

## The role of the United States military in the development of vector control products, including insect repellents, insecticides, and bed nets

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**ABSTRACT:** Arthropod-borne diseases such as malaria, dengue, scrub typhus, and leishmaniasis continue to pose a significant threat to U.S. military forces deployed in support of operational and humanitarian missions. These diseases are transmitted by a variety of arthropods, including mosquitoes, ticks, chiggers, sand flies, and biting midges. In addition to disease threats, biting arthropods can cause dermatitis, allergic reactions, and sleep loss; therefore, monitoring of vector impact and integrated use of personal protective measures (PPM) and methods to reduce the vector populations are needed to protect service members. The U.S. military has played a vital role in vector identification tools and the development and testing of many of the most effective PPM and vector control products available today, including the topical repellent DEET and the repellent/insecticide permethrin, which is applied to clothing and bed nets. Efforts to develop superior products are ongoing. Although the U.S. military often needs vector control products with rather specific properties (e.g., undetectable, long-lasting in multiple climates) in order to protect its service members, many Department of Defense vector control products have had global impacts on endemic disease control. *Journal of Vector Ecology* 34 (1): 50-61. 2009.

**Keyword Index:** Vector control, U.S. military, bed nets, insect repellents, insecticides.

### INTRODUCTION

The impact of vector-borne disease on military operations is well known (Engelman and Joy 1975, Peterson 1995, Withers and Craig 2003). Combat experiences with yellow fever in the Spanish-American War led to the deployment, in 1900, of the Yellow Fever Commission to Cuba (Engelman and Joy 1975, Lang 1988). Major Walter Reed and other researchers were sent to Cuba to investigate the causes and transmission of yellow fever. By 1901, the commission proved that yellow fever was transmitted by the *Aedes* mosquito. Using this knowledge, Major William Gorgas implemented programs that eliminated yellow fever in the Panama Canal region and in the United States. The last case of yellow fever in the Panama Canal Zone was reported in May 1906 (Byerly 2005, Pierce and Writer 2005). Major Gorgas also succeeded in controlling malaria in the Panama Canal Zone, and this improvement in disease conditions made it possible for the United States to complete the canal (Engelman and Joy 1975). These initial successes in the field of medical entomology set the stage for subsequent efforts by the U.S. military to control vectors and prevent vector-borne diseases that expanded during World War II. Last minute delousing of service members using the newly-discovered insecticide DDT halted an epidemic of louse-borne typhus (Lang 1988). Problems with malaria in the Pacific theater led Army malaria control teams to use DDT for mosquito control in 1944 (Engelman and Joy 1975). These U.S. combat experiences led to the commissioning of military entomologists and the formation of vector-borne

disease control units (Lang 1988). Ultimately, this has grown into a career field and program that produces and supports world-class research in vector control.

### VECTOR-TRANSMITTED ENDEMIC DISEASE THREATS TO DEPLOYED U.S. SERVICE MEMBERS

The U.S. military is prepared to deploy service members anywhere in the world in support of humanitarian efforts and operational missions vital to national security. U.S. military personnel and civilian support staff are presently deployed in over 130 countries, including many places where diseases transmitted by arthropods (e.g., insects, chiggers, and ticks) are still prevalent. Therefore, U.S. personnel may be exposed to a wide range of vector-borne diseases to which they have no acquired natural immunity and for which U.S. Food and Drug Administration (FDA) licensed vaccines are not yet available (Dickens 1990). Biting and stinging arthropods can degrade mission readiness and combat effectiveness even in the absence of disease transmission, because persistent pests and itching bites are associated with lack of sleep, dermatitis, secondary infections, and death from allergic reactions.

A recently published quantitative algorithm, utilizing information from the U.S. Armed Forces Medical Intelligence Center (renamed the National Center for Medical Intelligence in 2008) to score the relative importance of various diseases by taking into account their severity and the likelihood of infection, found that arthropod-borne diseases account for 28 of the top 40 endemic disease threats impacting deployed

