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Toxicity Comparison Of Eight Repellents Against Four Species Of Female Mosquitoes

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TOXICITY COMPARISON OF EIGHT REPELLENTS AGAINST FOUR SPECIES OF FEMALE MOSQUITOES

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ABSTRACT. The relative toxicities of 8 repellents (DMP, Rutgers 612, DEET, IR3535, Picardin, PMD, AI3-35765, and AI3-37220) were evaluated by topical application against females of *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles quadrimaculatus*, and *An. albimanus*. Based on 24-h LD_{50} values, the most toxic repellent against all 4 mosquito species was AI3-37220, with values of 0.25, 0.20, 0.16, and 0.11 µg/mg for the listed 4 mosquito species, respectively. The least toxic of the 8 repellents tested was DMP, with LD_{50} values of 5.40, 4.72, 2.50, and 1.83 µg/mg, respectively. Based on the 24-h LD_{50} values, *An. albimanus* was the most susceptible species. The findings of the study reported herein provide a comprehensive examination of the toxicities of 4 currently used, 2 formerly used, and 2 experimental repellents against 4 mosquito species.

KEY WORDS *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles quadrimaculatus*, *An. albimanus*, repellent toxicity, topical application

INTRODUCTION

Females of the mosquito species *Aedes aegypti* (L.) transmit viral pathogens to humans, resulting in diseases such as yellow fever, dengue, and dengue hemorrhagic fever. These illnesses can cause severe human morbidity and mortality. The mosquito species *Culex quinquefasciatus* Say is a vector of the filarial parasite *Wuchereria bancrofti* (Cobbold) (Spirurida: Onchocercidae), which causes bancroftian filariasis in humans (Samuel et al. 2004). It is also a vector of the West Nile virus (WNV; Godsey et al. 2005), Japanese encephalitis (JE) (Nitatpattana et al. 2005), and Saint Louis encephalitis (SLE; Jones et al. 2002). *Anopheles quadrimaculatus* Say and *An. albimanus* Weidemann are anthropophilic species that are vectors involved in the transmission of the malarial parasite in humans (Richards et al. 1994).

Using repellents is a common personal protection method against mosquito bites. Since 1942, more than 40,000 compounds have been evaluated as toxicants and repellents against mosquitoes at the United States Department of Agriculture's Center for Medical, Agricultural and Veterinary Entomology (Xue et al. 2001). Some insect repellents have been reported to possess insecticidal activities against mosquitoes (Xue et al. 2003, Licciardi et al. 2006), suggesting that these compounds might also be used as toxicants for mosquito control. To compare the relative toxicities of different repellents accurately, the adult topical application bioassay was chosen to determine the relative toxicities of the following 8 compounds: 1) DMP (dimethylphthalate), a fly repellent formerly used since 1929; 2) EHD, Rutgers 612 (2-ethyl-1,3-hexanediol), 1st used in 1939; 3) DEET (N,N-diethyl-3-methylbenzamide); 4) IR3535 (3-[N-butyl-N-acetyl]-aminopropionic acid, ethyl ester), in use since the 1970s; 5) picaridin (KBR 3023, Bayrepel®, 2-[2-hydroxyethyl]-1-piperidinecarboxylic acid 1-methylpropyl ester), in use since the 1990s; 6) PMD (*para*-menthane-3,8-diol), coming into commercial use since 2000; 7) AI3-35765 (1-[3-cyclohexen-1-ylcarbonyl] piperidine), an experimental piperidine repellent synthesized in 1978; and 8) AI3-37220 (1-[3-cyclohexen-1-ylcarbonyl]-2-methylpiperidine), another piperidine repellent synthesized in 1978. Because different susceptibility of various mosquito species to different pesticides has been previously reported (Pampiglione et al. 1985, Campos and Andrade 2003, Somboon et al. 2003, Pridgeon et al. 2008), we chose 4 mosquito species (*Ae. aegypti*, *Cx. quinquefasciatus*, *An. quadrimaculatus*, and *An. albimanus*) for our adult bioassay. Our results presented here provide important information on the relative toxicities of 2 experimental and 6 commercial repellents (2 were formerly used and 4 are currently used).

