure activity relationships make up the 2 branches of the Portfolio (Toxicity Evaluation Program; Health Effects Research Program).[6] Technical experts in inhalational toxicity testing, dermal sensitization, mutagenicity and novel methods team with the SMEs to develop and execute good laboratory practices-compliant protocols using rodents and select sentinel species.

The Toxicology Portfolio is funded by a combination of core Defense Health Program funds and investments by other Department of Defense organizations in collaborative arrangements. As important as toxicity testing is, the contribution of the AIPH Toxicology Portfolio is not widely known. Years ago, new products and materials were fielded based on efficacy and the ability to meet military operational requirements. Only relatively recently have regulatory guidelines mandated that new military products be not only effective (can it function as designed?), but also safe for Soldiers. Some reports state that human male fertility in certain developed nations declined in the 20th century,[8] although others dispute the claim. Regardless, regulatory guidelines for safety assessment of pharmaceuticals and chemicals currently include screens for effects on reproduction and fertility, including stage-aware histopathological examination of the testis, as a sensitive method for detecting disturbances in spermatogenesis.[9-11] These are services which AIPH Toxicology Portfolio personnel routinely perform as part of their mission.

As the nation grows increasingly motivated to spend taxpayer dollars efficiently and to protect the environment to preserve our future, the need for phased and targeted toxicity assessment will most likely become a prerequisite for all R&D investments, and it is reasonable to expect that the AIPH Toxicology Portfolio will remain a leader in this effort.

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The New US Military Role in the European Union's Import Program: Strategic Implications Ensuring Safe Food for the European Theater

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It is known across the Department of Defense (DoD) that the Secretary of the Army is the Executive Agent for DoD Veterinary Public and Animal Health Services, the functional responsibilities for which are assigned to US Army Veterinary Services.[1,2] A critical component of the Veterinary Services mission is the assurance of safe food and water for DoD personnel and their dependents assigned throughout the world, even extending to some US embassies and consulates in the most austere environments.

However, much less is known about the behind-the-scenes, complex mechanisms required to provide those safe food and water sources, achieved only by the consistent and elaborate coordination among military and civilian public health authorities, medical and veterinary personnel, logistics personnel, and foreign customs authorities. It is probably safe to say that the average consumer at an overseas Defense Commissary Agency commissary or an Army and Air Force Exchange Service facility is only vaguely aware of the intensive efforts made on their behalf to ensure constant availability of US food items.

The US European Command (USEUCOM) area of responsibility is a prime example of the enormous complexities involved with exporting and shipping foodstuffs to DoD's multiple locations around the world. As DoD locations span Europe, US government-owned subsistence transits the breadth of the continent, crossing many borders before reaching outlying installations. To support that mission, the processes involved have historically required sound logistics, communication, and teamwork across multiple services. In recent years, however, it has also required strategic diplomacy to integrate those processes into compliance with the European Union (EU) Trade Control and Expert System (TRACES), designed to track animal and animal origin product EU imports.

This article highlights the progression of US military's incorporation into and compliance with TRACES. It outlines the broader strategic implications of United States engagement in that program to ensure food safety and quality assurance of public health for DoD personnel assigned throughout USEUCOM while, at the same time, ensuring, and even strengthening, diplomatic ties and trust with European partners.

As the EU was coming together as a fully functioning regulatory and authoritative body representing its member states, around the year 2000 the European Commission Directorate General for Health and Consumers began to pay attention to animal origin products from the US that were entering the EU bound for US military installations. Movement of such products had been ongoing decades, but the backdrop of a number of high impact, economically costly animal disease outbreaks in the EU, combined with emerging awareness of and concerns about the use of growth hormones, antibiotics, and genetically modified organisms by the US agricultural industry, served to elevate attention even further.

The first of these animal disease outbreaks in the EU began in 1996 with cases of bovine spongiform encephalopathy (BSE), otherwise known as "mad cow disease," occurring in Britain. The scientific community associated human cases of the variant form of Creutzfeld-Jakob disease with the consumption of BSE-contaminated beef, and the resultant economic loss to the United Kingdom (UK) reached an estimated $6.4 billion by early 2001.[3] The BSE outbreak was quickly followed by an epidemic of classical swine fever which began with the first case reported from Germany in January, 1997 and presumably spreading from there to the Netherlands, then on to Italy, Spain and eventually to Belgium.[4] In 2001, the UK's livestock industry was again hit, this time by a foot-and-mouth disease outbreak which crippled exports and cost the UK an estimated £8 to £8.6 billion ($12 to

$13 billion at current exchange rates) to resolve.[5,6] Collectively, these diseases resulted in devastating effects on health, economic, and consumer confidence. It had become obvious that there was enormous potential economic risk posed by the introduction of foreign animal diseases into the EU. This awareness, undoubtedly, contributed to shaping the comprehensive import regulations currently being developed and implemented.

There was, however, another significant change as a result of the 1996 association of human disease with the consumption of potentially BSE-contaminated beef. Historically, the US military had procured beef for the European theater's needs predominantly from US approved European sources (primarily from the UK as it was economically the most competitively priced). But once the protection of human health was at risk according to the best scientific information available, the US military categorically shifted to exclusively procuring beef from US sources via import, as the US beef industry largely escaped infection with BSE and was considered safe. This monumental change of sourcing was to have far-reaching effects approximately a decade later when EU attention focused on US military meat importation.

By 2008, the EU community's internal implementation and compliance with import regulation had reached maturity and the spotlight shifted to US military animal origin imports which were not in compliance with existing regulations. Historically, US military importation had been permitted with the recognition that its purpose was to supply military forces outside the European economy, so installations were treated as "foreign soil" versus host nation. But that "exemption" was coming under increasing scrutiny, and the United States was approached to initiate an effort to comply with new EU requirements. Initial meetings, panels, working groups, and senior leader engagements failed to make progress toward this end, however, and US representatives gradually concluded that the problem would be more difficult to solve than originally anticipated, despite the best intentions.

Preventing the construction of a solution framework was a significant legal hurdle. From the inception of the issue, legal experts in multiple organizations insisted that no governmental organization outside of the Office of the Secretary of Defense (OSD) or the Department of State had the authority to initiate any such agreement. Over many years, multiple legal opinions were issued that effectively prevented any work toward an achievable solution. The chief legal concern was that while the United States and each of the individual member states in the EU are themselves sovereign nations, the EU itself, while a legislative body, is not recognized as a sovereign body and thus has no standing from which to negotiate.

By 2011, with multiple initials attempts failed and no traction toward compliance in sight, the EU's patience was wearing thin and US animal origin product imports were being threatened with refusal at ports of entry. It was clear that the US military had not responded rapidly enough and that EUCOM had to step in to reach a resolution. Finally, in the fall of 2012, senior leaders from EUCOM and other agencies including the Principal Deputy Assistant Secretary of Defense for Logistics and Materiel Readiness and the Joint Staff's J4 and J5 met and concluded that this problem required urgent solution as product procurement was in imminent jeopardy. Ultimately, the OSD determined that because this issue primarily affected forces serving in the EUCOM area of responsibility, the EUCOM Directorate of Logistics could serve as the lead agency to develop and implement policy governing a successful solution.

Point of Entry: EU Border Inspection Posts

First, and most critically, a successful solution was required to address the arrival and acceptance of shipments at EU Border Inspection Posts (BIPs), the importation entry points located at ports, certain borders, and airports. Then, the EU required that US military shipments be tracked from entry to receipt at destination using the EU's electronic tracking database, TRACES. This system is used, along with the information provided in accompanying animal health certificates and other import documents, to produce the EU transit health certificate called a Common Veterinary Entry Document (CVED) which accompanies a shipment. Upon receipt at destination, a shipment is either processed by an exit BIP as having left the EU in the case of transiting goods or, in the case of goods bound for retail operations with consumer sales, its status updated to reflect arrival at its final tracking destination. Thirty days are allowed from the time a shipment is logged at the entry point until it must be closed out. Failure to meet requirements potentially jeopardizes future shipments for a specific importer as compliance history is monitored and a poor track record can result in subsequent refusals of entry.

Progress Towards Solution

Once authority was granted for the involved players to engage with the EU toward constructing a workable framework for US compliance, progress was slow, but at least there was movement. While the US would no longer be permitted to import goods with an entirely free rein, it was recognized that shipments intended for US installations were, in fact, not freely entering the EU

food economy as were most other imported goods. So, despite the fact that US military imported animal origin products that often do not meet EU standards (eg, are not from EU approved sources, are not antibiotic free or may contain unapproved growth hormone), the EU level veterinary authority made the determination that US import shipments would be considered to be transiting the EU until they arrived at designated US installations which were to be considered "non-EU" destinations. This decision took advantage of the existing allowances in EU regulations that permitted the legal transit of goods bound for actual non-EU countries, such as from EU ports through the EU, then out via an EU exit BIP where the shipment is closed out of the TRACES tracking system. This decision, however, did require the official designation of US installations as exit BIPs and the identification and training of personnel at these locations to be able to perform this function.

As the oversight and authority for importation of animal origin products falls under the purview of EU veterinarians and the DoD veterinary mission belongs to the US Army, the US Army Public Health Command Region-Europe (PHCR-E) was delegated authority by USEUCOM to provide direct support and program implementation. The PHCR-E controlled most veterinary assets in Europe and its deputy (the senior veterinary officer) was appointed the Competent Veterinary Authority and granted limited authority to engage the EU directly regarding the importation program, thus ensuring appropriate professional engagement with the European veterinary authorities.

A decision was also made regarding prime vendor warehouse facilities located in the EU which could not technically be considered US military installations. They were assessed and categorized as nonconforming warehouses (permitted under EU legislation). They could function to process transiting goods provided they maintained US government owned goods strictly isolated from goods intended for the EU, and they shipped only to final destinations that included ships' supply, US military installations, or other locations actually outside the EU, such as those under the US Central Command or Africa Command. European Union veterinarians continued to be involved with these facilities which function as both entry and exit BIPs as subsequent shipments are moved on EU generated transit paperwork ("daughter CVEDs" based on the original CVED), then closed out when they reach their ultimate destinations at US installations.

US Interagency Cooperation

Before an export shipment of animal origin product leaves the United States, the Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS)* certifies the goods and issues health certificates confirming their quality. The FSIS is the public health agency within the USDA responsible for ensuring the nation's commercial supply of meat, poultry, and egg products is safe, wholesome, and correctly labeled and packaged—in other words, safe for human consumption per US standards. Once approved by USDA, a shipment can be loaded at a supplier's location, then transported to the east coast for transoceanic movement to European BIPs.

The USDA Foreign Agricultural Service (FAS)† links US agriculture to the world, enhancing export opportunities and global food security, as well as expanding and maintaining access to foreign markets for US agricultural products by removing trade barriers and ensuring US rights under existing trade agreements. The FAS also works with foreign governments, international organizations, and the Office of the US Trade Representative to establish international standards and rules to improve accountability and predictability for agricultural trade. For the DoD, the US Foreign Agricultural Service Mission to the EU, headquartered in Brussels, Belgium, has played an absolutely key role in assisting with issues related to importation of animal origin products to the EU. They were critical in overcoming challenges with the implementation of TRACES and continue to assist with ongoing and emerging situations.

Approaching a Stable End State

In 2013, the multiyear-long process of US military integration into the EU import program culminated with the release of USEUCOM European Command Instruction 4506.01: USEUCOM Guidance Regarding the EU TRACES, which codified the rules of engagement for the working levels of all service components and affected agencies to participate in the European import program. This guidance serves as the cornerstone for ensuring animal origin products including fresh meat and meat products from the US are available at commissaries, dining halls, food courts, schools, day care centers, and other installation food sources.

Combatant commands such as EUCOM are organized and structured for strategic military oversight and direction. As such, it is unusual that EUCOM was designated as the lead to pull together multiple service components and both DoD and non-DoD agencies to safeguard the US European theater's access to US animal origin products. Although progress remains ongoing, it is far enough along to be assessed and considered a success story.