U.S. troop operations (resulting in lost manpower days, decreased unit morale, and medical costs), which suggests that improved vector control products are needed (Table 1) (Burnette et al. 2008). Malaria, dengue fever, and diarrhea (often caused by bacteria that can be transmitted by flies) are currently the top three threats and have been important threats in past wars. In late 1992 through early 1993, 131 U.S. military members contracted malaria, including at least 11 cases of *Plasmodium falciparum*, while deployed to Somalia (Sharp et al. 1995, Wallace et al. 1996). In 2003, 80 (28%) of 290 Marines who went ashore in Liberia to oversee a civil transition acquired malaria (Debboun et al. 2006). Because malaria can be a relapsing disease and because the female *Anopheles* mosquitoes capable of transmitting malaria exist in the United States, returning troops can transmit malaria to others (Brunetti et al. 1954). Malaria remains a continuing threat for troops deployed to Afghanistan and South Korea (Ciminera and Brundage 2007). Dengue fever is transmitted by *Aedes* mosquitoes in both rural and urban areas in some regions. Although rarely fatal in adults, this disease often necessitates hospitalization for supportive care. Diarrhea and/or vomiting have been a problem for some 60% of service members deployed to Iraq and Afghanistan in Operation Iraqi Freedom/Operation Enduring Freedom (OIF/OEF), although it is unclear how many of these cases are due to fly-transmitted disease (Monteville et al. 2006).

Other vector-borne diseases that have significantly affected recent U.S. military operations include leishmaniasis and sand fly fever (both transmitted by sand flies) (Aronson et al. 2006). Among hospitalized U.S. service members during the 1990-1991 Gulf War, there were 12 cases of visceral leishmaniasis (a potentially fatal infection affecting multiple organs) caused by *Leishmania tropica* protozoa. There have been more than 1,000 incident diagnoses/reports among U.S. service members deployed to OIF/OEF of cutaneous leishmaniasis, a potentially disfiguring parasitic skin disease; most of these cases were caused by *L. major* protozoa. (Approximately 1,000 cutaneous leishmaniasis cases occurred among U.S. service members in the Persian Gulf Command during World War II.) In addition, at least four service members in OIF/OEF have acquired visceral leishmaniasis caused by *L. infantum* protozoa (Myles et al. 2007). There is concern that asymptomatic latent leishmaniasis infection could be occurring in deployed troops that might cause subsequent disease in service members who later become immunosuppressed because of other diseases. Due to their small size and the fact that not enough is known about their biology, sand flies are difficult to control; to date, the use of air-conditioned quarters by service members in Iraq has proven to be the only effective countermeasure (Coleman et al. 2006, Sanders et al. 2005). Scrub typhus, a potentially life-threatening disease caused by *Orientia tsutsugamushi* bacteria that is transmitted to humans via bites from infected chiggers, has also impacted U.S. troops, particularly those engaged in jungle warfare in the Far East in World War II and the Vietnam War.

To manage vector-related threats, the U.S. military utilizes medical entomologists in preventive medicine teams

to assess impacts of vectors and vector-borne diseases and optimize use of available personal protective equipment and pest management practices by deployed forces. This effort receives input from the Armed Forces Pest Management Board and the U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) (Debboun et al. 2006). Research to develop new products to diagnose, prevent, and treat many of the vector-borne diseases affecting U.S. Department of Defense (DoD) personnel is funded by the Military Infectious Diseases Research Program (MIDRP). In addition, efforts to identify vectors and improve and replace currently available DoD vector control products continue; product evaluation often involves overseas laboratories. Repellents and pesticides used by the U.S. military at present must be U.S. Environmental Protection Agency (EPA) registered. However, the U.S. military has additional criteria (e.g., little to no odor, efficacy in multiple climates, efficacy against multiple arthropods, compatibility with U.S. military materials) and is therefore engaged in further testing of EPA-registered repellents to determine whether these products meet U.S. military needs.

#### BED NETS

Bed nets have been used as a means of protection against arthropod-borne diseases for hundreds and perhaps thousands of years. Bed net use became compulsory for U.S. soldiers in the Pacific during World War II following severe outbreaks of malaria and dengue fever (Sapero 1946, Bwire 2000). Since that time, the U.S. military has been involved in developing and testing improved versions of bed nets. Controlled trials in the 1980s demonstrated that bed net use could provide significant protection against malaria and, in particular, evaluated the effect on civilians of bed nets treated with permethrin, an insecticide/repellent often applied to bed nets to help further prevent insect bites (Lines et al. 1987). At present, only pyrethroid-based insecticides are registered by the EPA for application to bed nets. Current U.S. military research includes evaluation of synthetic pyrethroids (permethrin, deltamethrin, or alpha-cypermethrin) for optimum performance against sand flies, with the goal of incorporating the selected compound into future versions of the U.S. military bed net.

Research such as the trials mentioned above has helped lead to a greater role for bed nets in the implementation of malaria control programs worldwide. For example, international health groups are providing long-lasting, insecticide-treated nets (LLIN) to residents in malaria-endemic areas of underdeveloped countries, particularly in Africa. In such areas, regular use of insecticide-treated bed nets can reduce childhood mortality up to 20% and severe disease up to 50% (Lindsay et al. 1991, Gimnig et al. 2003). The use of LLINs is a key component of the U.S. President's Malaria Initiative, established in 2005 and coordinated by the U.S. Agency for International Development (USAID) and the U.S. Centers for Disease Control and Prevention (CDC). The goal of this program is to reduce deaths due to malaria by 50% in 15 African countries. This objective will

Table 1. Top forty infectious diseases of U.S. military significance<sup>a</sup>.