MATERIALS AND METHODS

Mosquitoes and repellents

All mosquitoes were reared in the insectary of the Mosquito and Fly Research Unit at the United States Department of Agriculture–Agricultural Research Service–Center for Medical, Agricultural, and Veterinary Entomology (USDA-ARS-CMAVE). We used the following 4 colony species: *Ae. aegypti* (Orlando, 1952), *An. quadrimaculatus* (Orlando, 1952), and *An. albimanus* (El Salvador, 1975), and *Cx. quinquefasciatus* (Gainesville, 1995). Only females were tested. Mosquitoes were reared in accordance with standard procedures (Reinert et al. 1997, McCall and Eaton 2001, Pridgeon et al. 2007) as

follows: Collected eggs were hatched in a flask and larvae were held overnight in the flask and then transferred to a plastic tray containing distilled water. Larval diet was added to each tray. Mosquitoes were reared in an environmental chamber programmed with a temperature profile that represented a simulated summer day regime (ranging from 22°C to 30°C) and 80% relative humidity (RH). Incandescent lighting was set to a crepuscular profile with a photoperiod of 14 h:10 h (L:D), including 2 h of simulated dawn and 2 h of simulated dusk. Adults were held in a screened cage and provided 10% sucrose *ad libitum*. Bovine blood in 1% heparin contained in a pig intestine and warmed to 37°C was provided to adults twice a week.

The 8 repellents were either synthesized or obtained from commercial sources. The experimental repellents AI3-37565 and AI3-37220 and former repellents PMD and EHD were available as purified synthetics from the USDA-ARS Beltsville Insect Chemical Ecology Laboratory (ICEL). Picardin (KBR 3023) was provided by Lanxess (Pittsburgh, PA), IR 3535 by Merck (Darmstadt, Germany), DEET (AI3-22542-Gz) by Virginia Chemical (Portsmith, VA) and PMD by Bedoukian Research (Danbury, CT). The chemical structures of the repellents used are shown in Fig. 1.

Adult bioassays and data analysis

To determine the relative toxicity of each repellent, each chemical was serially diluted in acetone and applied topically to individual mosquitoes. Prior to application, 5–7-day-old female mosquitoes were anesthetized for 30 sec with carbon dioxide and placed on a 4°C chill table (BioQuip Products, Rancho Dominguez, CA). A droplet of 0.5 µl of prepared repellent solution was applied to the dorsal thorax using a 700 series syringe and a PB 600 repeating dispenser (Hamilton, Reno, NV). Six concentrations providing a range of 0–100% of mortality were used on 25–30 females per concentration. Tests were replicated 3 times with a different stock population. Control treatments that consisted of 0.5 µl of acetone delivered alone resulted in mortality rates of <10%. After treatment, mosquitoes were held in plastic cups and provided 10% sucrose solution for 24 h before mortality was recorded. Temperature and humidity were maintained at 26 ± 1°C and 80 ± 1% RH, respectively. Bioassays were replicated 3 times. Correction of mortality compared to controls was performed with the use of a modified Abbott's formula (Abbott 1925). Bioassay data were pooled and probit dose response was analyzed with the use of PoloPlus probit and logit analysis software (LeOra Software, Petaluma, CA) as described previously (Pridgeon et al. 2008). Toxicities of repellents are considered significantly different when the 95% confidence intervals of LD_{50} values fail to overlap ($P \leq 0.05$).

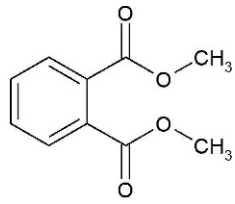
RESULTS

Topical application bioassays of the 8 selected repellents were performed to determine the susceptibility of 4 mosquito species to each repellent. The bioassay results for *Ae. aegypti* are summarized in Table 1. Of the 8 repellents tested, the 2 experimental (noncommercial) repellents, AI3-37220 and AI3-35765, were the most toxic to *Ae. aegypti*, with LD_{50} values of 0.25 and 0.30 µg/mg, respectively. The formerly used repellent, DMP, was the least toxic repellent against *Ae. aegypti*, with LD_{50} value of 5.40 µg/mg. On the basis of 24-h LD_{50} values after topical application, the activity order of the 8 repellents as toxicants was: AI3-37220 ≥ AI3-35765 > DEET ≥ KBR 3023 > IR3535 ≥ PMD > EHD > DMP (Table 1).