*http://www.fsis.usda.gov/About_FSIS/index.asp

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Evaluation of Postdeployment Cancers Among Active Duty Military Personnel

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Surpassed only by heart disease, cancer is the second highest cause of all deaths, accounting for 1 in every 4 deaths in the United States. According to the American Cancer Society, there will be more than 1.66 million new cancer diagnoses and an estimated 590,000 Americans will die of cancer in 2015.[1] These figures are similar to those reported by the Surveillance, Epidemiology, and End Results Program for 2014.[2] In its most recent Cancer Trends Progress Report – 2011/2012 Update, the National Cancer Institute reports that death rates for the 4 leading types of cancer as well as all cancers combined have been declining, yet incidence rates of some cancers are on the rise.[3] Worldwide, cancer is a leading cause of both morbidity and mortality, with approximately 14 million newly diagnosed cases and more than 8 million deaths attributed to cancer in 2012.[4]

The evidence indicating a connection between occupational and environmental exposures and cancer has been growing in recent years.[5] This is of particular concern because such cancers are theoretically avoidable, as measures can be taken to avoid these nongenetic risk factors. The World Health Organization estimates that 19% of all cancers are attributed to environmental factors, accounting for 1.3 million deaths per year.[6]

The military population presents a unique opportunity to study links between environmental exposures and cancer. Advantageous aspects of studying cancer among military personnel include well characterized person-time, occupation, and, though not always the case, environmental hazards. Access to routine healthcare including recommended cancer screenings at no cost to the service member and robust electronic medical record systems also facilitate assessments of cancer outcomes in the military population. Furthermore, exposures associated with military deployments may influence cancer risk among military personnel.[7] Possible deployment-related exposures have been documented by the Department of Defense,[8,9] to include potential carcinogens (eg, industrial solvents, jet fuel, air pollution, radiation). Behavioral changes during deployment, such as increased tobacco use, have also been documented.[10] It is thus plausible that military deployment and associated exposures may be risk factors for subsequent cancer among warfighters.

Cancer in the Military

Vietnam War

Historically, there has been concern regarding military service-related hazards and potential long-term health implications following military deployment. Postdeployment cancer risk is often at the forefront of the issue, as was the case after the Vietnam War.[11-12] As Richards describes in an article reviewing responses to military-associated environmental and occupational exposures:

> During the latter half of the 20th Century, medical knowledge of and concern about carcinogens grew, and human experimentation guidelines became more stringent. During the Vietnam era, concern for troop exposure to environmental contaminants evolved beyond acute exposures and experimentation to encompass long-term occupational and environmental hazards encountered on the battlefield.[13]

By far, the most prominent exposure in terms of health concern generated during this conflict is the herbicide commonly referred to as Agent Orange. Many veterans of the Vietnam conflict between 1965 and 1972 attribute poor postdeployment health outcomes, including rare cancers, to 2,3,7,8-Tetrachlorodibenzodioxin, an extremely toxic dioxin compound that contaminated one of the compounds used to make the herbicide Agent Orange.[14] The scientific evidence linking postdeployment cancer to Agent Orange exposure during the Vietnam War varies. Some studies have not found higher rates of mortality for outcomes such as soft tissue sarcomas,[15] Hodgkin's disease,[16] non-Hodgkin lymphoma, or testicular cancer in Vietnam veterans.[17,18] Another study of participants of the Agent Orange Registry had similar results, showing no difference in prevalence for any type of cancer when comparing Vietnam veterans to non-Vietnam veterans.[17] However, the CDC Selected Cancer Study reported a higher risk of non-Hodgkin's lymphoma among Vietnam veterans when compared to other men.[19] Frumkin summarized the existing literature on Agent Orange and cancer, reporting consistent

to fairly consistent negative results for increases of soft tissue sarcomas, Hodgkin's disease, and gastrointestinal and brain cancers, but inconsistent results of increases in respiratory and prostate cancers among Vietnam veterans.[20] Still yet, in the current Institute of Medicine Report of the health effects of herbicides used in Vietnam, *Veterans and Agent Orange: Update 2012*,[21] the committee found sufficient evidence of an association between soft tissue sarcomas, non-Hodgkin lymphoma, chronic lymphocytic leukemia, and Hodgkin lymphoma, and limited/suggestive evidence of an association with laryngeal, lung, bronchus, trachea, and prostate cancers as well as multiple myeloma.

1991 Gulf War

Similar to those of the Vietnam conflict, many veterans of the 1991 Gulf War are also concerned about the specter of cancer and possible links to hazards associated with their deployment. Notable hazards of concern to service members during the Gulf War include depleted uranium, petroleum products, pesticides, and chemical and biological warfare agents.[22] However, scientific literature shows mixed findings regarding potential associations between Gulf War exposure and postdeployment cancer risk. A particular exposure event of interest during the Gulf War was the destruction of chemical munitions at Khamisiyah, Iraq. While Bullman et al indicated an increased risk of brain cancer mortality among US Army Gulf War veterans who were potentially exposed to low-level chemical warfare agents at Khamisiyah when compared to Gulf War veterans who were not exposed,[23] a later study by Young et al found no excess in brain cancer.[24] In his report on a study on testicular cancer following Gulf War deployment, Levine stated:

> …testicular cancer was found to be the only significantly increased malignancy among deployed Persian Gulf War veterans. The increase became apparent 2 to 3 years after the Persian Gulf War and peaked 4 to 5 years afterward.[11]

Yet, Knoke et al found that although there was an initial increase in testicular cancer immediately following deployment among Gulf War veterans compared to nondeployed Gulf War era veterans, the difference was no longer observed by 4 years postdeployment.[25] Kang et al described "very small rate differences (less than 1.0%) between Gulf veterans and non-Gulf veterans" for both skin cancer and other cancers, with higher rates among the Gulf War veterans.[26] Kang and Bullman reported

> ...no significant excess of overall cancer deaths or deaths from cancer at any specific site among Gulf veterans compared with non-Gulf veteran controls.[27]

In a 2005 report, *Gulf War and Health*, an Institute of Medicine committee found sufficient evidence of an association between combustion products and lung cancer and limited/suggestive evidence of an association between combustion products and nasal, oral, laryngeal, and bladder cancers and between hydrazines and lung cancer. There was inadequate/insufficient evidence to support conclusions regarding potential associations between fuels, combustion products, hydrazines, and nitric acid for numerous types of cancers.[28]

Operations Enduring and Iraqi Freedom

Deployment-related exposures are now causing the same concerns regarding cancer among service members following support of Operations Enduring Freedom (OEF) and Iraqi Freedom (OIF). Since 2001, in excess of 2 million US military personnel have deployed to Southwest Asia,[29,30] with environmental hazards including but not limited to pollutants from local industry; military-produced exhaust from vehicles, machinery, and generators; open air burn pit emissions and fumes from fires; high levels of indigenous ambient particulate matter; munitions and weapons; depleted uranium; and radiation.[7,31-39] Potential relationships between exposures in theater and cancer diagnoses subsequent to deployment are again a priority for researchers and public health professionals in the military community.

Baseline Cancer Rates

In the population of OIF and OEF veterans, one expects a certain amount of cancer to occur, irrespective of deployment history and associated deployment-related environmental exposures. Understanding baseline rates of cancer in the military population is useful when evaluating whether cancer among service members with a history of deployment in support of OIF and/or OEF occurs at excessive rates. Cancer investigations in military populations typically focus on specific types of cancer or are specific to a single service branch. This was the case when Yamane reported on cancer incidence from 1989-2002 among Airmen. In comparison to the general US population, he found standardized incidence ratios for all cancers to be lower than expected among male Air Force service members and as expected among female Air Force service members.[40] Zhu et al later compared incidence rates of a select group of cancers (lung, colorectal, prostate, breast, testicular, and cervical cancers) across the military to US civilians. The authors reported lower incidence rates of colorectal, lung, and cervical cancers, and higher rates of prostate and breast cancers.[7] Although these comparisons provide valuable information, knowledge of rates across all service branches for all types of cancers is important.

In June 2012, the Armed Forces Health Surveillance Center published a report describing incident diagnoses of cancers and cancer-related deaths in active duty

military personnel from 2000-2011. Results for the 12-year surveillance period showed a crude incident rate of 55.2 per 100,000 person-years, with the lowest annual incidence rate of 50.3 per 100,000 person-years occurring in 2003 and the highest annual incidence rate of 60.1 per 100,000 person-years occurring in 2009. The data indicated no apparent increasing or decreasing trends in overall or site-specific incident cancer diagnoses. Of note, rates of cancer diagnoses among active duty military members have remained stable since 2000.[41]

Identifying Carcinogens

More than 900 agents have been evaluated by the International Agency for Research on Cancer for determination of potential to cause cancer. A group of four different categories is utilized to classify every agent: carcinogenic to humans (Group 1), probably or possibly carcinogenic to humans (Group 2A and Group 2B, respectively), unclassifiable as to carcinogenicity in humans (Group 3), and probably not carcinogenic to humans (Group 4). In excess of 125 agents have been classified into Group 1.[42] It is suspected or known that some of these environmental carcinogens can be found in the deployment environment.

Identifying Cancers

The concern for postdeployment cancer due to potential exposure environmental carcinogens in theater has been raised by service members and veterans alike, as demonstrated by advocacy groups such as Burnpits360 and Operation Purple Heart, which allow for self-reported cancer diagnoses on website registries.[43,44] While these concerns are reasonable and recognized by public health professionals in the military community, they have yet to be supported by epidemiologic studies using appropriate populations and suitable comparison groups. However, there are many factors that should be considered when approaching a study intended to establish whether a history of deployment in support of OIF or OEF is associated with subsequent incidence of postdeployment cancer.

Age

Age is an important factor to consider when designing any epidemiologic investigation pertaining to postdeployment cancers among service members and veterans. Incidence rates of many types of cancers are known to increase with age. As pointed out by the Armed Forces Health Surveillance Center, generally speaking, US military personnel are younger than the general population.[41] When focused on a chronic disease such as cancer that is known to increase with age, in a younger population, priority should be given to cancers that typically occur with highest incidence falling during the young adult years.

Latency Periods

The empirical latent period for cancers consists of 2 parts: an induction period ranging from the time between the action of a given component cause (ie, an exposure of interest) and the action of the last causal component (ie, biological onset of the cancer) and a subsequent period ranging from the biological onset of the cancer to the clinical detection of the cancer. Minimum empirical latency periods must be taken into account when deciding which cancers to evaluate in service members and veterans postdeployment, as they must be consistent with study hypotheses. Latency periods vary by different type of cancer of interest, with some cancers having a typical latency period of 15 to 20 years or longer, while some cancers typically have latency periods that are considerably shorter. In the former, these types of cancers would be better suited for postdeployment cancer evaluations among veteran populations of wars that occurred at least that far in the past, such as Vietnam or the first Gulf War, yet they would not be appropriate for OIF/OEF veterans as that much time has not yet passed since exposure. On the other hand, it would be prudent to study the latter types of cancers in a population of OIF/OEF deployed service members because time since deployment and typical latency periods align.

Biologic Plausibility

When selecting cancer outcomes of interest, the focus should be on cancers that are biologically plausible. For example, the following cancers were selected for an upcoming collaborative study between the US Army Public Health Command, the Navy and Marine Corps Public Health Center, and the Department of Veterans Affairs: melanoma, leukemia, lymphoma, and brain, thyroid, testicular, and breast cancers. Those cancers have peak incidence during young adult years, which matches the demographics of our service members with potential exposure(s) of interest.[45] These selections were also made based on suspected or known occupational or environmental risk factors.[46-49] The latent periods of these cancers are also in accordance with investigating the association between in-theater environmental exposures and postdeployment cancer among service members formerly deployed to OIF or OEF.[50,51]

Karshi-Khanabad: An Example

Recent efforts to understand possible associations between environmental exposures in theater and postdeployment cancer diagnoses include an investigation conducted at the US Army Public Health Command, which explored multiple cancer outcomes among service members formerly deployed to Karshi-Khanabad, an air base located in southeastern Uzbekistan used to support missions in neighboring Afghanistan during OEF.[39] Active

duty members of the US armed forces were located at the Karshi-Khanabad Air Base, also known as K2 or Camp Stronghold Freedom, between 2001 and 2005. General conditions were known to be harsh. Historically, the site was used by the Soviet military to support operations in Afghanistan in the late 1970s. During this time, the Soviet Air Force maintained a fleet of various bomber aircraft at K2, which required an underground fuel distribution system. Furthermore, construction of military equipment (including missiles) in the Soviet era used materials such as asbestos and radioactive material. An occupational and environmental survey performed at K2 in November 2001 by the Center for Health Promotion and Preventive Medicine-Europe.found underground jet-fuel plumes and surface dirt contaminated with asbestos and radioactive uranium.[38] Periodic high levels of dust and other particulate matter (PM) in the air due to seasonal dust storms was also noted.

