Field Evaluations of Topical Arthropod Repellents in North, Central, and South America

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J. Med. Entomol. 51(5): 980–988 (2014); DOI: http://dx.doi.org/10.1603/ME14075

ABSTRACT Recently, vector-borne diseases have been resurging in endemic areas and expanding their geographic range into nonendemic areas. Such changes have refocused attention to the potential for major public health events, as naïve populations are exposed to these pathogens. Personal topical repellents, recommended by the United States Centers for Disease Control and Prevention and World Health Organization, remain a first line of protection against infection. The current study evaluated the repellent efficacy of four new U.S. Environmental Protection Agency-registered topical repellent products, two with picaridin as the active ingredient and two with IR3535, against a standard DEET (*N*,*N*-diethyl-3-methylbenzamide)-based product. All products were evaluated against a wide range of vector species under field conditions across the Americas. Human volunteers were used to evaluate product efficacy as compared with a well-known DEET-based formulation and determine suitability for use by the U.S. military. Findings demonstrated the new formulations performed as well as the standard U.S. military repellent and could be recommended for use.

KEY WORDS topical repellent, human volunteer, efficacy, vector-borne disease, biting fly

During the 20th century, scientific advances and new, effective pesticides rendered insignificant several major arthropod-borne diseases that had frequently plagued temperate zones of the Western hemisphere. Most significant of these was yellow fever and malaria (De La Rocque et al. 2011). As a result, concern over arthropod-borne diseases waned, and many were classified as "tropical diseases" that persisted in more remote or underdeveloped parts of the world (De La Rocque et al. 2011). Since 2000, vector-borne diseases have been elevated on the public health agenda due to the emergence and reemergence of these diseases across the temperate zones of Europe and North America (Zell 2004, De La Rocque et al. 2011) and their spread into higher elevations of Africa, Latin America, and Asia (Epstein 2001). Dengue fever and dengue hemorrhagic fever have resurged dramatically in Latin America (Zell 2004). In North America, West Nile virus has impacted significantly the health and welfare of humans and other animals since it was introduced into the United States in 1999. Only 3 years later, it had been detected in all but for four states across the continental United States (DiMenna et al. 2006). While climate change, globalization, and land use patterns have all been cited as contributing factors for occurrence of diseases in new geographical regions or recurrence of diseases in regions where the disease had been eliminated (Berns and Rager 2000, Epstein 2001, Zell 2004, De La Rocque et al. 2011), the immediate and future impacts on public health in these regions must be addressed.

For most of these diseases, there is no vaccine or chemoprophylaxis that can prevent their transmission, thus necessitating a continual need for additional measures to reduce disease risk, such as the use of adequate personal protective measures. The World Health Organization (WHO), the United States Centers for Disease Control and Prevention (CDC), and the U.S. Environmental Protection Agency (EPA) all recommend the use of personal, topical repellents to prevent bites from arthropod vectors and thus reduce the possibility of pathogen transmission (Rozendaal 1997, CDC 2012, EPA 2013). DEET (*N*,*N*-diethyl-3-methylbenzamide) is considered the gold standard topical arthropod repellent, and it is recommended for use as a positive control in scientific evaluations of other topical repellents (WHO 2009, EPA 2010). It is

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Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
SEP 2014		**00-00-2014 to 00-00-2014**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Field Evaluations of Topical Arthropod Repellents in North, Central, and South America	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Walter Reed Army Institute of Research,Entomology Branch,Silver Spring,MD,20910**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Recently, vector-borne diseases have been resurging in endemic areas and expanding their geographic range into nonendemic areas. Such changes have refocused attention to the potential for major public health events, as na??ve populations are exposed to these pathogens. Personal topical repellents, recommended by the United States Centers for Disease Control and Prevention and World Health Organization, remain a ?rst line of protection against infection. The current study evaluated the repellent ef?cacy of four new U.S. Environmental Protection Agency-registered topical repellent products, two with picaridin as the active ingredient and two with IR3535, against a standard DEET (N,N-diethyl-3-methylbenzamide)-based product. All products were evaluated against a wide range of vector species under ?eld conditions across the Americas. Human volunteers were used to evaluate product ef?cacy as compared with a well-known DEET-based formulation and determine suitability for use by the U.S. military. Findings demonstrated the new formulations performed as well as the standard U.S. military repellent and could be recommended for use.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**Same as Report (SAR)**	**9**	

Table 1. Distribution of species across total mosquitoes collected from human volunteers at Orange Walk Town, Belize (19–21 September 2007)