Disease	Arthropod-borne
1. Malaria	Yes ( <i>Anopheles</i> mosquitoes)
2. Diarrhea - bacterial	Yes (flies)
3. Dengue fever	Yes ( <i>Aedes</i> mosquitoes)
4. Rift Valley fever	Yes (mosquitoes)
5. Gonorrhea/chlamydia	No
6. Chikungunya	Yes ( <i>Aedes</i> and <i>Culex</i> mosquitoes)
7. Leptospirosis	No
8. HIV/AIDS	No
9. Meningococcal meningitis	No
10. Crimean-Congo hemorrhagic fever	Yes (ixodid ticks)
11. Brucellosis	No
12. Diarrhea - protozoal	No
13. Sand fly fever	Yes (sand flies)
14. Hepatitis E	No
15. Schistosomiasis	No
16. O'nyong-nyong	Yes ( <i>Anopheles</i> mosquitoes)
17. Sindbis (Ockelbo) virus	Yes (multiple species of mosquitoes)
18. Hemorrhagic fever - renal syndrome	No
19. Scrub typhus	Yes (mites)
20. Leishmaniasis - visceral	Yes (sand flies)
21. Diarrhea - cholera	Yes (flies)
22. Rickettsioses (spotted fever group)	Yes (ticks)
23. Tick-borne encephalitis	Yes ( <i>Ixodes</i> ticks)
24. Japanese encephalitis	Yes ( <i>Culex</i> mosquitoes)
25. Murine typhus	Yes (fleas)
26. Q fever	Yes (argasid ticks)
27. Plague	Yes (rodent fleas)
28. Venezuelan equine encephalitis	Yes ( <i>Culex</i> and <i>Psorophora</i> mosquitoes)
29. Oropouche virus	Yes (biting midge <i>Culicoides paraensis</i> )
30. Leishmaniasis - cutaneous	Yes (sand flies)
31. Tularemia	Yes (multiple tick vectors)
32. Trypanosomiasis - gambiense	Yes (tsetse flies)
33. West Nile fever	Yes (mosquitoes)
34. Lassa fever	No
35. Leishmaniasis - cutaneous/mucosal	Yes (sand flies)
36. Trypanosomiasis - rhodesiense	Yes (tsetse flies)
37. H5N1 avian influenza	No
38. Hantavirus pulmonary syndrome	No
39. Lyme disease	Yes ( <i>Ixodes</i> ticks)
40. Mayaro virus	Yes (mosquitoes)

<sup>a</sup>From Burnette et al. 2008

be met in part by ensuring that at least 85% of children less than 5 years of age and of pregnant women have access to and routinely use LLINs.

The U.S. military standard insect bed net that has been used for many years (it is not impregnated with an arthropod repellent) requires four 36-inch poles to be set up for use with military cots and must be completely tucked in on all sides of the cot to prevent biting insects from entering. The Self-Supporting Low-Profile (SS-LP) bed net is a smaller bed net recently developed for short-term use by highly mobile forces (e.g., Infantry, Rangers, Special Forces) by the Walter Reed Army Institute of Research (WRAIR) in collaboration with Breakthrough Technologies. This lightweight bed net constructed of flame-retardant materials and rip-stop fabric includes an internal, self-supporting flexible vinyl ester/fiberglass frame, a waterproof floor, two zippered doors, and a mesh top. The frame pops open automatically from a 12-inch-diameter backpack package so that the bed net is ready for immediate use on bare ground or over a standard military cot or hospital bed (Frances et al. 2003). The tightly woven mesh is intended to exclude small insects such as midges and sand flies, and all fabric surfaces are impregnated with EXPEL, an EPA-registered permethrin-based insecticide. A comparison of the SS-LP bed net with the Australian Defence Force (ADF) mosquito bed net found that both bed nets provided greater than 97.9% protection compared to unprotected personnel (Frances et al. 2003), and the insecticide-untreated SS-LP bed net provided better night-time protection than the untreated ADF bed net. However, the small dimensions can provoke claustrophobia and the fine mesh of the SS-LP bed net limits air flow, which can cause overheating and noncompliance in hot climates (Coleman et al. 2006).