Although efforts for remediation of the environmental health risks present at K2 were made (eg, covering the contaminated areas with clean soil and declaring these areas "off-limits"), exposure to the toxicants mentioned above during deployment to K2 was plausible. In other settings, exposure to jet fuel plumes, asbestos-contaminated soil, radioactive materials, and/or dust and PM have resulted in documented adverse health outcomes, including both acute and chronic disease. As such, this investigation focused on identifying the frequency of postdeployment medical encounters for health outcomes consistent with exposure to the identified toxicants, with an emphasis on cancer due to the various types among personnel previously deployed to K2.[52-61]

At the request of a US Central Command Force Health Protection Officer, an evaluation of health outcomes among active duty military personnel with a history of deployment to K2 was conducted to address concerns for exposure(s) of health consequence among Army, Air Force, and Marine Corps personnel deployed to the air base. The Army Public Health Command subsequently conducted a comparative health assessment using one year of postdeployment medical follow-up. In the context of the above discussion regarding latency periods for cancer outcomes, the US Army Special Operations Command Surgeon later requested that the original analysis be repeated to incorporate up to 10 years of follow-up, using all available postdeployment medical encounter data. In response to this request, a retrospective cohort study was conducted in order to assess postdeployment health status among service members formerly deployed to K2. This was accomplished by linking K2 deployment rosters from 2001-2005 with postdeployment inpatient and outpatient medical records from 2001-2011. Additionally, a reference group of personnel stationed in South Korea during the same period was selected for comparison. The results are presented in the Table.

The results of this analysis are somewhat mixed, with relative risks lower than one for about half of the specific cancer type outcomes and relative risks higher than one for the other half. The only statistically significant findings were for malignant melanoma and neoplasms of lymphatic and hematopoietic tissues (excluding Non-Hodgkin Lymphoma and Leukemia; highlighted in bluein the Table), indicating a risk approximately 3.7 times greater and 5.6 times greater among those deployed to K2 compared to those stationed in Korea. Concern for postdeployment cancer at K2 is warranted, given the relative risks above one, irrespective of statistical significance and the limitations of this particular analysis. Although the environmental hazard risk profile may differ somewhat between K2 and other OIF/OEF locations, these results bolster the rationale for conducting broader studies to evaluate incidence of cancers following military deployment.

Challenges and Limitations

Long latency periods, low incidence rates of most types of cancer, and appropriate selection of nondeployed controls present challenges for investigators wishing to evaluate postdeployment cancer risk. Only very recently has a sufficient amount of time elapsed in order to assess cancer incidence following OIF and OEF deployments. Given the low incidence rates of most types of cancers, researchers must take care to ensure that study sample sizes are large enough to provide adequate statistical power to detect associations, should they exist. Epidemiologic studies comparing cases to controls with respect to OIF/OEF deployment status presents a challenge due to a high prevalence of deployment for any military personnel serving between 2001 and 2014. As such, a large well-powered study is imperative.

Additional challenges include a lack of data on individual environmental exposures over time as well as a lack of exact locations of each service member during military deployments. As a result, deployment in general is typically used as a proxy for deployment-associated exposures. Also limiting to epidemiologic studies such as these is the lack of information on behavioral habits such as smoking, which can have significant effects on certain types of cancer.

Cancer case definitions are often based on ICD-9-CM coded medical encounter data from military medical record databases. Using administrative records to

Age-Adjusted Relative Risks and Corresponding 95% Confidence Intervals for Cancer Outcomes, Comparing US Military Personnel Deployed to K2 to US Military Personnel Stationed in Korea

Outcome	K2				Korea					
	Age				Age					
	Young		Old		Young		Old		Age-Adjusted*	
	n	%	n	%	n	%	n	%	RR	95% CI
All cancer	11	0.39	50	1.21	41	0.28	133	1.00	1.23	0.92-1.65
Brain cancer	1	0.04	4	0.10	0	0.00	8	0.06	2.04	0.68-6.09
Cervical cancer	0	0.00	0	0.00	1	0.01	0	0.00	0	--
Leukemia	0	0.00	1	0.02	5	0.03	4	0.03	0.43	0.05-3.63
Malignant melanoma	1	0.04	7	0.17	3	0.02	5	0.04	3.68	1.35-10.04
Neoplasm of bone/connective tissue/skin/breast	1	0.04	3	0.07	5	0.03	9	0.07	1.06	0.35-3.22
Neoplasm of colon/rectum	2	0.07	3	0.07	2	0.01	9	0.07	1.6	0.57-4.51
Neoplasm of digestive organs/peritoneum	0	0.00	1	0.02	1	0.01	6	0.05	0.48	0.06-3.95
Neoplasm of female breast	1	0.04	3	0.07	1	0.01	9	0.07	1.35	0.43-4.24
Neoplasm of genitourinary organs	1	0.04	4	0.10	2	0.01	8	0.06	1.74	0.60-5.08
Neoplasm of lip/oral cavity/pharynx	1	0.04	3	0.07	0	0.00	6	0.05	2.18	0.64-7.49
Neoplasm of lung/bronchus	0	0.00	4	0.10	0	0.00	0	0.00	--	--
Neoplasm of lymphatic and hematopoietic tissue	2	0.07	5	0.12	6	0.04	0	0.00	5.64	1.70-18.70
Neoplasm of respiratory/intrathoracic organs	0	0.00	0	0.00	0	0.00	2	0.02	0	--
Neoplasm of testis	1	0.04	2	0.05	8	0.05	12	0.09	0.57	0.17-1.91
Non-Hodgkin lymphoma	0	0.00	3	0.07	4	0.03	8	0.06	0.89	0.25-3.26
Prostate cancer	0	0.00	4	0.10	0	0.00	18	0.14	0.71	0.24-2.10
Neoplasm of other and unspecified sites	0	0.00	3	0.07	3	0.02	27	0.20	0.33	0.10-1.09
Neoplasm of uncertain behavior (plasma cells)	0	0.00	0	0.00	0	0.00	0	0.00	--	--

*RR indicates relative risk. CI indicates confidence intervals.

ascertain cancer cases may result in false positives. For example, not only are some cancers not well defined, but some require several encounters, sometimes with multiple specialists or requiring different medical procedures, in order to make a definitive diagnosis. In such circumstances, an ICD-9-CM code may reflect a true case of cancer or the medical encounter may signify that a patient is in the process of fulfilling diagnostic evaluations necessary to rule out cancer. Using medical encounter data for case ascertainment presents another limitation of this study: whereas medical encounter data capture is complete for service members who remain in service, the same cannot be said for personnel who leave military service. This becomes particularly problematic when studying chronic health outcomes such as cancer, with the latency periods often years after exposure, beyond the average time of military service. Investigators are currently attempting to establish methodology for linking medical encounter records from military service with medical encounter records from the Veterans Administration (VA) in order to minimize loss of follow up due to attrition from military service. However, this methodology will still fail at perfect case capture, as a certain portion of veterans are not VA beneficiaries or simply choose to obtain healthcare services outside the VA health system. It has been suggested that state cancer registries be used as additional sources of data in postdeployment cancer studies, however, the feasibility of this approach has yet to be explored.

Although many challenges are presented to researchers seeking to determine whether or not cancer incidence is elevated among military service members and veterans formerly deployed in support of OIF and OEF relative to personnel without a history of deployment, it is an important topic that is worthy of public health efforts and resources.

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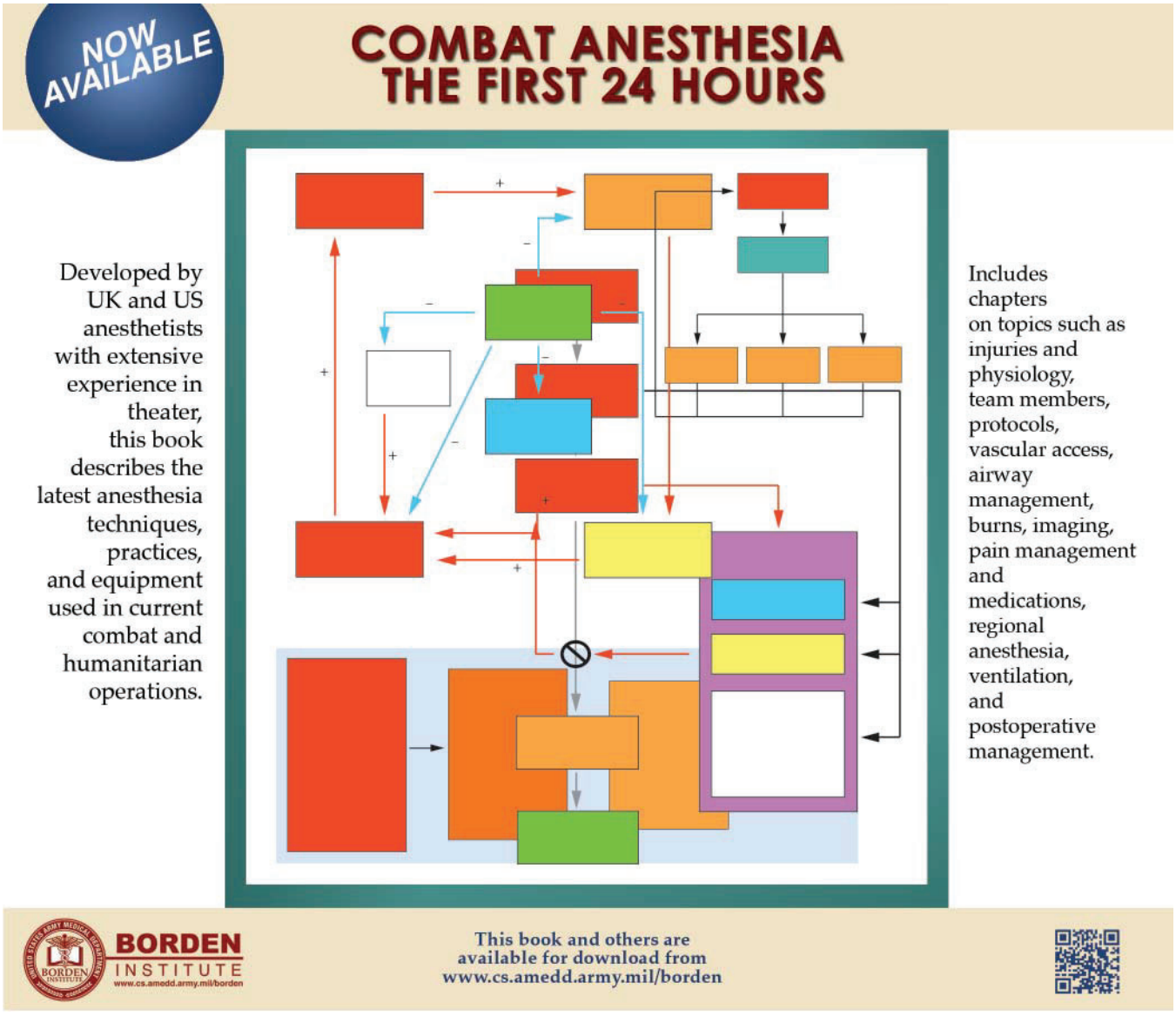

A Preliminary Analysis of Noise Exposure and Medical Outcomes for Department of Defense Military Musicians

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Abstract

Noise exposure is a known occupational health hazard to those serving in the military. Previous military epidemiology studies have identified military occupations at risk of noise induced hearing loss (NIHL); however, musicians have not been specifically mentioned. The focus of military NIHL studies is usually on those service members of the combat arms occupations. This project was a preliminary examination of Department of Defense (DoD) active duty military musicians in regard to their noise exposure, annual hearing test rates, and hearing injury rates using available data sources. The analysis concluded that DoD military musicians are an underserved population in terms of hearing conservation efforts. Noise surveillance data extracted from the Defense Occupational and Environmental Health Readiness System-Industrial Hygiene showed that every musician similar exposure group (SEG) with noise survey data from 2009 to 2013 exceeded the occupation exposure level adopted by *DoD Instruction 6055.12*. However, only a small percentage of all DoD active duty military musicians (5.5% in the peak year of 2012) were assigned to a SEG that was actually surveyed. Hearing test data based on Current Procedural Terminology coding extracted from the Military Health System revealed that the percentage of musicians with annual hearing tests increased over the 5 years studied in all services except the Air Force. During 2013, the data showed that the Navy had the highest percentage of musicians with annual hearing tests at 70.9%, and the Air Force had the lowest at 11.4%. The Air Force had the highest percentage of hearing injuries of those musicians with annual hearing tests for all 5 years analyzed. Although noise surveillance and annual hearing tests are being conducted, they occur at a much lower rate than required for a population that is known to be overexposed to noise.