Species	Number collected	%
Anopheles albimanus	1,063	24.84
Aedes taeniorhynchus	1,052	24.58
Anopheles vestitipennis	555	12.97
Psorophora confinnis	371	8.67
Aedes (Ochlerotatus) sp.	331	7.73
Aedes scapularis	189	4.42
Mansonia titillans	162	3.79
Anopheles galbaldoni	148	3.46
Anopheles (Anopheles) sp.	98	2.29
Coquillettidia nigricans	50	1.17
Anopheles sp.	40	0.93
Anopheles (Nyssorhynchus) sp.	35	0.82
Psorophora albipes	29	0.68
Culex (Culex) sp.	25	0.58
Culex erraticus	22	0.51
Anopheles apicimacula	21	0.49
Culex nigripalpus	19	0.44
Anopheles crucians	17	0.40
Culex (Melanoconion) sp.	16	0.37
Psorophora sp.	10	0.23
Anopheles punctimacula	8	0.19
Aedes serratus	6	0.14
Culex sp.	4	0.09
Psorophora ferox	2	0.05
Psorophora (Psorophora) sp.	2	0.05
Aedes fulvus	2	0.05
Aedes aegypti	1	0.02
Culex coronator	1	0.02
Coquillettidia venezuelensis	1	0.02
Total	4,280	100.00

EPA-registered and recommended for use by the CDC. However, two other active ingredients are also recommended by the CDC (CDC 2012) as an alternative to DEET: picaridin (1-(1-methyl-propoxycarbonyl)-2-(2-hydroxy-ethyl)-piperidine) and IR3535 (3{*N*-butyl-*N*-acetyl}-amino propionic acid, ethyl ester).

Both picaridin and IR3535 have demonstrated comparable efficacy to DEET in several field studies against a wide range of mosquito species in the United States and other parts of the world (Yap et al. 1998, Thavara et al. 2001, Barnard et al. 2002, Costantini et al. 2004). However, these studies were primarily conducted using technical grade active ingredient and not formulated products. Both active ingredients have been available in commercial compounds in foreign markets for over 20 yr, but until only recently (1999 for IR3535 and 2005 for picaridin) have become available in commercial products in the United States. The products marketed in the United States all contain lower concentrations (<10%) of the active ingredient compared with those sold in other parts of the world. New formulations that contain higher percentages of these active ingredients are EPA registered, and preliminary data indicate that they exhibit comparable efficacy to DEET against a wide range of arthropod vectors. For example, these new products performed as well as Ultrathon (3M, St. Paul, MN), a popular formulation of DEET, against nymphal *Amblyomma americanum* (L.) ticks (Carroll et al. 2010). Additional laboratory studies demonstrated that these products are effective against both Old World and New World Leishmania vectors, *Phlebotomus papatasi* (Scopoli) and *Lutzomyia longipalpis* (Lutz and Neiva, 1912), respectively (K.L.L., unpublished data).

The current study was designed and executed as a set of independent evaluations to assess the efficacy and duration of two lotion formulations (20% picaridin, 10% IR3535) and two spray formulations (20% picaridin, 20% IR3535) under field conditions over 12 h postapplication against Ultrathon (34% DEET) in Belize, South Carolina, and Peru. Ultrathon was selected as the positive control because it is the standard U.S. military repellent (NSN 6840-01-284-3982; Armed Forces Pest Management Board [AFPMB] 2001) and is also one of the top-rated repellents in the consumer market (Consumer Reports [CR] 2006).

The evaluations were conducted with the support of the Military Infectious Disease Research Program (MIDRP), which along with the AFPMB, has an interest in evaluating the efficacy of non-DEET repellents for military adoption to provide additional choices for service members and DoD personnel, especially given evidence that many service members have negative perceptions of DEET (Sanders et al. 2005, Vickery et al. 2008). The military has long had an interest in reduction of arthropod-borne diseases because historically, these diseases have had major impacts on the health and capacity of troops serving in other parts of the world (Fukuda et al. 2011). Increasingly, the U.S. military has been called on to provide support and humanitarian assistance, often in the wake of a natural disaster or political turmoil (Fukuda et al. 2011, Armed Forces Health Surveillance Center [AFHSC] 2012). Topical repellents are often the only means of protection against arthropod-borne diseases in these environments and locations, especially when

Table 2. Percent protection over time of each of the five insect repellent formulations in Orange Walk Town, Belize (19–21 September 2007)