WRAIR is working with several companies to develop a new erectable, durable, low-weight bed net that is sufficiently tall to allow a soldier to sit up on a cot within the bed net. The DoD is evaluating long-lasting net products that are already commercially available as well as new long-lasting netting materials being developed by companies supported by military Small Business Innovation Research (SBIR) Program funding. The goal is a bed net constructed of high-denier polymer fabric with integrated insecticide to help protect against small biting insects, but with a mesh large enough to permit adequate air flow. Use of battery-operated fans to improve air flow within the new bed net and improve vector control has been considered, but employing products that require batteries increases the logistical burden on deployed troops. The revised bed net could be useful to civilians as well as the U.S. military. However, some troops decline to use any bed nets because of concerns that they will not be able to escape blast injuries while confined in a bed net. Development of a repellent-impregnated fabric belt to be worn over pants around the waist area (thereby avoiding skin contact issues) could provide an alternative means of vector control for such individuals.

## INSECT REPELLENTS AND INSECTICIDES

Many cultures use plants or plant material to repel insects. The smoke produced by burning plant material is thought to work by increasing heat, lowering humidity (thereby reducing mosquito sensory input), and masking human-produced carbon dioxide (Davis and Bowen 1994). Plants can also be used more directly. Many plants (e.g., eucalyptus, citronella, catnip, sage, lavender, basil, thyme, and the tea tree) contain oils that repel arthropods when applied to skin, hair, or wood (that keeps insects out of an area where humans or animals dwell) (Moore et al. 2007). In fact, the use of plants as natural repellents can be an inexpensive and aesthetically appealing option. However, both of these methods have drawbacks that limit their use in military situations. Smoke has adverse health effects and can reveal the locations of deployed personnel to potential adversaries. Similarly, strong plant odors emanating from natural oils can reveal personnel locations. In addition, insect repellents are only effective in the vapor phase and plant-related repellent compounds are generally highly volatile. Therefore, application to human skin results in an initially strong but often short-lived repellent effect. Although it may be possible to lengthen repellent activity by incorporating these natural oils from plants into ointment or gel formulations, at least one drawback remains. Deployed service members do not always have access to clean running water for hand washing and showers and thus often do not wish to apply sticky or oily repellents to already oily skin.

Plants with components that can kill insects include nicotine, *Mentha* (mint) species, and pyrethrum daisies. Pyrethrum, a natural plant oil with minimal mammalian toxicity derived from two species of pyrethrum daisies (*Tanacetum cinerariifolium* and *Tanacetum coccineum*), has been used for centuries to control pests. Pyrethrins, the insecticidal components of pyrethrum, are located in tiny oil-containing glands in the flower head. Pyrethrum was probably introduced into Europe circa 1300 by Marco Polo, and pyrethrum powders were used by military troops from the time of Napoleon to kill head and body lice. Because insects avoid pyrethrum, pyrethrum also has repellent effects. Major Gorgas used pyrethrum in Cuba to control yellow fever and malaria by burning it inside sealed dwellings. Spiral-shaped burnable mosquito repellents containing mixtures of pyrethrum powder have been marketed since 1902 and these items are still widely used in Asia. Pyrethrum daisies were grown in the United States from approximately 1870 to the early 1900s and then imported from Japan until World War II, at which time the United States supported efforts to grow pyrethrum crops in Kenya to make pyrethrum products available to Allied troops.

More potent and photostable pyrethroids (synthetic analogs of pyrethrum) were developed in Europe beginning in the 1930s (Moore and Debboun 2007, Moore et al. 2007). Permethrin was first synthesized in 1972 in the United Kingdom and registered for agricultural use in 1979 by the EPA. Permethrin is an odorless, water-based, and ultimately

biodegradable compound with low mammalian toxicity that will not damage plastics, is harmless to natural and synthetic fabrics, and is somewhat resistant to degradation by sunlight (Hossain et al. 1989, Macedo et al. 2007). Although useful as a topical treatment for human head lice and scabies and as an insecticide/repellent when applied to horses, permethrin is ineffective as an insecticide/repellent when applied to human skin because the compound does not bond to human skin.

Important early research on permethrin was conducted by Wellcome Research Laboratories. The DoD began evaluating permethrin for clothing treatments in collaboration with the U.S. Department of Agriculture (USDA) in 1979. In a three-day field evaluation at Camp Lejeune, NC, 21 subjects participated in studies that demonstrated that permethrin-treated clothing protected against chigger mites (Breedon et al. 1982). The effectiveness of pressurized sprays of permethrin on clothing for protection against the lone star tick (*Amblyomma americanum*) has been demonstrated (Schreck et al. 1982). Pressurized sprays of commercially available permethrin (0.5%) or DEET (20% or 30% concentrations) applied to military field uniforms were evaluated in May 1984 as protectants against bites by the *Ixodes dammini* tick in Massachusetts. A one-minute application of permethrin to the exterior surface of pants and jackets provided 100% protection against attack by all life stages of the tick, whereas application of DEET provided much less protection. Field evaluations in Harford County, MD, in the summer of 1988 revealed similar results in terms of protecting service members from bites of local ticks.