Background

During the fall of 2014, a US medical liaison officer from the Office of The Surgeon General of the Army, stationed in the United Kingdom, made an inquiry to the Army Hearing Program (AHP), US Army Public Health Command (USAPHC) regarding noise induced hearing loss (NIHL) in military musicians (W. Startz, e-mail, September 16, 2014). The USAPHC AHP conducted a literature search for studies on noise exposure and hearing loss in military band members; however, the search yielded limited specific information on hearing loss in military musicians. As a result, a multidisciplinary team formed at the USAPHC to carry out a preliminary analysis on hearing loss in military band members.

Noise exposure is a known occupational health hazard to those serving in the military.[1,2] The effect of hazardous noise, however, can vary significantly depending on the type of noise (impulse versus steady state), intensity and duration of exposure, and the degree of effort to mitigate the effects. As a result of the variability in noise exposure, some military occupations may be more at risk for NIHL than others. For example, it has been estimated that Soldiers serving in combat arms units have a 30% chance of experiencing a hearing loss.[3,4] Previous epidemiology studies have shown that infantry, gun crews, and seamanship specialists are 1.4 to 2 times more likely to suffer a significant threshold shift (change in hearing) than other military occupations.[5,6]

Although several military occupations have been identified in previous military epidemiology studies, musicians have not been specifically mentioned. Previous noise measurements collected in rehearsal halls and during performance venues suggest that noise exposure for musicians can range from 83 to 120 A-weighted decibels (dBA).[7-14] We presume that military musicians will be at risk for similar noise exposures as their civilian counterparts and may be more at risk for NIHL than other military occupations.

Noise exposure in the performance of duties as a military musician varies depending on the type of instrument, composition of the band or orchestra, venue,

duration of exposure and proximity to other musicians. Also, as the number of years of military service increases, the likelihood of developing a noise-induced hearing loss increases. There is evidence of NIHL of 15 dB or greater at 4000 or 6000 Hertz in at least one ear in 45% of student (nonmilitary) musicians aged 18 to 25 years. Student musicians who practiced more than 2 hours a day were more likely to exhibit a decrease in hearing at some frequency than those who reported practicing for less hours.[15] Another recent study examining incidence of hearing loss among professional musicians in Germany, suggests musicians have 3.51 times higher incidence rate of noise induced hearing loss and 1.45 times higher incidence rate of tinnitus than the general German population.[16] The incidence of hearing loss for musicians increases with length of time of exposure.[17,18] Professional symphony orchestra musicians in Denmark were found to have better hearing than the general population, but were considered at risk for occupational noise-induced hearing loss after prolonged exposure.[19] Brazilian military musicians were found to be 14.54 times more likely to experience hearing loss when compared to their nonexposed counterparts, with a further decline in hearing noted as years of music exposure increases.[20] However, among British Army musicians with 8 to 12 years of military service, the risk of developing hearing loss did not appear to be any greater than their nonmusician counterparts.[21]

Despite conflicting results of studies with regard to the effects of music on hearing,[11,22] musicians are generally considered to be at risk for NIHL and efforts to prevent hearing loss among this group of military personnel is essential. The military musician serves not only in their chosen occupation but also performs other military duties, such as weapons firing, placing them at even greater risk of hearing loss from noise exposure compared to nonmilitary musicians.

Purpose

A multidisciplinary team consisting of audiologists, industrial hygienists, and a statistician formed to analyze NIHL in military musicians based on a review of available data sources. The purpose of this project was to determine the noise exposure of Department of Defense (DoD) military musicians, the percentage of DoD military musicians receiving annual hearing tests, and the percentage of DoD military musicians that received an annual hearing test and was diagnosed with a hearing injury.

This collection of information and its analysis was initiated and completed as a component of operational public health investigations and was not, therefore, subject to review by a human protections board such as an Institutional Review Board.

Methods

Population

The population used for the analysis consisted of active duty musicians serving in the Air Force, Army, Marine Corps, and Navy as identified in Defense Enrollment Eligibility Reporting System (DEERS) by DoD occupational codes 145000 (enlisted) and 271400 (officer and warrant officers) during calendar years 2009 to 2013. The DEERS data were extracted using the Military Health System (MHS) Management and Analysis Reporting Tool (M2).

Ascertainment of Noise Exposure

The Defense Occupational and Environmental Health Readiness System-Industrial Hygiene (DOEHRS-IH) was queried for the personal noise dosimetry conducted during calendar years 2009 to 2013 by the installation industrial hygiene program. The noise survey results were converted to an 8-hour time weighted average (TWA) using a 3 dB exchange rate as required by the *DoD Instruction 6055.12*[23] to identify personnel who were at risk of occupational exposure to hazardous noise. The DOEHRS-IH personal noise survey results were considered to be representative samples of the similar exposure groups (SEG) in which the sampled musicians were assigned. The use of SEGs is a well-established strategy employed by industrial hygienists to conduct occupational exposure assessments. A SEG is a group of workers who have the same general exposure profile for an agent, such as noise, because of the similarity and frequency of the tasks they perform.[24] The assigned SEG population identified in the DOEHRS-IH was confirmed for active duty status, catchment area, and DoD occupational code through DEERS.

Ascertainment of Audiology Procedures

Data were extracted from the MHS using the M2 for the population using the Current Procedural Terminology (CPT) codes captured during direct care encounters. The CPT codes and their definitions are listed in Table 1.

Table 1. Current Procedural Terminology Codes for Hearing Test Surveillance

CPT Code	Definition
92552	Pure tone audiometry (threshold)
92555	Speech audiometry threshold
92556	Speech audiometry threshold with speech recognition
92557	Comprehensive audiometry threshold evaluation and speech recognition
92559	Audiometric test of groups

If one or more of these CPT codes were indicated in the first 5 procedures for an encounter, the patient was considered to have had an audiogram. Persons with multiple encounters with audiograms within the calendar year were counted once for having an annual hearing test.

Ascertainment of Hearing Injury

Data for the population were extracted from the MHS direct care via the M2 using the International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9 CM) codes identified as a diagnosis of a hearing injury. The ICD-9 CM codes identified for hearing injuries and their corresponding definitions and hearing injury categories are listed in Table 2. If one of the hearing injury-identified ICD-9 CM codes was indicated in the first 5 diagnoses for the direct care encounter, the diagnosis was identified for the person for the calendar year. A person diagnosed with one or more hearing injury ICD-9 CM codes within a calendar year is considered to have a hearing injury and was counted once for the calendar year. A person with one or more hearing injury ICD-9 CM codes within a hearing injury category (NIHL, tinnitus, sensorineural hearing loss (SNHL), and significant threshold shift (STS)) is counted once for each category for the calendar year. For this project, hearing injuries were analyzed for those who had an annual hearing test as identified through CPT codes.

Analysis

Noise Surveillance

From 2009 to 2013, a total 174 personal noise dosimetry samples were taken across the services. These 174 samples were representative of 38 different SEGs for musicians ranging from general characterization such as "band" to more specific characterization of a specific musical genera of "rock," "marching," and "concert" bands. The population of a SEG ranged from one to 165 personnel. Consistent with SEG assessment strategy, if one of the personal noise dosimetry samples of a given SEG is determined to be over the occupational exposure limit (OEL) of 85 dBA 8-hour TWA adopted by *DoD Instruction 6055.12*,[23] then all personnel assigned to the SEG are considered to be over the OEL and at risk of exposure to hazardous noise. All DoD personnel exposed to noise levels greater than the OEL are identified on the command's roster for inclusion in the Hearing Conservation Program (HCP); therefore, requiring personnel to be placed in a hearing testing surveillance program and have an audiogram conducted at least annually.[23]

Table 2. ICD-9 CM Codes for Hearing Injury

Hearing Injury Category	ICD-9	Definition
Noise induced hearing loss	388.10	Noise effect-ear not otherwise specified (NOS)
	388.11	Acoustic trauma
	388.12	Hearing loss D/T noise
Tinnitus	388.30	Tinnitus NOS
	388.31	Subjective tinnitus
	388.32	Objective tinnitus
Sensorineural hearing loss	389.10	Sensorineural hearing loss NOS
	389.11	Sensory hearing loss, bilateral
	389.15	Sensorineural hearing loss, unilateral
	389.16	Sensorineural hearing loss, asymmetrical
	389.17	Sensory hearing loss, unilateral
	389.18	Sensorineural hearing loss, bilateral
Significant threshold shift	794.15	Abnormal auditory function study

Hearing Test Surveillance

Hearing test data were compared across services within a given year. A chi-square test, followed by the Marascuilo procedure, was used to determine if the Navy, Marines Corps, or Air Force had a significantly different proportion of service members with an annual hearing test compared to the Army. Statistical significance was defined as $P<.05$. The Army was chosen as the reference group because *Army Pamphlet 40-501*[25] requires every Soldier to undergo an annual hearing test that is recorded in both DOEHRS-Hearing Conservation (HC) and the MHS during a direct care encounter. Data from the DOEHRS-HC system is not linked into military treatment data; therefore, this project used the CPT coding as a surrogate for the DOEHRS-HC hearing test data.

Hearing Injury

Hearing injury data analysis was restricted to those in the population that received an annual hearing test. Hearing injuries were compared across the services and evaluated at the DoD level for total injuries and for each diagnosis.

Results

Noise Surveillance

All of the 38 different SEGS had at least one personal noise dosimetry sample over the OEL, resulting in all those assigned to the 38 SEGs being classified as being "overexposed." The percentage of DoD military musicians assigned to the 38 SEGs during the 5-year period represents both those musicians under noise surveillance and those classified as being overexposed. Figure 1 shows the percentage of the DoD military musicians by each service that was assigned to SEGs during the noise dosimetry testing. There was limited information available for the personnel assigned to SEGs in 2009 because DOEHRS-IH was still being incorporated into DoD-wide

use. From a surveillance perspective, the percentage of musicians being surveyed is far below what is expected given that all SEGs were over the OEL. The 2 services with the highest percentage of personnel in SEGs with noise surveillance were the Air Force and the Army, with peaks of 12% for the Air Force in 2013 and 8% for the Army in 2012. The percentage of military musicians under noise surveillance DoD wide (Figure 2) showed a steady increase from 2009 to 2013; however, the highest percentage in the 5 years was 5.5%. Given that all SEGS within all years were considered overexposed, it appears that regardless of how many SEGs or which SEG the industrial hygienists surveys, they will be classified as overexposed.

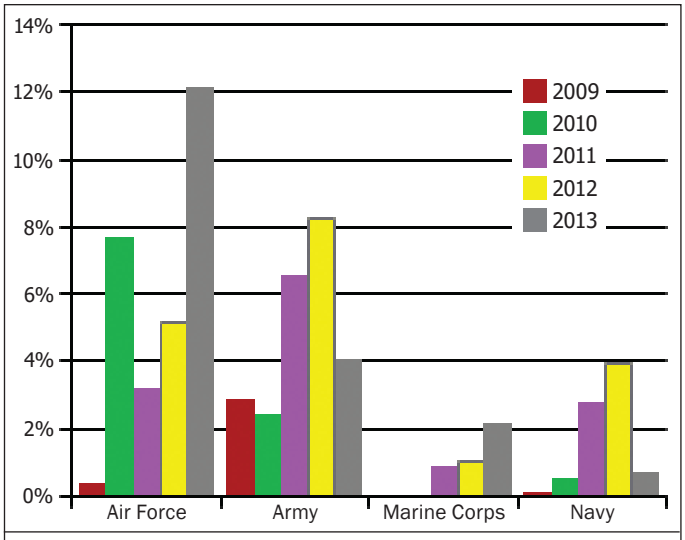

Figure 1. Percentage of active duty musicians by military service assigned to SEGs during noise surveillance.