Hours post application	Ultrathon			10% IR3535 lotion			20% IR3535 spray			20% Picaridin lotion			20% Picaridin spray		
	PP (%)	Lower 95%	Upper 95%	PP (%)	Lower 95%	Upper 95%	PP (%)	Lower 95%	Upper 95%	PP (%)	Lower 95%	Upper 95%	PP (%)	Lower 95%	Upper 95%
2	93.5	75.6	111.3	99.0	81.1	116.8	97.1	79.3	114.9	100.0	82.2	117.8	94.9	77.1	112.8
4	99.2	81.3	117.0	82.6	64.7	100.4	82.3	64.4	100.1	97.5	79.7	115.3	85.1	67.3	103.0
6	99.6	81.8	117.4	92.5	74.6	110.3	90.0	72.2	107.8	90.4	72.6	108.3	92.9	75.1	110.8
8	91.6	73.8	109.4	58.9	41.1	76.8	73.1	55.3	90.9	76.6	58.7	94.4	82.9	65.0	100.7
10	91.9	74.0	109.7	73.4	55.5	91.2	79.9	62.1	97.8	82.0	64.2	99.8	74.8	57.0	92.7
12	89.3	71.4	107.1	62.4	44.6	80.3	59.3	41.4	77.1	70.6	52.8	88.5	48.1	30.3	66.0

Table 3. Linear mixed modelling (Belize)

Model effect	Time-adjusted vs time-specific	*F*-test	*P* value (2-sided)	Formulation comparison	*P* value (2-sided)
Time-by-formulation (20 df)	–	1.05	0.4063	–	–
Formulation (4 df)	Adjusted for time	1.13	0.3639	Ultrathon vs KBR 3023 All-Family Insect Repellent Spray	0.1137
				KBR 3023 All-Family Insect Repellent Lotion	0.3712
				Bug Repell IR3535 20% Spray	0.1254
				Bug Repell IR3535 10% Lotion	0.0788
Formulation (4 df)	At time = 2 h	0.09	0.9848	–	–
	At time = 4 h	0.86	0.4922	–	–
	At time = 6 h	0.18	0.9459	–	–
	At time = 8 h	1.83	0.1306	–	–
	At time = 10 h	0.67	0.6175	–	–
	At time = 12 h	**2.92**	**0.0255**	Ultrathon vs KBR 3023 All-Family Insect Repellent Spray	**0.0017**
				KBR 3023 All-Family Insect Repellent Lotion	0.1455
				Bug Repell IR3535 20% Spray	**0.0202**
				Bug Repell IR3535 10% Lotion	**0.0372**

Values in bold indicate significant differences in percent protection when compared to Ultrathon.

other vector control measures are not possible or when the speed of operations prevents the use of available chemoprophylaxis or vaccines.

Materials and Methods

Study Sites. *Belize.* Belize (formerly British Honduras) is a Central American country with a population of ≈290,000 people (48.7% mestizo) in a geographic area of 22,966 km^2 (Central Intelligence Agency [CIA] 2013). The climate and general ecology of Belize are favorable for year round transmission of malaria. Extensive marshes, swamps, and rivers provide continuous larval habitats for malaria vector species, even during the dry seasons. Malaria incidence is significantly higher in the southern and western prov-