Studies involving the U.S. Army's laboratories in Natick, MA, demonstrated that long-lasting permethrin clothing impregnation can be achieved via an industrial dye bath process with both polyester-cotton and nylon-cotton uniform fabrics. Four different methods used by the DoD to impregnate clothing have been registered by the EPA, and factory permethrin-treated clothing was approved for DoD use in 1990 and civilian use in 2003 (Gupta et al. 1987, Gupta et al. 1990, Hossain et al. 1989, Asidi et al. 2004). Permethrin's insecticidal effect on clothing can last two to six weeks despite weekly launderings but is promptly removed by dry cleaning. A limited cumulative "herd effect" has been noted when large groups of personnel wearing permethrin-treated uniforms are congregated.

U.S. service members are trained on and provided with methods to impregnate loose-fitting combat uniforms with permethrin and given a long-lasting DEET formulation, for application to exposed skin, as part of an integrated risk mitigation strategy when deployed to regions where vector-borne diseases exist (Curtis et al. 1996, Young and Evans 1998). The U.S. Marine Corps now issues uniforms that have been factory-treated with permethrin.

Although there have been reports of reduced efficacy of permethrin as an insecticide for some vectors in some areas, permethrin is still a useful clothing repellent (Etang et al. 2004). Another issue impacting U.S. troops is that because the fabrics of currently used fire-resistant uniforms do not absorb permethrin evenly, the risk of vector-borne diseases

in OIF/OEF personnel could increase. Given that the threat of explosive devices in OIF/OEF is substantial, the use of fire-resistant uniforms is now mandated for U.S. Army ground convoy personnel.

A need for non-plant-based synthetic insect repellents and insecticides for U.S. troops was identified during World War II, when Japan and Japanese-controlled countries were the major sources for pyrethrum as well as quinine. Just as the non-availability of quinine during the World Wars prompted development of synthetic antimalarial drugs by U.S. military scientists, the enormous burden of disease suffered by Allied troops (especially malaria, dengue, and scrub typhus) motivated research on synthetic insect repellents (Sapero 1946).

The U.S. military relied on the residual insecticide dichloro-diphenyl-trichloroethane (DDT) beginning in 1943 after insecticidal properties of this compound were discovered by the Swiss chemist Paul Hermann Müller, and USDA helped develop usable products (Bwire 2000). Between 1942 and 1945, over 20,000 potential repellent/insecticide compounds (including many synthetic compounds) were tested by USDA with U.S. War Department support. Dimethyl phthalate (DMP) and Indalone (Butyl-3,3-dihydro-2,2-dimethyl-4-oxo-2H-pyran-6-carboxylate) demonstrated significant protection against chiggers in Louisiana and New Guinea (Moore and Debboun 2007). Benzyl benzoate provided protection against mites/lice. Application of these substances to clothing and skin significantly reduced the incidence of scrub typhus among troops in the Pacific theater during World War II (Whayne 1955). In 1943, USDA researchers Lyle Goodhue and William Sullivan developed small aerosol cans pressurized by liquefied fluorocarbon gas to spray vector control agents.

After World War II, a repellent known as 6:2:2 (better known as 6-2-2 or M-250) that contained 6 parts DMP, 2 parts Indalone, and 2 parts ethyl hexanediol became popular in the United States. However, products containing ethyl hexanediol were voluntarily removed from the U.S. and Canadian markets in 1991 following identification of exposure-related developmental toxicity (Moore and Debboun 2007).

The development of DEET (N,N-diethyl-metaltoluamide) was an important achievement (McCabe et al. 1954). DEET is a broad-spectrum insect repellent that is effective against mosquitoes when applied to skin. DEET products became available to the general public in 1957 (before the creation of the EPA in 1970). The EPA subsequently registered DEET, and this product remains the most widely used and widely studied repellent. DEET has been used for half a century with minimal reported adverse effects, many of which involved excessive or inappropriate use of this repellent. DEET toxicology issues have been carefully scrutinized and it has been deemed safe for human use, including children and pregnant women (Robbins and Cherniack 1986).