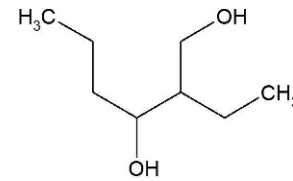
To investigate whether the 8 repellents have similar toxicities against other mosquito species, topical application bioassays were performed with females of *An. quadrimaculatus*, *An. albimanus*, and *Cx. quinquefasciatus*. The bioassay results are presented in Tables 2–4, respectively. Our results revealed that AI3-37220, the most toxic repellent against *Ae. aegypti*, was also the most toxic against the other 3 mosquito species, with LD_{50} values ranging from 0.11 to 0.20 µg/mg (Tables 2–4). DMP, the least toxic repellent against *Ae. aegypti*, was also the least toxic repellent against the other 3 mosquito species with LD_{50} values ranging from 1.83 to 4.72 µg/mg (Tables 2–4). However, the activity orders of the other 6 repellents as toxicants against these mosquito species differed from that of *Ae. aegypti*. For *An. quadrimaculatus*, the activity order of the 8 repellents was: AI3-37220 > DEET ≥ AI3-35765 > KBR 3023 > PMD ≥ EHD ≥ IR3535 ≥ DMP (Table 2). The activity order against *An. albimanus* was: AI3-37220 > AI3-35765 ≥ DEET > KBR 3023 > PMD ≥ EHD ≥ IR3535 ≥ DMP (Table 3). The activity order against *Cx. quinquefasciatus* was AI3-37220 > AI3-35765 ≥ DEET >> KBR 3023 ≥ EHD ≥ PMD > IR3535 > DMP (Table 4).

DISCUSSION

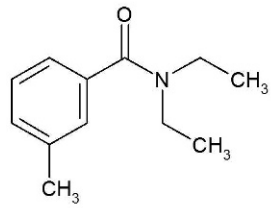
It has been reported that some repellents possess insecticidal activities against mosquitoes. For example, Xue et al. (2003) has reported that 16 commercial insect repellents (6 botanical and 10 synthetic organic products) in spray formulations produced significant adult knockdown (KD) and 24-h mortality against laboratory-reared female *Ae. aegypti*, *Ae. albopictus*, and *An. quadrimaculatus*. Furthermore, they have



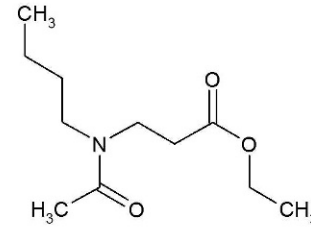
DMP



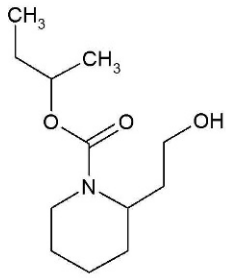
EHD



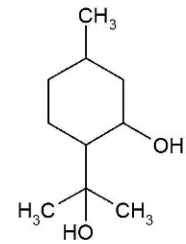
DEET



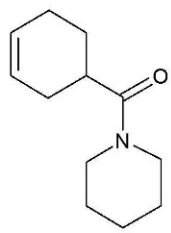
IR3535



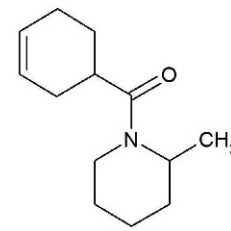
Picaridin



PMD



AI3-35765



AI3-37220

Fig. 1. Chemical structures of the 8 repellents.

reported that the synthetic organic repellents induced faster KD with higher magnitude to adult mosquitoes than most botanical repellents, suggesting that repellents could also be used as toxicants for mosquito control in some situations. Consistent with their finding, our results also revealed that some insect repellents possess high insecticidal activity against mosquitoes. For

Table 1. Toxicities of 8 repellents against female *Aedes aegypti* by topical application.[1]