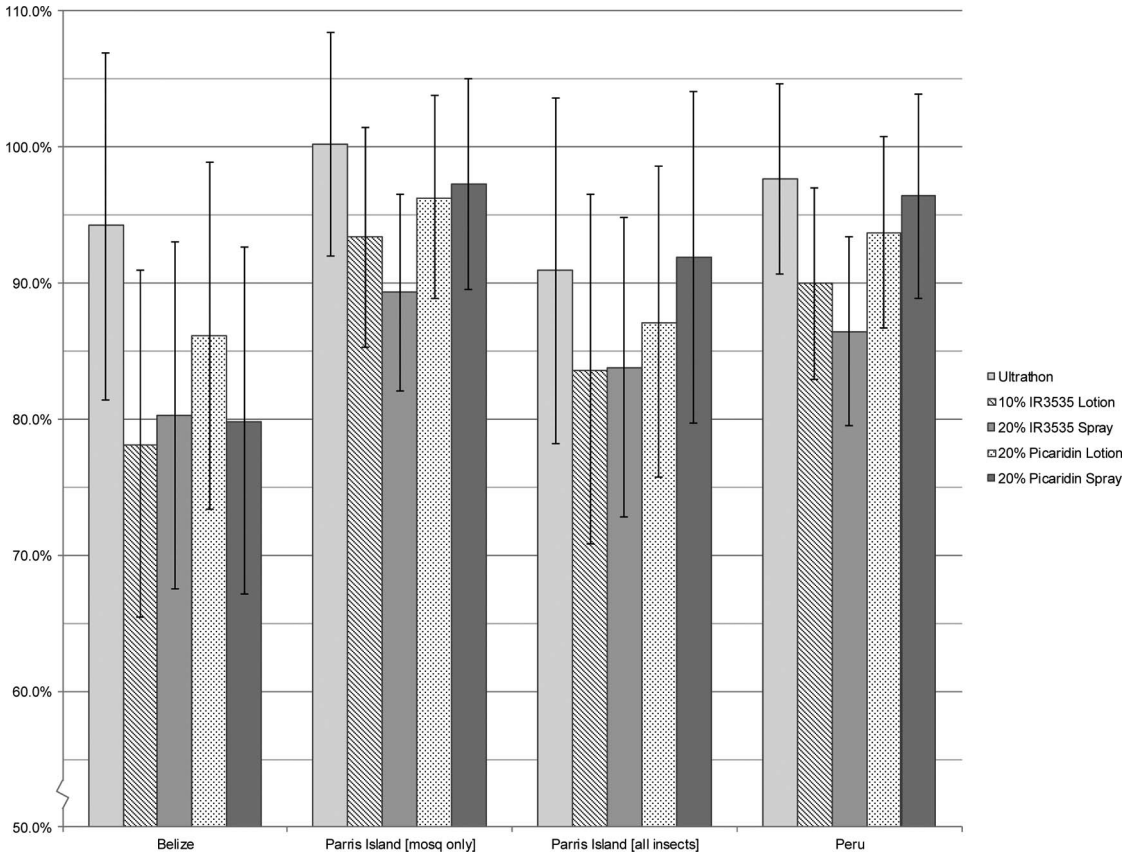

Fig. 1. Percent protection including upper and lower confidence intervals adjusted for time of each of the five insect repellent formulations in the three locations.

Table 4. Distribution of species across total insects collected from human volunteers at Parris Island, SC (25–31 August 2009)

Species	Number collected	%
Culicoides furens	4,206	56.40
Aedes taeniorhynchus	3,084	41.35
Culicoides melleus	127	1.70
Psorophora ciliata	14	0.19
Culicoides sp.	11	0.15
Aedes sp.	10	0.13
Culex (Culex) sp.	4	0.05
Psorophora columbiae	1	0.01
Anopheles sp.	1	0.01
Total	7,458	100.00

inces of Toledo, Cayo, and Stann Creek than in the northern provinces of Corozal and Orange Walk (Hakre et al. 2004). The study was conducted in the town of Orange Walk Town in the northern Belize province of Orange Walk from 19 to 21 September 2007. The study site in Orange Walk Town was located in a large open field (≈300 m^2) and bordered on one side by a semiwooded area.

Parris Island. The Marine Corps Recruit Depot (MCRD) at Parris Island, located in Beaufort County, SC, is ≈8,047 acres, over much of which is salt marsh with smooth cordgrass (*Spartina alterniflora* Loisel) (Breidenbaugh et al. 2009). The remaining 3,000 acres of dry land is composed of open and forested areas. The island is surrounded by Archers Creek, the Beaufort River, Port Royal Sound, and the Broad River. The city of Charleston, SC, is 90 km to the north and Savannah, GA, is 50 km to the south. Mosquitoes (Culicidae) and biting midges (Ceratopogonidae) are serious pests for those stationed at Parris Island and for the Marine recruits attending basic training. Disease transmission, including West Nile Virus (Adler and Wills 2003) and Eastern equine encephalitis (Ortiz et al. 2003), by mosquitoes has been detected and also poses a significant threat. The main medical concern with *Culicoides* in the United States stems from allergic reactions to their bites, and while they do not pose a disease threat in the United States, they are vectors of pathogens that cause disease in humans, particularly in Central and South America, parts of the Caribbean and Africa. The primary species of mosquito and biting midge present at Parris Island are *Aedes taeniorhynchus* and *Culicoides furens*, respectively. The diel activity pattern for both species indicates peak activity from ≈1800–2200 hours (Breidenbaugh et al. 2009).

Peru. The study was conducted in a rural area near the city of Iquitos, the major urban area in the Department of Loreto, northeastern Peru. Iquitos currently reports a population of ≈340,000. Peru's largest department, Loreto is situated in the Amazon basin and has an estimated population of 920,000. The study area is in low jungle at the headwaters of the Amazon River where the average temperature is 27°C and yearly rainfall is 3.2 m. The population is predominantly a mix of European and Amerindian descent, and Spanish is spoken as the first language. The major occupations are small-scale agriculture, fishing, logging, and small-scale businesses. Padre Cocha is a town of 2,500 on the Nanay River, and its residents are a mix of farmers and artisans (Aramburu Guarda et al. 1999, Bautista et al. 2006). It is ≈30 min from Iquitos by car and boat (it faces the city of Iquitos from across the Nanay River) and has been used as a center of entomologic collection by the Naval Medical Research Unit-6 (NAMRU-6) in the past.