The original 75% DEET/ethanol liquid formulation (that earned the nickname "bug juice") was developed

by USDA and supported through DoD funding. This formulation was painful when applied to skin areas with cuts or abrasions because of the ethanol content and was effective for only one to two hours under warm, humid conditions. Subsequently, the U.S. Army and USDA collaborated in developing the Extended Duration Topical Insect and Arthropod Repellent (EDTIAR). EDTIAR is a multipolymer, extended-duration formulation containing 33% DEET along with polymers that slow absorption and evaporation of the repellent (Rutledge et al. 1986). This extended-duration DEET formulation, manufactured by 3M and registered by the EPA in 1991, is used by the U.S. and British militaries. It provides six to 14 hours of protection under varying environmental conditions (Rutledge et al. 1996, Schreck et al. 1995). Despite its excellent track record as a repellent, DEET does have drawbacks. It dissolves plastic on eyeglasses, watch crystals, and protective mask eyepieces and can harm synthetic fabrics. Evidence suggests that some DEET users find the oily sensation and chemical odor unpleasant and, in particular, service members have expressed doubts about its effectiveness and safety (Sanders et al. 2005). This has led to renewed efforts in repellent research by the U.S. military.

#### VECTOR AND VECTOR-BORNE INFECTIOUS DISEASE IDENTIFICATION TOOLS

Effective control of vectors and arthropod-borne diseases necessitates development of reliable means of recognizing key vector species. British and U.S. military officers identified vectors of malaria and yellow fever in the late nineteenth century (Essig 1931, Gilbert and Hamilton 1990). In 1961, when a major study of the mosquitoes in Thailand was undertaken by the U.S. Army Medical Component, Southeast Asia Treaty Organization (SEATO) in Bangkok, the need for a broader, more detailed investigation beyond the capability of the Bangkok SEATO laboratory (which became the Armed Forces Research Institute of Medical Sciences in 1977) became evident. The consensus was that this project could best be conducted at the Smithsonian Institution's National Museum of Natural History (SI NMNH) in Washington, D.C., supported by a contract from the U.S. Army Medical Research and Development Command. The Section of Entomology in the SINMNH's Department of Systematic Biology was identified as the most appropriate partner owing to its interest in the project, the presence of extensive mosquito collections, and the active participation of mosquito taxonomists. Various titles have been used to identify this endeavor: "The Army Mosquito Project," the "Southeast Asia Mosquito Project," and the "Medical Entomology Project." The Walter Reed Biosystematics Unit (WRBU), now located at the Museum Support Center of the Smithsonian Institution in Suitland, MD, replaced the MEP beginning in 1981.

The WRBU is an internationally renowned center that is dedicated to studying the systematics and taxonomy of medically important arthropods. The WRBU has developed interactive, Web-based guides to the medically important

mosquitoes of the CENTCOM (Central Command) and PACOM (Pacific Command) regions, which can be accessed via the WRBU Web site ([www.wrbu.org](http://www.wrbu.org)). Military preventive medicine specialists deployed in an operational setting can conduct surveillance and trap arthropods capable of transmitting endemic diseases to humans, identify many of these vectors via laptop computers, and utilize this information to assess the likelihood of human disease, plan and develop optimal countermeasures, and monitor effectiveness of control efforts. The WRBU also supports epidemiological studies, vector research activities such as arboviral assays, and repellent and insecticide evaluations.

The WRBU's staff includes three research entomologists (including an active duty Army officer) and two museum specialists. There are also opportunities for temporary staff and collaborators—including visiting scientists, research associates, and National Research Council fellows. Salaries are paid by the MIDRP vector research program. WRBU office and laboratory space is provided by the Smithsonian Institution with the expectation that the mosquito collection will be enlarged and maintained at the highest standards.

The identification guides to mosquitoes of the U.S. Pacific Command Area of Responsibility and U.S. Central Command Area of Responsibility are nearing completion. To date, 189 new species of mosquitoes and 61 new species of biting midges have been described. Anticipated future projects include the development of Web-based mosquito keys for medically important African mosquitoes and Southwest Asian sand flies. Over 12,000 pages have been published in 392 publications (senior authorship attributed to 14 military officers, 11 civilian employees, and 31 adjunct personnel). A total of 58 revisionary studies and major identification keys/guides have been published (Faran 1980, Glick 1992, Harbach 1985, Huang 2004, Pecor et al. 1992, Peyton and Scanlon 1966, Rueda 2004, Rutledge et al. 1996, Sallum et al. 2005, Wilkerson and Peyton 1990, Wilkerson et al. 1990).