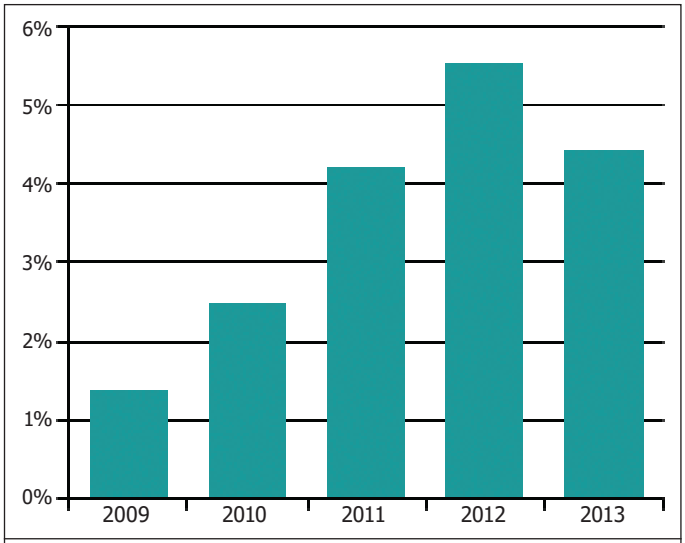

Figure 2. Percentage of active duty DoD musicians assigned to SEGS during noise surveillance.

Hearing Test Surveillance

As shown in Table 3, the Army consistently had the highest (or nearly the highest) proportion (54% to 69%) of musicians with annual hearing tests from 2009 to 2013. In these 5 years, Army had a significantly greater proportion of musicians with an annual hearing test compared to the Air Force and Marine Corps ($P<.05$). Both the Navy (31% to 71%) and Marine Corps (21% to 51%) had an increased proportion of musicians with annual hearing tests from 2009 to 2013, noting that from 2011 to 2013, the Navy had nearly identical proportions as the Army. The Air Force started with the lowest percentage tested in 2009 (18%) with no improvement observed from 2010 to 2013.

The 5-year trend is presented in Figure 3. Although some services, notably the Army and Navy, had higher testing rates, all services were not in compliance with the 100% testing requirement. No service had above 71% of its musicians tested within a given year.

Hearing Injury

As shown in Table 4, hearing injury rates are highest in the Air Force for all 5 years; however, the Air force has the least percentage of musicians with annual hearing tests. The Army showed the second highest injury rates followed by the Marine Corps and Navy, respectively. The most common hearing injury diagnosis among DoD military musicians is SNHL, with tinnitus as the second most common diagnosis. Significant threshold shift comes in third and NIHL fourth. The rates of these diagnoses fluctuated between 2009 and 2013, but only marginally.

The comparison of each diagnosis across the services (Figures 4, 5, 6, 7), revealed that the Air Force had much higher injury rates for NIHL, SNHL, and tinnitus than the other 3 services for all 5 years.

Table 3. Number and Percentage by Service of DoD Military Musicians With an Annual Hearing Test from 2009 to 2013

Military Service	2009		2010		2011		2012		2013	
	Count[a]	%	Count[a]	%	Count[a]	%	Count[a]	%	Count[a]	%
Air Force	134	17.7%[b]	136	18.4%[b]	95	13.3%[b]	89	13.1%[b]	78	11.4%[b]
Army[c]	1076	54.6%	1268	61.6%	1129	55.1%	1170	58.5%	1363	69.0%
Marine Corps	206	21.1%[b]	352	36.1%[b]	398	43.1%[b]	368	41.3%[b]	455	51.2%[b]
Navy	223	31.1%[b]	326	44.6%[b]	405	55.7%[b]	412	58.3%	498	70.9%

Data from the Military Health System Management and Analysis Reporting Tool.

[a]Count indicates the number of persons with one or more of the designated CPT codes during the calendar year.
[b]Significantly different proportion of hearing injuries compared to the reference group (Army).
[c]Reference Group

The Air Force's injury rate for SNHL was at least 3 times those in the Army. With exception of 2012, the Air Force's injury rate for tinnitus was 4 times the Army injury rate. The injury rates for all the services for NIHL, SNHL, and tinnitus had little fluctuation during the 5 years.

The noise surveillance data from DOEHRS-IH demonstrates that a portion of the DoD musicians are exposed to hazardous occupational noise related to their jobs as musicians in the military. A large percentage of DoD military musicians were not assigned to a SEG and not under noise surveillance; however, the noise surveillance documented shows overexposure. The annual hearing test results do not reflect that which would be expected for a population with known hazardous occupational noise exposure. *DoD Instruction 6055.12*[23] requires personnel in hazardous noise environments to have an annual hearing test. Among the services, the Army and the Navy showed the highest percentage of musicians receiving their annual hearing test; however, even these 2 services are well below 100%. The Air Force's injury rates for hearing injuries in general and for NIHL, SNHL, and tinnitus specifically were higher than the other services. Additionally, the Air Force had the lowest percentage of musicians with annual hearing tests.

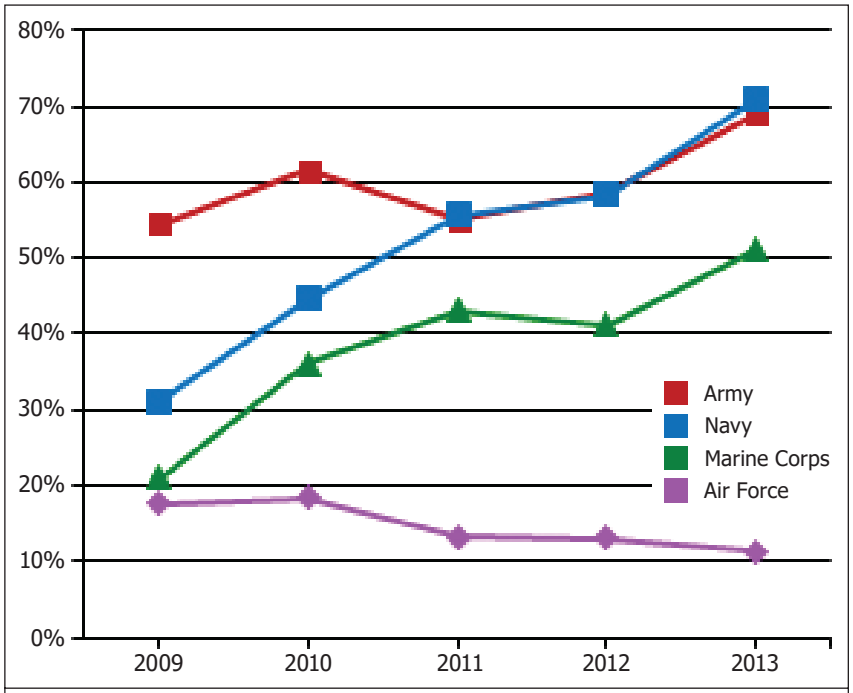

Figure 3. Percentage of military musicians within each service with an annual hearing test, 2009 to 2013.

There are multiple strengths of this analysis. The interdisciplinary team brought different perspectives that enhanced the public health performance evaluation methods promulgated by the Institute of Medicine.[26] The data sources used (DEERS, MHS, and DOEHRS-IH) provided a broad-spectrum approach using the best available data, which provided an analysis that incorporated multisource data integration techniques including demographic details data, noise exposure data, medical procedures data (as a surrogate for hearing test surveillance), and medical outcome diagnoses data associated with procedure data.

There are limitations of the analysis as well. National Guard and Reserve service member data were not included and the injury data do not include purchase care visits. The 5 years of data was not determined to be sufficient to determine trends in the longitudinal data. Since the data set in this analysis is limited, the sample may not truly be representative of the entire population of military musicians; therefore, generalization to the entire population may not be feasible.

Table 4. Number and Percentage of Injuries by Service and Diagnosis Among DoD Musicians Who Received an Annual Hearing Test from 2009 to 2013

Military Service	2009[a]		2010[a]		2011[a]		2012[a]		2013[a]	
	Count	%	Count	%	Count	%	Count	%	Count	%
Air Force	25	18.7%	31	22.8%	25	26.3%	17	19.1%	17	21.8%
Army	73	6.8%	91	7.2%	92	8.1%	101	8.6%	93	6.8%
Marine Corps	13	6.3%	16	4.5%	25	6.3%	33	9.0%	33	7.3%
Navy	11	4.9%	14	4.3%	19	4.7%	30	7.3%	24	4.8%
Diagnosis										
NIHL	12	0.7%	8	0.4%	10	0.5%	13	0.6%	7	0.3%
SNHL	88	5.4%	92	4.4%	90	4.4%	110	5.4%	117	4.9%
STS	38	2.3%	66	3.2%	72	3.6%	54	2.6%	35	1.5%
Tinnitus	74	4.5%	85	4.1%	79	3.9%	101	5.0%	106	4.4%

Data from the Military Health System Management and Analysis Reporting Tool.

[a]Count and percentages are representative only of those injuries that occurred among military personnel who had an annual hearing test. Denominator for counts and percentages is the total number of military personnel tested.

Conclusion and Recommendations

This article demonstrates that DoD military musicians are an underserved population with regard to hearing conservation efforts. While noise surveillance and annual hearing tests are conducted, they occur

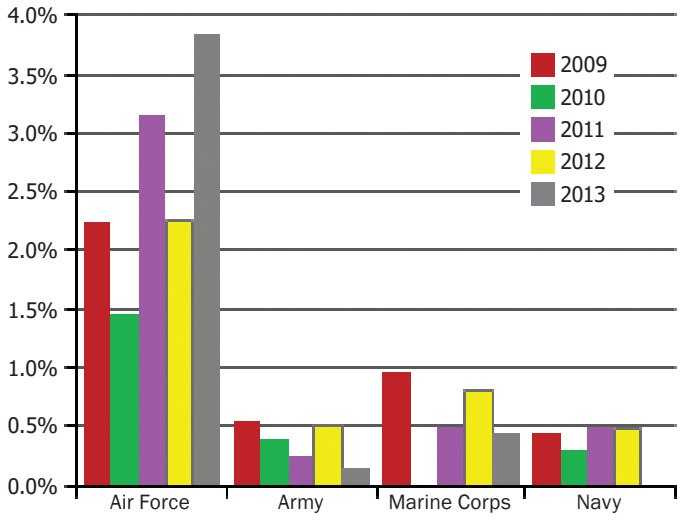

Figure 4. Percentage of DoD military musicians by service with annual hearing test and NIHL diagnosis.

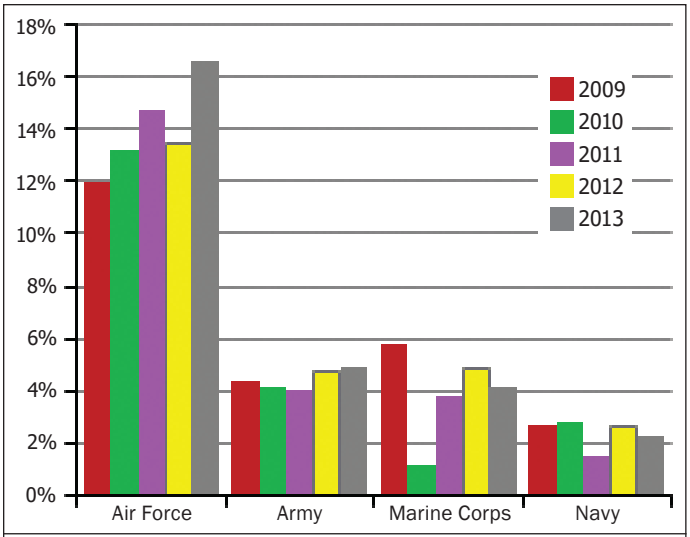

Figure 5. Percentage of DoD military musicians by service with annual hearing test and SNHL diagnosis.

at a much lower rate than required for a population that is overexposed to noise. Although the focus of this analysis was on a single population that is relatively small in comparison with other military occupations, a systematic approach is recommended to improve the hearing conservation efforts that would affect DoD military musicians.