Volunteers. *Belize.* Thirty volunteers were recruited, screened, and enrolled (17–18 September 2007) under a human-use protocol reviewed and approved by the Walter Reed Army Institute of Research (WRAIR) Institutional Review Board (IRB; WRAIR Protocol no.1345). Volunteers were recruited from the villages of Orange Walk Town and August Pine Ridge with the assistance of Belizean field liaisons who spoke both English and Spanish. Interested parties were briefed on the nature of study participation, and those who agreed to volunteer provided written informed consent before any study-related procedures in accordance with research guidelines for studies involving humans (Human Research Protection Office, United States Army Medical Research and Material Command, Ft. Detrick, MD).

Parris Island. Twelve volunteers were recruited, screened, and enrolled (24–25 August 2009) under a human-use protocol reviewed and approved by the WRAIR IRB (WRAIR Protocol no. 1486). Volunteers were recruited from the WRAIR, the U.S. Department of Agriculture–Agricultural Research Service (USDA-ARS) Center for Medical, Agricultural and Veterinary Entomology (CMAVE) and the Navy Entomology Center of Excellence (NECE) with the assistance of the study investigators. Interested parties were briefed on the nature of study participation and those who agreed to volunteer provided written informed consent before any study-related procedures in ac-

Table 5. Percent protection over time of each of the five insect repellent formulations in Parris Island, SC (25–31 August 2009) (mosquitoes only)

Hours post application	Ultrathon			10% IR3535 lotion			20% IR3535 spray			20% picaridin lotion			20% picaridin spray		
	PP (%)	Lower 95%	Upper 95%	PP (%)	Lower 95%	Upper 95%	PP (%)	Lower 95%	Upper 95%	PP (%)	Lower 95%	Upper 95%	PP (%)	Lower 95%	Upper 95%
2	100.0	79.2	120.9	99.6	82.5	116.6	98.4	83.6	113.2	100.0	85.5	115.0	100.0	85.2	114.8
4	100.0	85.2	114.8	98.9	84.1	113.7	85.9	72.6	99.1	100.0	86.8	113.2	95.8	81.0	110.6
6	99.5	84.7	114.3	100.0	85.2	114.8	98.3	83.6	113.1	100.0	85.2	114.8	100.0	85.2	114.8
8	100.0	83.0	117.1	96.4	79.3	113.5	94.9	81.7	108.2	89.6	74.8	104.3	99.4	84.6	114.2
10	100.0	82.9	117.1	93.8	76.7	110.8	80.0	65.2	94.8	94.0	73.2	114.8	100.0	85.2	114.8
12	99.1	84.3	113.9	73.2	58.4	88.0	79.1	64.3	93.9	92.0	78.8	105.2	88.3	73.5	103.1

Table 6. Linear mixed modeling (Parris Island mosquitoes only)

Model effect	Time-adjusted vs time-specific	*F*-test	*P* value (2-sided)	Formulation comparison	*P* value (2-sided)
Time-by-formulation (20 df)	–	0.53	0.9449	–	–
Formulation (4 df)	Adjusted for time	1.31	0.3048	–	–
Formulation (4 df)	At time = 2 h	0.01	0.9998	–	–
	At time = 4 h	0.78	0.5404	–	–
	At time = 6 h	0.01	0.9998	–	–
	At time = 8 h	0.30	0.8759	–	–
	At time = 10 h	1.17	0.3339	–	–
	At time = 12 h	1.96	0.1110	–	–

cordance with research guidelines for studies involving humans.

Peru. Thirty volunteers were recruited, screened, and enrolled (20–21 September 2009) under a human-use protocol reviewed and approved by the WRAIR IRB (WRAIR Protocol no. 1553). Volunteers were recruited from the villages of Padre Cocha with the assistance of Peruvian field liaisons who spoke both English and Spanish. Interested parties were briefed on the nature of study participation and those who agreed to volunteer provided written informed consent before any study-related procedures in accordance with research guidelines for studies involving humans.

Test Materials. Five repellent formulations each containing one of three different active ingredients were included in this evaluation: 1) Ultrathon; 2) KBR 3023 All-Family Insect Repellent Spray; 3) KBR 3023 All-Family Insect Repellent Lotion; 4) Bug Repell IR3535 20% Spray; and 5) Bug Repell IR3535 10% Lotion. Ultrathon (3M, EPA reg. no. 58007-1) is a 34.34% DEET lotion formulation commercially marketed in the United States. KBR 3023 All-Family Insect Repellent Spray (Lanxess Corp., Pittsburgh, PA, EPA reg. no. 39967-53) is a 20% picaridin pump spray formulation. KBR 3023 All-Family Insect Repellent Lotion (Lanxess Corp., EPA reg. no. 39967-50) is a 20% picaridin lotion formulation. Bug Repell IR3535 20% Spray (EMD Chemicals, Inc., Darmstadt, Germany, EPA reg. no. 79759-3) is a 20% IR3535 spray formulation. Bug Repell IR3535 10% Lotion (EMD Chemicals, EPA reg. no. 79759-2) is a 10% IR3535 lotion formulation. These products were selected for testing and evaluation due to being EPA registered, having U.S. commercial potential and price comparability to Ultrathon.