Diagnostic assays that identify human-disease-causing pathogens in vectors help U.S. military preventive medicine service members mitigate vector-borne disease outbreaks. A malaria sporozoite antigen panel assay for detection of malaria parasites in mosquitoes has been developed and marketed by VecTOR Test Systems, Inc. with Army SBIR funding, and overseas DoD laboratories have tested the assay for sensitivity and specificity. A similar assay (called VecTest™) was previously manufactured by Medical Analysis Systems, Inc. (Sattabongkot et al. 2004), and development of a VecTest™ used to identify West Nile virus and St. Louis encephalitis viral antigens in mosquitoes was also supported by U.S. Army SBIR funding. Other fielded non-commercial assays used by the U.S. military to detect infectious diseases in vectors since 2004 include Rift Valley fever virus conventional and real-time (RT) polymerase chain reaction (PCR) assays (used during the 2006-2007 outbreak); chikungunya and O'nyong-nyong conventional and RT PCR assays; sand fly fever conventional PCR assays (for Toscana, Naples, and Sicilian strains); Ndumu, Babanki, Bunyamwera, and Dugbe virus conventional

PCR assays; and eastern equine encephalitis virus reverse transcription PCR assays (O'Guinn et al. 2004). The U.S. Air Force has developed deployable, field-sustainable, reverse transcription PCR assays for rapid screening and serotype identification of dengue virus in mosquitoes (McAvin et al. 2007).

Detecting endemic infections in service members via rapid testing helps alert preventive medicine teams to the need to increase vector countermeasures. Although diagnosis of malaria in humans has traditionally relied on labor-intensive microscopy that is difficult to accomplish in field conditions, WRAIR and overseas laboratories have been involved in testing the BinaxNOW® rapid immunodiagnostic assay for detecting malaria in humans following 510(k) clearance by the FDA (Murray et al. 2008). The *Leishmania* Diagnostics Laboratory at WRAIR has also utilized the College of American Pathologists-certified SmartCycler®-based RT PCR *Leishmania* assay for diagnosis of cutaneous leishmaniasis cases in theater, and efforts are ongoing to obtain U.S. FDA licensure for this product (Wortmann et al. 2005). A rapid diagnostic assay to detect scrub typhus in humans is in development.

#### CURRENT U.S. MILITARY VECTOR CONTROL RESEARCH EFFORTS

In addition to the development of new bed nets and continuing diagnostic efforts, U.S. military researchers are evaluating even safer and more effective candidate repellents as eventual replacements for DEET. (It should be noted in this connection that attractive packaging for insect repellents may be important to encourage use.) Several compounds show promise as alternatives to DEET, including optically active (1S, 2S)-methylpiperidinyl-3-cyclohexen-1-carboxamide (SS220), Picaridin (KBR 3023, commercial name Saltidin®), IR3535®, and PMD (p-menthane-3,8-diol).

In recent years, there has been a U.S. military focus on SS220, which had been specially formulated into compounds for WRAIR repellent field and laboratory tests. Although studies have demonstrated that SS220 is an effective repellent (Debboun et al. 2000, Klun et al. 2004, Watanaporn Dheranetra, unpublished data), it is expensive to produce and not EPA registered, and no business is currently working to market SS220 products. Research focus has therefore shifted to formulations containing Picaridin and IR3535®, both recommended for arthropod-borne disease prevention by the World Health Organization in 2005.

Insect repellent 3535 (IR3535®) (3-[N-Butyl-N-acetyl]-aminopropionic acid, ethyl ester) was developed in 1975 by Merck. Because this product is a substituted  $\beta$  amino acid structurally similar to naturally occurring  $\beta$ -alanine, IR3535® was registered by the EPA as a biopesticide in 1999 (Moore and Debboun 2007). Formulations containing IR3535® have been available on both the U.S. and international markets for several years, and the efficacy of IR3535® as an insect repellent is generally comparable to that of DEET (Thavara

et al. 2001). Formulations with higher concentrations of IR3535® (10% and 20%) have recently been produced for the U.S. market and registered by the EPA.