The ultimate responsibility for compliance with annual hearing tests and the requirement for the HCP lie with the commanders. The hearing conservation efforts should be a high-priority item evaluated during all command safety assessments and inspector general inspections. Making hearing conservation efforts a leadership priority will require commanders to engage the professional fields responsible for the different aspects of hearing conservation (industrial hygiene, preventive medicine, occupational health, hearing conservation, audiology, and safety). Command situational awareness and command emphasis that identifies and characterizes hearing health challenges sets a foundation for hearing injury prevention planning and execution at all levels.

Acknowledgments

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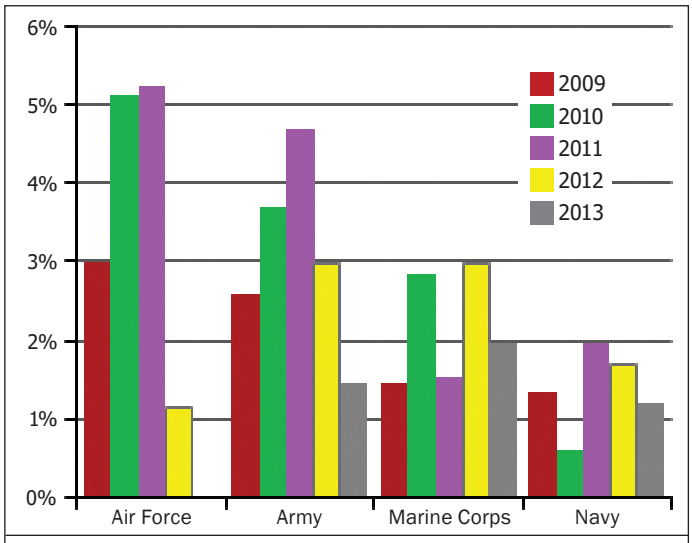

Figure 6. Percentage of DoD military musicians by service with annual hearing test and STS diagnosis.

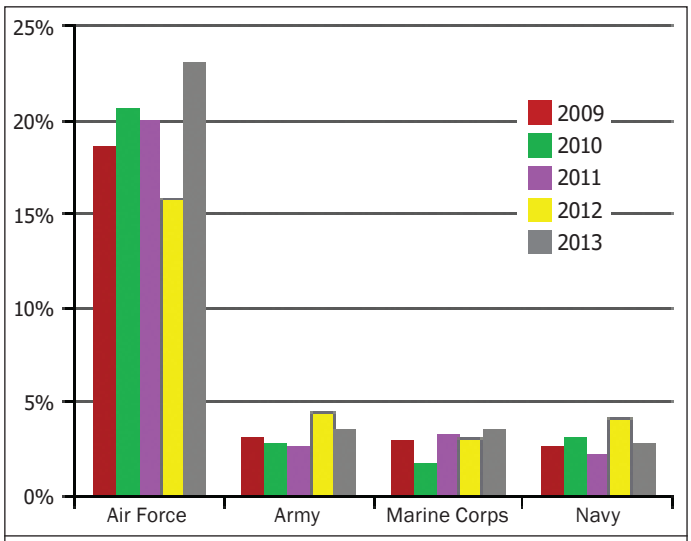

Figure 7. Percentage of DoD military musicians by service with annual hearing test and Tinnitus diagnosis.

The Benefits of Deploying Health Physics Specialists to Joint Operation Areas

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Abstract

Preventive Medicine Specialists (military occupational specialty [MOS] 68S) with the health physics specialist (N4) qualification identifier possess a unique force health protection skill set. In garrison, they ensure radiation exposures to patients, occupational workers and the public from hospital activities such as radioisotope therapy and x-ray machines do not to exceed Federal law limits and kept as low as reasonably achievable. Maintaining sufficient numbers of health physics specialists (HPSs) to fill authorizations has been a consistent struggle for the Army Medical Department due to the rigorous academic requirements of the additional skill identifier-producing program. This shortage has limited MOS 68SN4 deployment opportunities in the past and prevented medical planners from recognizing the capabilities these Soldiers can bring to the fight. In 2014, for the first time, HPSs were sourced to deploy as an augmentation capability to the 172nd Preventive Medicine Detachment (PM Det), the sole PM Det supporting the Combined Joint Operations Area-Afghanistan. Considerable successes in bettering radiation safety practices and improvements in incident and accident response were achieved as a result of their deployment. The purposes of this article are to describe the mission services performed by HPSs in Afghanistan, discuss the benefits of deploying HPSs with PM Dets, and demonstrate to senior medical leadership the importance of maintaining a health physics capability in a theater environment.

Historically, health physics support to the Combined Joint Operations Area-Afghanistan (CJOA-A) was administered by a nuclear medical science officer (NMSO) assigned to Task Force-Medical built around a medical brigade. This officer was responsible for managing the theater health physics program and had no other health physics experts to serve as assistants. In late spring 2013, the NMSO (military occupational specialty [MOS] 72A) position was eliminated to meet force size reduction requirements. The US Forces Afghanistan (USFOR-A) Safety Office initiated a hiring action for a civilian radiation safety professional at this time, but the hiring action was never approved. An NMSO working as the senior medical planner at the International Security Assistance Force Joint Command Headquarters for most of 2013 was available to provide consultation assistance. However, the loss of the dedicated NMSO position left the CJOA-A without a full-time health physics expert when a surge in the health physics mission workload and a corresponding heightening of ionizing radiation exposure risks occurred in early 2014.

The reasons for the surge in health physics mission requirements in 2014 were varied. A primary cause was the acceleration of retrograde operations which led to progressively larger quantities of radioactive commodities arriving for processing and shipping at the Bagram Airfield (BAF) and Kandahar Airfield (KAF) Redistribution Property Accountability Team (RPAT) yards. The arrival rates of these commodities far exceeded removal rates, thus overtaxing the RPAT yard's management capabilities and creating problems in storage of such items. Another leading cause was a dramatic increase in base closures and increase in demolitions of structures. These activities led to the discovery of orphan sources (unwanted and uncontrolled radioactive materials) on the installations and also necessitated radiological surveys of foreign military equipment on some of the closing bases to clear for demilitarization and removal. Additionally, safety concerns about exposures to x-ray and gamma radiation sources in mobile vehicle and cargo inspection systems (MVACISs) found at installation entry control points spurred a requirement to begin monitoring and inspecting the operation of these systems.

Filling the health physics capability gap left by the departure of the NMSO forced medical planners in the CJOA-A to devise a solution within the mandated force management level constraints at the time. Under these constraints, reestablishing the lost NMSO billet was not deemed a viable option due to the General Officer level of approval necessary for such action. The developed solution was to substitute 2 health physics specialists (HPSs) for preventive medicine specialists projected to arrive in June of 2014 with the incoming 172nd Preventive

Medicine Detachment (PM Det). As a stopgap measure, an HPS who had deployed as a Battle Noncommissioned Officer with the 31st Combat Support Hospital (Task Force 31) in February 2014, was employed to perform crucial health physics mission requirements on a part-time base until arrival of the 172nd PM Det.

The substitution of HPSs for PMS was made possible by the fact that HPSs are former preventive medicine specialists. The only difference between the 2 types of specialists from a qualifications standpoint is the HPSs have completed a 20-week course to earn N4 skill identifier. The HPS may be considered MOS qualified to perform PMS tasks and as such executed these tasks on a routine basis in Afghanistan. However, HPSs are consistently in short supply due to the rigors of their academic training and are usually assigned to health care facilities to support health physics programs. Their small number and high garrison demand have made long-duration deployments rare and deprived them the opportunity to prove their value in the combat theater environment. Therefore, their deployment to the CJOA-A beginning in 2014 was a unique event providing an opportunity for lessons to be learned about how to best employ them.

Mission Overview

The HPSs assigned to the 172nd PM Det served on PM teams at BAF and KAF. The HPS at BAF supported Regional Command (RC)-North, RC-East, RC-West, and RC-Capital, while the other supported RC-South and RC-Southwest. When not performing health physics missions, they were engaged in standard preventive medicine technician duties such as sanitation inspections and water quality monitoring. The most common health physics and radiation safety missions performed are summarized in the Table. It is important to note these missions could have been executed by a NMSO. The medical imaging oversight mission was performed by a 2-person team comprised of a NMSO and a HPS from US Army Public Health Command (USAPHC) brought in for a 3-week period. The remaining missions shown in the Table were performed by the HPSs assigned to the PM Det.

MVACIS Inspections

Many entry control points used MVACIS to image local national vehicles for weapons and explosives prior to permitting their entry onto installations. The Nuclear Regulatory Commission (NRC) regulates the MVACIS radioactive sources in the United States. The Communications-Electronics Command (CECOM) holds the NRC license for these sources before they are shipped overseas. Although the NRC does not have jurisdiction, the MVACIS sources are managed by an Army Radiation Authorization given to USFOR-A by CECOM. Appointments as Radiation Safety Officers for NRC licenses and Army Radiation Authorizations are routinely held by NMSOs, civilian health physicists, or very specialized trained individuals.

United States and International Security Assistance Force (ISAF) uniformed personnel assigned security responsibilities for installations served as the operators of these systems. Many operators had no experience with MVACIS operations prior to their deployments and had never been enrolled in a radiation dosimetry program. Contractors were used by ISAF and USFOR-A to maintain and service these systems. The contractors would position contract service representatives (CSRs) at major bases and designate coverage areas for the representatives to support. The CSRs would travel to the bases to administer safety and operator training, and issue and collect thermoluminescent dosimeters. They also visited entry control points at a specified frequency to examine the daily exposure by reading records kept by operators, collect and reissue dosimeters, and troubleshoot any problems. Upon the completion of the visit, they would offer recommendations and advice to the site operators on improving work practices. The effectiveness of their visits was limited since they lacked the authority to hold operators accountable for failing to follow proper safety procedures.

Health Physics and Radiation Safety Missions

Type	Frequency	Description
Mobile Vehicle and Cargo Inspection System (MVACIS) inspections	Quarterly	Quarterly inspections of MVACIS at entry control points to ensure safe system operation.
Radioactive commodity retrograde support	Continuous	Consultation on the establishment and operation of a consolidated storage locations, offering radiation safety training to the workforce, leak testing turned-in items, and surveying packaged items prior to shipment.
Radiation surveys	As needed	Survey with specialized measurement equipment areas where a suspected radiation release and/or exposure has occurred, followed by an assessment of health risks.
Orphan source management	As needed	Provide assistance and consultation on how to manage items unexpectedly found on installations. These discoveries could prompt a radiation survey mission.
Medical imaging system oversight	Annually	Inspection of HCF imaging equipment that emits ionizing radiation.

In order to address radiation exposure concerns and improve radiation safety operational practices, in mid-2014 USFOR-A issued a directive implementing MVACIS inspection program (Figure 1). The inspection checklist was developed with input from the HPSs, USAPHC health physics experts, and the civilian radiation safety officer employed by the largest MVACIS contractor in Afghanistan. Prior to starting the quarterly inspections, an initial site assistance visit was completed at each ECP by the HPSs or uniformed PM personnel at bases not supported by the 172nd PM Det. The non-HPS inspectors received training by the CSRs on how to perform these inspections before initiating the initial site assistance visits. The scrutiny and attention garnered by the inspections improved operator adherence to safety policies and procedures. The inspections also improved the completeness of occupational and environmental health site assessments since preventive medicine elements had previously not been populating the Defense Occupational and Environmental Health Readiness System's (DOEHRS) DoD Deployment Surveillance Portal with information concerning the occupational hazards associated with MVACIS radiation emissions.

Figure 1. A nuclear medical science officer examining a mobile vehicle and cargo inspection systems at Bagram Airfield on March 28, 2015. (Photo courtesy of the authors.)