Study Design and Procedure. Peak biting activity of the targeted vectors occurred between 1800 and 2000 hours in all three locations. It was desired to test repellents at 2-h intervals up to 12 h postapplication to match previous topical repellent evaluations (Lawrence et al. 2009, Carroll et al. 2010). Therefore, a staggered application design was employed so that all postapplication challenge time points (2, 4, 6, 8, 10, and 12 h) could be measured during the peak vector biting time period. Repellents were applied to the volunteers at 0800 hours (10, 12 h), 1200 hours (6, 8 h), and 1600 hours (2, 4 h). Volunteers were rotated through each application time to ensure each was tested at all three postapplication time points for their repellent.

A 600-cm^2 treatment area from just above the ankle to just below the knee was measured for each volunteer. Five equally spaced circumference measurements were taken along each lower leg, averaged and divided into 600 to get the length of the exposed area, and then marked above and below by an indelible marker by a trained staff member. This marked area, from ankle to knee, on one leg was treated with the assigned topical repellent and the same marked area on the opposite leg was left untreated to serve as a

Table 7. Linear mixed modeling (Parris Island all insects)

Model effect	Time-adjusted vs time-specific	*F*-test	*P* value (2-sided)	Formulation comparison	*P* value (2-sided)
Time-by-formulation (20 df)	–	1.11	0.3567	–	–
Formulation (4 df)	Adjusted for time	0.45	0.7686	Ultrathon vs KBR 3023 All-Family Insect Repellent Spray	0.9998
				KBR 3023 All-Family Insect Repellent Lotion	0.9704
				Bug Repell IR3535 20% Spray	0.7744
				Bug Repell IR3535 10% Lotion	0.8022
Formulation (4 df)	At time = 2 h	0.12	0.9740	–	–
	At time = 4 h	0.75	0.5589	–	–
	At time = 6 h	0.89	0.4751	–	–
	At time = 8 h	0.17	0.9513	–	–
	At time = 10 h	1.03	0.3962	–	–
	At time = 12 h	**2.70**	**0.0372**	Ultrathon vs KBR 3023 All-Family Insect Repellent Spray	0.2206
				KBR 3023 All-Family Insect Repellent Lotion	0.8716
				Bug Repell IR3535 20% Spray	0.6647
				Bug Repell IR3535 10% Lotion	**0.0387**

Values in bold indicate significant differences in percent protection when compared to Ultrathon.

Table 8. Distribution of species across total mosquitoes collected from human volunteers at Padre Cocha, Peru (22–24 September 2009)

Species	Number collected	%
Culex vomerifer	317	40.69
Mansonia indubitans/titillans	175	22.46
Culex pedroi	92	11.81
Culex coronator	43	5.52
Coquillettidia venezuelensis	38	4.88
Mansonia humeralis	21	2.70
Psorophora cingulata	18	2.31
Culex quinquefasciatus	14	1.80
Culex (Melanoconion) sp.	14	1.80
Culex gnomatus	13	1.67
Ochlerotatus serratus	13	1.67
Anopheles triannulatus	8	1.03
Anopheles nuneztovari	5	0.64
Culex portesi	3	0.39
Ochlerotatus fulvus	3	0.39
Anopheles oswaldoi	1	0.13
Coquillettidia hermanoi	1	0.13
Total	779	100.00

control. The U.S. EPA Product Performance Test Guidelines (EPA 2010) recommend using 1.0 g of DEET lotion over 600 cm^2 of skin surface area for testing repellents. To maintain consistency throughout all of our repellent studies and to facilitate comparisons among products, 1.0 g of the lotion formulations and 1.0 ml of the spray formulations were spread evenly on the treatment area of each volunteer by a trained staff member. This application procedure was repeated in the same manner on subsequent nights of testing. Treatment application alternated between the left and right legs of every volunteer each night of the trial to minimize the number of bites on the skin of the leg that was used as the control.

During each repellent challenge, volunteers were covered (long sleeve shirts, long pants, mesh jackets with hood, gloves, and footwear) except for the exposed experimental areas (treatment and unprotected skin control) on each leg. All insects landing in the marked areas of the exposed lower legs were mouth aspirated by the volunteers during a 50-min test period (30 min in Belize) and placed into screen-topped cartons individually marked with date, time of collection, and collector number (to correspond to postapplication time). All collected insects were killed on-site, labeled, and stored with silica gel until identification. Specimen identifications from Belize and Parris Island were performed by the Walter Reed Biosystematics Unit (Suitland, MD) and those in Peru by the experienced study team at the NAMRU-6.