Picaridin (1-piperidinecarboxylic acid, 2-[2-hydroxyethyl]-1-methylpropylester; commercial name Saltidin®) was developed by Bayer in the 1980s based on molecular modeling of piperidine. Saltidin® (EPA registration no. 3125-512, December 7, 2000) has minor impacts on plastic and is a colorless, odorless compound with low mammalian toxicity similar to that of DEET. The CDC has proposed use of Picaridin as well as DEET for West Nile fever and malaria prevention. Picaridin performed as well as DEET against *Culex spp.* and *Aedes spp.* in Malaysia (Yap et al. 1998). The Australian Defence Force (ADF) determined that a 19.2% Picaridin compound (Autan®, manufactured by Bayer) outperformed the ADF's 35% DEET gel in terms of longer-lasting efficacy and acceptability for soldiers deployed to East Timor in 2001. Half of respondents using ADF 35% DEET gel reported that the compound was uncomfortably oily or caused irritation (Frances and Cooper 2002, Frances et al. 2002). Recent studies showed that Picaridin provided the highest percent repellency compared to DEET and IR3535® against mosquitoes in the Everglades National Park (Barnard et al. 2002) and against the *Anopheles gambiae* complex in Africa (Costantini et al. 2004). However, this product may be a less effective repellent than DEET for a few insects such as *Culex annulirostris* in northern Australia (Frances et al. 2004). Insect repellents containing 7% Picaridin are currently available in the United States, and 20% formulations (pump spray and lotion) have recently been registered by the EPA for commercial use.

In addition to evaluating the compounds discussed above, the U.S. military and USDA are working to identify potential new arthropod repellents and insecticides. Much of this work has been funded by the DoD through MIDRP and the Deployed Warfighter Protection (DWFP) program. Via efforts aimed at identifying the molecular mechanisms of known repellents and insecticides such as DEET and permethrin, a 3D pharmacophore model has been developed to predict the repellent and insecticidal potency of novel compounds (Bhattacharjee et al. 2005). The Department of Vector Control of the Division of Entomology at WRAIR has conducted *in vitro* screening of new repellent compound candidates identified by the model and has provided data to support the model's further development. Other researchers supported by military funding have designed novel N-acylpiperidine compounds (some of which exhibited prolonged mosquito repellency) by using an artificial neural network model to analyze USDA archival data (Katritzky et al. 2008).

Finally, recent developments in spatial repellent technology are resulting in novel products that can be used to protect service members from vector-borne diseases. One such product is the ThermaCELL® Mosquito Repellent system, consisting of a butane-fueled generator that heats a metal plate to volatilize d-cis/trans allethrin (a pyrethroid) from an impregnated pad. In a field trial in Turkey, the ThermaCELL® system reduced biting rates of sand flies

and mosquitoes by at least 90% for 6 h out to a distance of approximately 7 m (Alten et al. 2003). In addition, research supported by DWFP, the Army SBIR program, and MIDRP has focused on developing spatial repellents into wearable repellent devices and using spatial repellents in field situations as barrier treatments.

### CONCLUSION

The U.S. military has worked closely with a variety of partner organizations to develop a range of vector control and vector identification techniques. Partner organizations include friendly military forces, other U.S. governmental organizations such as USDA, and businesses. In many instances, the U.S. military has supplied funding and motivation to other organizations (e.g., USDA) to develop products and has subsequently tested and fielded these products. Many of these products have played a significant role in protecting deployed U.S. military forces from arthropod-borne diseases. Continued research and development with a range of partners is essential, as no single vector control product can completely control all vectors under all conditions. The most effective control of vectors often requires the simultaneous use of multiple products and measures. In addition to protecting deployed forces, many of the vector control products whose development was supported by the U.S. military are also widely used by nonmilitary organizations and have had global impacts on endemic disease control (Table 2).

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Table 2. U.S. Military contributions to vector control products/methods.

Product/Method	Background	U.S. Military Contributions
Bed Nets	Used since ancient Egypt.	Bed net use compulsory for U.S. soldiers in Pacific regions during World War II; U.S. Army has tested and utilized permethrin-impregnated bed nets.
Insecticides	Pyrethrum has been used since ancient Persia; synthetic version (permethrin) developed in UK in 1972.	DoD tested efficacy and safety of permethrin-coated field uniforms beginning in 1979.
Repellents	USDA screened chemicals to identify repellents beginning in 1942; developed DEET with DoD funding.	U.S. Army and USDA collaborated in developing extended-duration version of DEET given U.S. EPA approval in 1991; a combined camouflage face paint/DEET product was fielded in 2002.
Vector Identification	British Surgeon Major Ronald Ross discovered that the <i>Anopheles</i> mosquito transmitted malaria in 1897.	U.S. Army researchers determined in 1900 that the mosquito species now known as <i>Aedes aegypti</i> transmits yellow fever; 1961 study of mosquitoes in Thailand by U.S. Army Medical Component, Southeast Asia Treaty Organization showed that more related work was needed, so Walter Reed Biosystematics Unit was created in 1981.
Identification of pathogens transmitted by vectors that cause disease in humans	Diagnosis of diseases such as malaria in humans has traditionally relied on labor-intensive methods.	U.S. military researchers have been involved in the development and testing of diagnostic assays to identify multiple pathogens associated with human disease in vectors and humans.

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