Radioactive Commodity Retrograde Support

Over the course of 13 years of continuous military operations, significant quantities of US equipment containing radioisotopes had been brought into Afghanistan. Examples of some of the more common items included weapon system optics, compasses, and chemical agent detection alarms. The processes and procedures for retrograding items varied based on the manager of the commodity and the type of radioisotope it contained. Many items had to be tested through the collection and submission of wipe tests to the US Army Test, Measurement, and Diagnostic Equipment laboratory in Pirmasens, Germany, to prove they had been surveyed with a radiation detection device and were free of leaks before final packaging and shipment. Prior to arrival of the HPSs, there was only one contractor in all of Afghanistan stationed at KAF qualified to perform wipe sampling.

When the push to retrograde commodities began, management and shipping processes were not sufficiently mature to handle the influx of turned-in items. The problem was further magnified by a lack of trained personnel to support these processes. Unlike the drawdown from Operation Iraqi Freedom, there was no Army Contaminated Equipment Team, a special team deployed by the Army Material Command, to lead the radioactive commodities retrograde effort in Afghanistan. Efforts to overcome personnel shortfalls were further hampered by delays in civilian hiring actions; stringent force management level constraints governing the number of contractors, service members, and Department of Defense employees permitted in country; and difficulties in modifying inflexible scopes of work to permit contractors already involved with incountry retrograde operations to participate in radioactive commodity retrograde support activities.

By necessity, the HPSs were used to support the retrograde effort even though retrograding activities are doctrinally the responsibility of logistics rather than medical authorities. Their assistance was broad in scope and evolved throughout the deployment as new challenges were identified and previous problems were solved. The effect of their assistance was greatly amplified by their exceptional knowledge base in health physics, strong oral and written communication skills, and the credibility boost offered by their ranks as noncommissioned officers. Those areas of assistance where the HPSs had the most meaningful effect on retrograde support were in the administration of safety awareness training to retrosort yard personnel, reviewing and coauthoring pertinent standard operating procedures and policy documents, relieving the wipe sample collection backlog through additional sampling, surveying prepped shipments, participation in installation radiation safety working groups, and providing consultative services to aid the establishment of a consolidated radioactive commodities storage area on BAF.

Radiation Surveys

During the course of their deployment, the HPSs performed surveys of areas and equipment possibly contaminated with radioactive material. Some of these surveys were of an urgent nature and required prompt execution, while others were less time-sensitive. The

urgent surveys were typically high profile and garnered intense interest from senior commanders. One such incident resulted after a report was received about possible acute radiation exposures from a malfunctioning X-ray emitting MVACIS received by North Atlantic Treaty Organization (NATO) soldiers manning an entry control point at the ISAF Headquarters in downtown Kabul. The guards were evacuated to a nearby NATO-run hospital and kept under medical observation for symptoms of acute radiation sickness after it was discovered that one of the 3 x-ray tubes of the MVACIS was not functioning properly, and high radiation readings were allegedly read from a radiation detection meter.

Within one hour of notification of the incident, a HPS was flown by air ambulance from BAF to the scene to investigate. Much to the relief of all parties involved, this investigation conclusively proved that no medically significant radiation releases had occurred. The malfunctioning x-ray tube was determined to be burned-out, meaning it could not emit any x-ray radiation. The alleged high radiation measurements were due to a 3 order of magnitude instrument reading error. As an additional precautionary measure, the HPS performed a radiation survey of the entry control point and found no radiation readings above normal background levels.

Another high-profile radiation survey was performed at the site of an MI-17 helicopter fire within a hangar at the New Kabul Afghanistan International Airport (Figures 2 and 3). The Russian-made helicopter was the property of the Afghanistan Air Force (AAA) and was equipped with instrumentation that contains radioisotopes. The hangar where the helicopter was parked was an important rotary wing aircraft maintenance location and also served as a training area where US military experts administered hands-on training to AAA maintenance recruits. Senior AAA and ISAF leadership wanted to remove the burned out helicopter hulk and resume hangar operations as soon as possible; however, a radiation survey was first required to assess the dangers posed from any radiation contamination. The survey results from the HPS found the hangar free of contamination, thus clearing the way for recovery operations.

Figure 2. Smoke pours from a burning Afghan MI-17 helicopter inside of a maintenance hangar at the Kabul International Airport. The helicopter in foreground is another MI-17 not involved in the fire. (Photo courtesy of the authors.)

Most radiation surveys performed by the HPS were not as spectacular as the two previous examples. The majority of surveys supported retrograde efforts and included surveys checking for contaminated areas at radioactive commodity storage locations and the aforementioned surveys of items prepped for shipment out of the CJOA-A. The HPSs also surveyed items, often of foreign make, earmarked for demilitarization to certify those items were radioisotope free.

Orphan Source Management

There were several instances where the HPSs were called upon to provide assistance and consultative support in dealing with orphan sources discovered on ISAF installations. Examples of such orphan sources included a Soviet-era ice detector containing strontium-90, a beta emitter, found at BAF (Figure 4); thorium nitrate, an alpha emitter, encapsulated in concrete within a 5 gallon bucket in an abandoned building that once served as an East German Pharmaceutical Plant on Camp Phoenix (Figure 5); and 2 ion chamber survey meters improperly discarded, presumably by a contractor, in a dumpster at BAF. Though all the orphan source discovery incidents were judged by the HPS to pose low health risks, they were, nevertheless, documented and archived within DOEHRS.

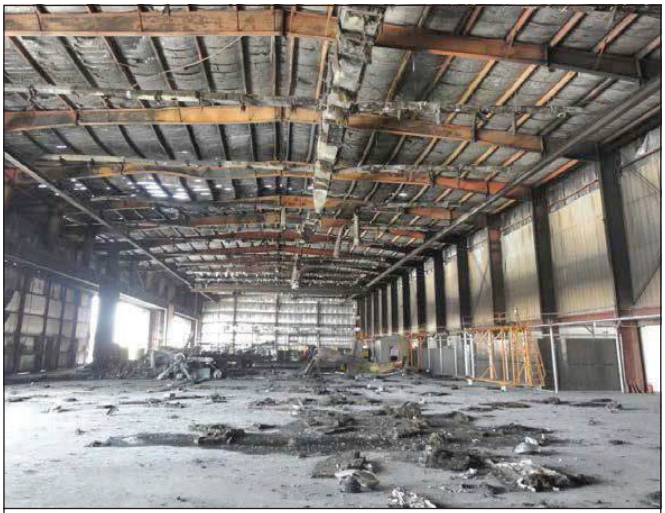

Figure 3. Damage inside of the maintenance hangar at the Kabul International Airport following removal of burned Afghan MI-17 helicopter. (Photo courtesy of the authors.)

Figure 4. A Soviet aviation ice warning device containing strontium-90, a beta particle emitter, found at Bagram Airfield. (Photo courtesy of the authors.)

Figure 5. A metal bucket containing thorium nitrate which had been encapsulated in concrete at the now closed Camp Phoenix in the Kabul base cluster. (Photo courtesy of the authors.)

Medical Imaging System Oversight

One of the basic responsibilities for HPSs in a nondeployed environment is to ensure medical imaging systems (x-rays, fluoroscopy machines, and computed tomography scanners) at medical treatment facilities, dental clinics, and veterinary clinics are functioning properly. The highly specialized equipment necessary to perform these checks was not available in Afghanistan. As such, the oversight role of medical imaging systems for HPSs within Afghanistan was limited to checking on TLD wearing and monitoring system operator work practices to determine their adherence to as low as reasonably achievable radiation exposure work practices. In order to satisfy the regulatory requirement for an annual check of imaging systems, a request for assistance was submitted by USFOR-A in collaboration with US Army Central Command to the US Army Medical Command for a team to perform these checks. This team spent 3 weeks in country checking 17 systems at 6 installations.

Recommendations

The health physics support provided by the HPSs proved to be of great benefit across multiple staff support areas (safety, logistics, and force health protection). As a first of its kind deployment, there was initially some confusion as to how best utilize them. The confusion abated as duties and responsibilities became better defined and beneficial partnerships with other organizations and commands were established. The following paragraphs offer several recommendations on how to best prepare, equip, and employ HPs should they be used for future long-term deployments.

Preparation

Since HPSs are not assigned to PM Dets in garrison, they should be afforded an opportunity to participate in the PM Det's predeployment certification training exercise. Doing so allows them to meet their deployment teammates, relearn basic PMS tasks, and advance their understanding of health physic mission requirements in the deployed environment before arriving at the deployment destination. The HPSs selected to deploy should be knowledgeable in performing medical imaging system surveys and conducting contamination surveys, and skilled in decontamination practices. Given the strong possibility of being asked to provide retrograde operation support and MVACIS inspections, completion of Class 7 shipment training and familiarization with MVACIS radiation safety fundamentals prior to deployment is advisable. In addition, they should have the necessary rank, experience, and initiative to successfully establish and maintain an effective health physics program in the absence of a NMSO or other officers well versed in health physics.

Equipment

With the exception of the medical imaging radiation surveys, all other surveys performed by the HPSs required use of an AN/PDR 77 radiac set plus its accessories to measure and detect radiation levels. This radiac set is not an item found in the modified table of organization and equipment of a PM Detachment. One of the 2 sets used in Afghanistan by the HPSs was a loaner from a USAPHC, while the other was acquired through logistic channels after submitting an operational needs statement. Had these radiac sets not been available, the scope and the overall effectiveness of the services provided by the HPSs would have been severely diminished. A commercial off-the-shelf portable gamma spectroscopy

instrument will also be useful in a theater of operations since it can identify common radioisotopes, is more sensitive than the AN/PDR-77 in detecting gamma-emitters, and can detect some neutron emissions.

Employment

Since the bulk of the HPS mission services occurred at the retrograde hubs at BAF and KAF, this was logically the best location to station them. While stationed here, they had ample time to gain an understanding of the retrograde processes and learn where their services were needed most to support retrograde efforts. Also, BAF and KAF were installations where the 172nd PM Det had the lead for providing most PM support. This meant the HPSs were able to perform PMS mission work when there were lulls in the health physics missions.

Currently, there is only one HPS for the 224th PM Det which replaced the 172nd PM Det. This soldier is located at BAF where the majority of the health physics operations are concentrated. The USFOR-A is preparing to designate an Air Force Industrial Hygienist as their Radiation Safety Officer (RSO), in absence of an NMSO, and the HPS as the alternate RSO. A NMSO recently arrived in Kuwait and provides long-distance support to Afghanistan and other countries in the area of operations. The NMSO serves as a bridging solution until the safety community develops a permanent solution for the Radiation Safety Program.

Conclusion

Maintaining a capability to execute health physics missions is critical and likely to grow in urgency and magnitude during the drawdown phase of an overseas military deployment operation. In the absence of an available NMSO, medical planners should give strong consideration to deploying HPSs to assist with filling health physics and radiation safety capability gaps. An ideal place to assign HPSs is within a deploying PM Det. As members of the detachment, they are well-positioned to execute their health physics missions while also being available to perform routine PMS duties. The HPSs deployed to the CJOA-A in 2014 executed a multitude of crucial missions. Some of the missions were extremely high profile with significant diplomatic implications. Based on the notable success of their Afghanistan deployment, the HPSs have proven their worth many times over and should be considered for future deployments.

Authors

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A New Volume in the Borden Institute Textbooks of Military Medicine Series

Forensic and Ethical Issues in Military Behavioral Health

COL (Ret) Elspeth Cameron Ritchie, MC, USA
Senior Editor

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COL (Ret) Edward Lindeke, USA

The US Army has made education of its Soldiers regarding behavioral health one of its foremost considerations. This emphasis has resulted in the Borden Institute's publication of the fourth Textbook of Military Medicine in the past 20 years to address the behavioral health of the Soldier: *Forensic and Ethical Issues in Military Behavioral Health.*

The writing of the first two behavioral health books, *Military Psychiatry: Preparing in Peace for War* (1994) and *War Psychiatry (1995)*, was led by COL (Ret) Franklin D. Jones. These volumes were published soon after the end of the Gulf War in 1991. COL (Ret) Elspeth Ritchie was the Senior Editor for *Combat and Behavioral Health*, published in 2011, as well as this newly published volume. COL Ritchie's books were published as Operations Iraqi Freedom and Enduring Freedom were winding down. All four of these books present insights into the emotional aspects of war and the role of behavioral health care providers within the context of the US Army. Each volume reinforces the understanding that the stresses of military life can be significant. *Forensic and Ethical Issues* differs from the other books in that it focuses on the practice of forensic psychology and psychiatry and their application to medical issues arising within the military legal system. Covering a broad range of topics, this book provides an easily accessible reference for readers wishing to understand the implications of a Soldier's behavior and how care providers specializing in psychological and psychiatric care can help Soldiers in trouble.