Analysis. For all three field studies, average repellency (95% CI) for each formulation at each time point was estimated using a linear fixed model (SAS Mixed procedure) with Percent Protection (PP) as the outcome and Formulation (five levels), Time (six levels), and the Time × Formulation interaction as fixed effects (Lawrence et al. 2009). Percent Protection was calculated as—$PP = 100 \times [(LRC - LRP)/(LRC)]$ where LRC represents the landing rate for the bare skin control and LRP represents the landing rate for the repellent formulation. The least squares means within levels of Time × Formulation were used to estimate average repellency (95% CI) for each formulation at each postapplication time point. The same model was then used to look at the significance of the Time × Formulation interaction, as well as time-specific formulation effects. In all three data sets, the Time × Formulation interaction was not significant. Therefore, the time-adjusted (overall) formulation effects were evaluated in a main effects only model, with PP as the response variable and the main effects of Formulation and Time as fixed effects. Least squares means (95% CI) were used to estimate the PP for each repellent formulation, as well as for differences between each formulation and Ultrathon. The data analyses for all three data sets and locations were performed in SAS version 9.1.3 (SAS Institute, Cary, NC).

Results and Discussion

Belize. During the three nights of evaluation, >4,200 mosquitoes were collected from the human volunteers with *Anopheles albimanus* (24.84%) and *Ae. taeniorhynchus* (24.58%) constituting the majority of those identified (Table 1). Ultrathon provided the highest level of protection (>89%) throughout all 12 h of testing (Table 2); however, there was no significant difference among repellent formulations at any time point except at 12 h (Table 3). At 12 h, KBR 3023 All-Family Insect Repellent Spray, Bug Repell IR3535 20% Spray, and Bug Repell IR3535 10% Lotion were all significantly different from Ultrathon ($P < 0.0017$, 0.0202, and 0.0372, respectively). There was no significant Time × Formulation interaction ($F = 1.05$, df = 4,20, $P = 0.4063$; Table 3); therefore, we estimated overall (averaged over time) PP for each formulation. When collapsed over time, average PP across repellent formulations was at least 78% with Ultrathon providing

Table 9. Percent Protection over time of each of the five insect repellent formulations in Padre Cocha, Peru (22–24 September 2009)

Hours post application	Ultrathon			10% IR3535 lotion			20% IR3535 spray			20% picaridin lotion			20% picaridin spray		
	PP (%)	Lower 95%	Upper 95%	PP (%)	Lower 95%	Upper 95%	PP (%)	Lower 95%	Upper 95%	PP (%)	Lower 95%	Upper 95%	PP (%)	Lower 95%	Upper 95%
2	99.9	80.5	119.2	100.0	82.3	117.7	100.0	82.3	117.7	100.0	82.3	117.7	94.9	75.5	114.2
4	100.0	82.3	117.7	80.0	62.3	97.7	97.9	80.2	115.6	100.0	82.3	117.7	100.6	75.6	125.5
6	100.0	82.3	117.7	100.0	82.3	117.7	100.0	82.3	117.7	99.7	80.4	119.1	100.0	82.3	117.7
8	92.5	74.8	110.2	94.4	76.8	112.1	87.9	70.2	105.6	100.0	82.3	117.7	100.0	80.7	119.4
10	92.6	74.9	110.3	86.3	67.0	105.7	48.6	30.9	66.3	77.4	59.7	95.0	90.7	73.1	108.4
12	100.0	82.3	117.7	79.7	60.4	99.1	84.6	65.3	104.0	84.2	64.9	104.0	91.7	74.0	109.3

Table 10. Linear mixed modeling (Peru)

Model effect	Time-adjusted vs time-specific	*F*-test	*P* value (2-sided)	Formulation comparison	*P* value (2-sided)
Time-by-formulation (20 df)	–	0.89	0.6001	–	–
Formulation (4 df)	Adjusted for time	1.82	0.1558	Ultrathon vs KBR 3023 All-Family Insect Repellent Spray	0.9971
				KBR 3023 All-Family Insect Repellent Lotion	0.8375
				Bug Repell IR3535 20% Spray	**0.0880**
				Bug Repell IR3535 10% Lotion	0.3416
Formulation (4 df)	At time = 2 h	0.06	0.9941	–	–
	At time = 4 h	0.94	0.4439	–	–
	At time = 6 h	0.00	1.0000	–	–
	At time = 8 h	0.32	0.8626	–	–
	At time = 10 h	**4.06**	**0.0041**	Ultrathon vs KBR 3023 All-Family Insect Repellent Spray	0.8835
				KBR 3023 All-Family Insect Repellent Lotion	0.2297
				Bug Repell IR3535 20% Spray	**0.0007**
				Bug Repell IR3535 10% Lotion	0.6354
	At time = 12 h	0.74	0.5689	–	–

Values in bold indicate significant differences in percent protection when compared to Ultrathon.

the highest PP at 94% (95% CI = 81.4–106.9; Fig. 1); however, these differences were not significant (Table 3).

Parris Island. Over 7,400 insects were collected from the human volunteers during seven nights of evaluation. *C. furens* (56.40%) and *Ae. taeniorhynchus* (41.35%) constituted the majority of those identified (Table 4). Ultrathon provided the highest level of protection against mosquitoes (at least 99%) for the duration of the study (Table 5), but there were no significant differences between repellents at any of the time points (Table 6). There was no significant Time × Formulation interaction ($F = 0.53$, df = 4,20, $P = 0.9449$; Table 6); therefore, we estimated overall (averaged over time) PP for each formulation. When averaged over time, there were no significant differences among the formulations and PP was at least 89% for all of the formulations (Fig. 1). We originally planned to evaluate average PP separately for biting midges (*Culicoides*); however, the data were too sparsely distributed and/or contained null counts (i.e., 0 midges collected) to allow convergence to a model solution. Therefore, we analyzed the data for mosquitoes and midges combined. For mosquitoes + midges, there was no significant difference in average PP among the formulations through 10 h postapplication (Table 7). At 12 h, PP of Bug Repell IR3535 10% Lotion was significantly less than Ultrathon ($P = 0.0387$). Despite this difference, there was no significant Time × Formulation interaction ($F = 1.11$, df = 4,20, $P = 0.3567$; Table 7). When averaged over time, there were no significant differences in PP among any of the repellent formulations with all repellents providing at least 83.6% PP (Fig. 1).

Peru. During the three nights of collections, 779 mosquitoes were collected with *Culex vomerifer* (40.69%), *Mansonia indubitans/titillans* (22.46%), and *Cx. pedroi* (11.81%) constituting the majority of those identified (Table 8). Ultrathon and KBR 3023 All-Family Insect Repellent Spray provided at least 90% protection throughout all 12 h of testing (Table 9). Bug Repell IR3535 10% Lotion provided >80% protection through 10 h while Bug Repell IR3535 20% Spray and KBR 3023 All-Family Insect Repellent Lotion provided >87% protection through 8 h. The only significant difference between formulations occurred at 10 h where Bug Repell IR3535 20% Spray was significantly lower than Ultrathon ($P = 0.0007$; Table 10). However, there was no significant Time × Formulation interaction ($F = 0.89$, df = 4,20, $P = 0.6001$; Table 10), so we estimated overall (averaged over time) PP for each formulation. When collapsed over time, average PP was at least 90%, except for Bug Repell IR3535 20% Spray, with Ultrathon and 20% picaridin spray providing the highest levels of repellency (Fig. 1).

Cases of traveler-imported arthropod-borne disease and occasional reports of autochthonous transmission (Zucker 1996, Sunstrum et al. 2001, Agarwal et al. 2012) continue along with occasional outbreaks and localized epidemics (e.g., dengue in southern Florida; Richards et al. 2012). A recent outbreak of locally acquired chikungunya in the Caribbean (WHO 2013) is evidence that this disease, once thought limited to areas of Asia and Africa, continues to expand its geographic range. It is transmitted by *Ae. aegypti* and *Ae. albopictus*, both of which are widespread across the Americas, and is therefore a major cause for concern. It is possible that chikungunya, and even dengue fever, could follow a similar pattern to that of West Nile virus in the United States—that is, after introduction, it could become endemic because the vectors are present across a broad geographic range not only in the United States but elsewhere in the Americas (Pan American Health Organization [PAHO] 2011). Our findings demonstrate that there are several repellent formulations and active ingredients that work as well as DEET against a wide range of vector species and geography in the Americas. These newer, commercially available topical repellent formulations provide a broader product choice for the average consumer and, in particular, for the U.S. military whose personnel operate in endemic locations and represent a naïve at-risk population.

Acknowledgments

We express our appreciation to the volunteers whose cooperation and patience were essential to these repellent evaluations, to Ireneo Briceno and Russell King (Ministry of Health, Belize), and Victor López-Sifuentes and Karin Escobedo-Vargas (NAMRU-6) for their field assistance and coordination, and to Jim Pecor (Walter Reed Biosystematics Unit) for his identification of insect specimens. These evaluations were funded by the Military Infectious Disease Research Program.

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Received 5 May 2014; accepted 9 July 2014.