The men and women who serve in the military responded without hesitation to the challenge of terrorism provoked by the events of September 11, 2001. Yet, now that military operations conducted in response are winding down, many Soldiers and their families have been left with the psychological wounds of war. Our military specialists in behavioral health focus on helping Soldiers cope with these mental injuries and associated behaviors.

In this book, psychiatrists, psychologists, scientists with expertise in behavioral health, and lawyers who specialize in military law, from all the military services, have coauthored chapters detailing how the sciences of psychology and forensic psychiatry apply to behavioral issues in the context of the legal system. The intent is to show the insight and understanding that forensic specialists add to the military justice system as Soldiers face great challenges in their lives. Throughout the book are descriptions of the very broad roles that behavioral health professionals play in attempting to help jurists better understand why Soldiers commit crimes. The work of these professionals adds an element of fairness to the evaluation of Soldiers in crisis. The content of each chapter reflects topical issues in today's military world.

Suicide, sexual assault, and posttraumatic stress disorder (PTSD) are the primary behavioral health issues facing the military. To begin addressing the first of these, the authors discuss ways to stop a determined individual from committing suicide. When a suicide occurs, the authors detail the military's investigative plan, which provides a "psychological autopsy" of the events leading to the act. When suicides are perceived to occur in clusters within a military unit or location, an epidemiologic review of the cases and identification of common forces (if any) that drive these events are described.

Sexual assaults are discussed in a similarly straightforward manner. The need for the sexual education of men, particularly where it applies to the issue of competent consent, and the underlying psychological environment

leading to a culture in which sexual assaults occur is recognized and addressed.

The authors also provide an understanding of the psychiatric diagnosis of PTSD. Flashbacks, sleep disturbances, and a labile mood in the context of previous combat experience form the core of the diagnosis. Yet the chapter proceeds to address unresolved questions about PTSD, with the recognition that it is a very difficult injury to treat; available therapies are not as effective as we would wish. Not infrequently Soldiers with PTSD come to the attention of care providers because of legal difficulties, during struggles in navigating the Veterans Administration Disability System, or following accusations of malingering. Although there is a recognition that PTSD may invoked as a possible defense for crimes, no clear conclusion is presented to show whether a diagnosis is likely to affect trial outcomes. However, this defense strategy may be a more powerful mitigating factor in a military, as opposed to a civilian, courtroom. Furthermore, care providers continue to struggle with optimal PTSD therapy, and their numerous, widely disparate approaches help us realize how difficult overcoming this illness can be. Resilience training, cognitive behavioral therapies, and medications are all a part of care. Not infrequently, alternative and complementary methods are also tried. Although PTSD is labelled an anxiety disorder, overlapping therapies of antipsychotics and antidepressants remain the primary approach to medical therapy.

The training of forensic psychologists and psychiatrists, both within and outside of the military, is outlined, providing insight into the formal education of these care providers. These experts must be prepared to help Soldiers interact with the sanity board process, help recognize mitigating factors in the defense of a Soldier facing criminal charges, and explain behavioral health issues in a Soldier's disability hearing by describing how he or she has faced mental health and disciplinary issues. In addition, the role of these care providers in two specialized and specific criminal arenas is discussed. The first is the role of the forensic specialist in cases where Soldiers are charged with substance abuse; the second is the rare but intense situation when capital murder is alleged and the death penalty is sought.

Another chapter draws a clear line defining the expected (and required) behavior of psychiatrists in their role as care providers of detainees. A key point is that the psychiatrist or psychologist is not an interrogator, but he or she may act as a behavioral health care provider to the detainee or a as consultant to the interrogators (never serving in both roles). In the latter role, the focus is to help interrogators better recognize features of mental illness and the psychological effects of interrogation techniques.

The book also discusses how to design a safe and secure psychiatric facility. A clear description of how to house those with long-term forensic psychiatric illnesses is detailed, as illustrated by the new Saint Elizabeths Hospital in Washington, DC, maximizing both security and therapeutic concerns. The principles presented can be used by all in the mental health field.

The book closes with an intriguing chapter addressing the relationship of mefloquine, a drug prescribed to Soldiers for malaria prophylaxis, with development of mood changes, psychosis, and in the context of underlying PTSD. The author recommends that all service members should be screened for the use of mefloquine. If the service member has been exposed to the medication and is facing legal charges, the situation should be articulated by the defense.

Forensic and Ethical Issues in Military Behavioral Health makes a significant contribution to the body of work identifying the lessons learned by Army care providers following the wars in Iraq and Afghanistan. Throughout the book, Dr Ritchie and the contributing authors identify and address many of the issues now in the forefront of care provided by the Army's behavioral medicine specialists. The authors show a strong understanding of the relationship between war and a Soldier's mental health and help us recognize approaches that must be in place as we go forward to treat service members of the next generation, lessons that will be relevant for many years to come.

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This book is available in PDF and will soon be available in iPad and Kindle formats for download on your electronic reader. The book can be ordered at no cost by those on active duty, in the National Guard, and in the Reserve by following the instructions on the Borden Institute website, http://www.cs.amedd.army.mil/borden/. It will soon be available for purchase from the US Government Printing Office.

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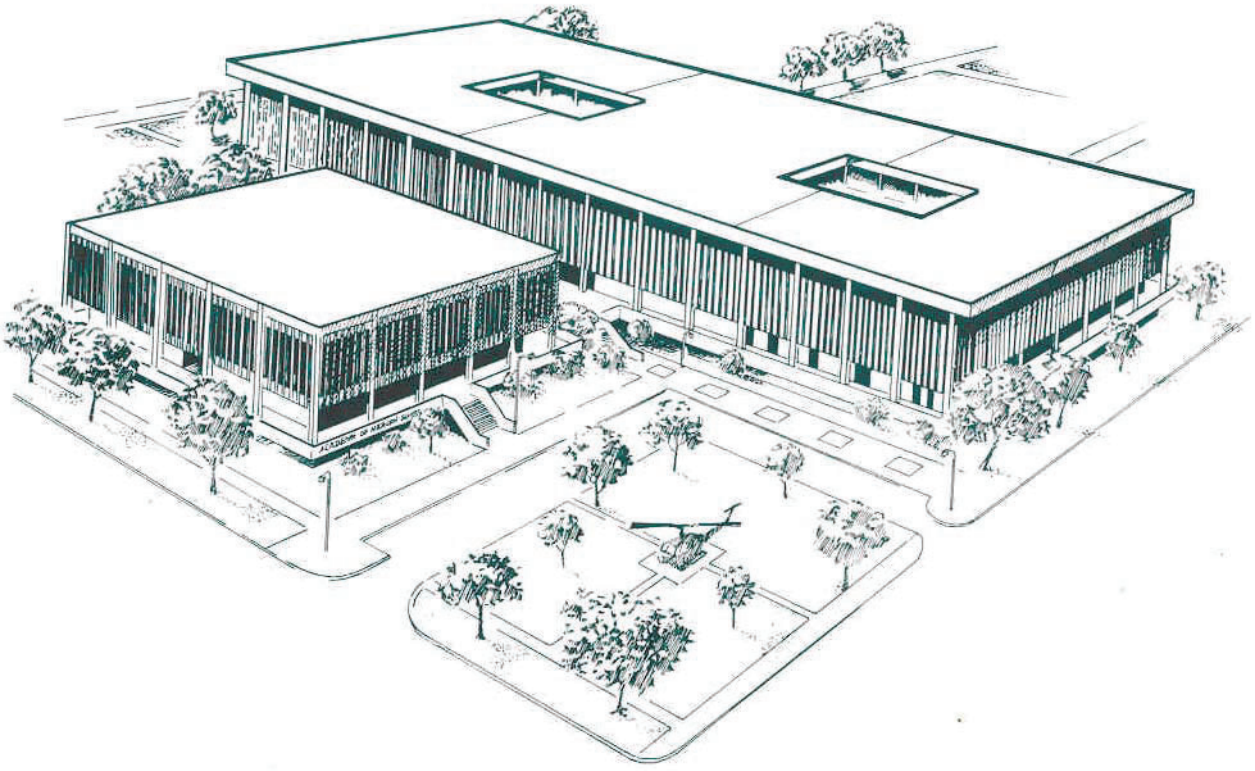

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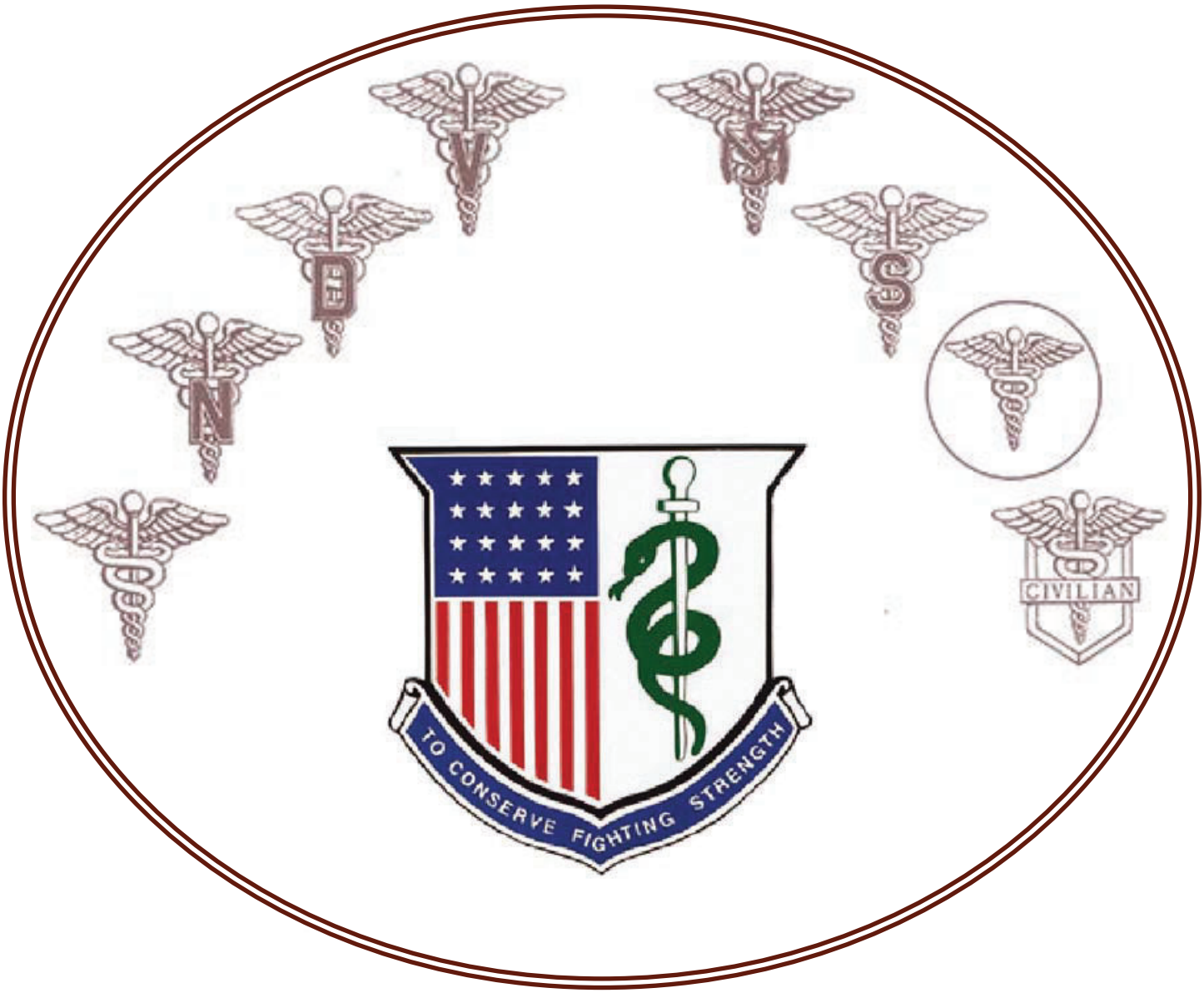

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