Repellent name	LD_{50}[2] (95% CI)[3,4]	LD_{95}[2] (95% CI)[3]	Slope (SE)	χ^2	*n*
IR3535	1.88 (1.55–2.23)c	4.91 (3.71–7.85)	3.88 (0.58)	1.78	420
DEET	0.94 (0.80–1.13)b	2.42 (1.85–3.80)	4.03 (0.60)	1.73	420
DMP	5.40 (5.10–5.77)e	7.39 (6.71–8.70)	12.09 (1.82)	1.68	420
AI3-37220	0.25 (0.21–0.31)a	0.75 (0.55–1.30)	3.44 (0.52)	0.33	420
Ethyl hexanediol	2.88 (2.64–3.08)d	4.41 (3.96–5.31)	8.88 (1.44)	0.45	420
AI3-37220G	0.31 (0.26–0.37)a	0.77 (0.60–1.15)	4.13 (0.61)	1.96	420
KBR 3023	1.09 (0.92–1.30)b	2.49 (1.94–3.91)	4.60 (0.77)	0.35	420
AI3-35765	0.30 (0.25–0.35)a	0.63 (0.50–0.94)	5.10 (0.85)	0.68	420
PMD	1.90 (1.59–2.21)c	5.90 (4.41–10.22)	3.35 (0.57)	1.18	420

[1] IR3535, 3-(N-butyl-N-acetyl)-aminopropionic acid, ethyl ester; DEET, N,N-diethyl-3-methylbenzamide; DMP, dimethylphthalate; AI3-37220, 1-(3-cyclohexen-1-ylcarbonyl)-2-methylpiperidine; AI3-37220G, AI3-37220 granular; KBR 3023, picaridin; AI3-35765, 1-(3-cyclohexen-1-ylcarbonyl) piperidine; PMD, *para*-menthane-3,8-diol.
[2] LD_{50} and LD_{95} values are in units of micrograms of pesticide per milligram of mosquito.
[3] CI, confidence interval. Toxicity of repellent is considered significantly different when the 95% CI fails to overlap.
[4] Same letters indicate that the toxicities of the repellents are not significantly different from each other because the 95% CI overlapped with each other. Different letters indicate that the toxicities of the repellent are significantly different from each other because the 95% CI fails to overlap.

Table 2. Toxicities of 9 repellents against female *Anopheles quadrimaculatus* by topical application.[1]

Repellent name	LD_{50}[2] (95% CI)[3,4]	LD_{95}[2] (95% CI)[3]	Slope (SE)	χ^2	*n*
IR3535	2.35 (2.09–2.68)d	5.33 (4.18–8.68)	4.63 (0.83)	1.68	420
DEET	0.40 (0.22–0.47)b	1.08 (0.84–2.63)	3.77 (1.12)	0.46	420
DMP	2.50 (1.78–5.57)d	19.60 (8.27–279.67)	1.84 (0.58)	0.22	420
AI3-37220	0.16 (0.11–0.20)a	0.83 (0.49–3.80)	2.29 (0.58)	0.86	420
Ethyl hexanediol	1.72 (1.38–2.31)d	7.70 (4.78–18.38)	2.52 (0.40)	0.05	420
AI3-37220G	0.14 (0.08–0.21)a	0.71 (0.39–5.16)	2.30 (0.37)	3.62	420
KBR 3023	0.63 (0.55–0.76)c	1.55 (1.13–3.14)	4.18 (0.84)	1.30	420
AI3-35765	0.45 (0.39–0.54)b	1.18 (0.87–2.10)	3.91 (0.67)	0.53	420
PMD	1.46 (0.99–2.16)d	4.79 (2.94–36.64)	3.30 (0.56)	4.23	420

[1] IR3535, 3-(N-butyl-N-acetyl)-aminopropionic acid, ethyl ester; DEET, N,N-diethyl-3-methylbenzamide; DMP, dimethylphthalate; AI3-37220, 1-(3-cyclohexen-1-ylcarbonyl)-2-methylpiperidine; AI3-37220G, AI3-37220 granular; KBR 3023, picaridin; AI3-35765, 1-(3-cyclohexen-1-ylcarbonyl) piperidine; PMD, *para*-menthane-3,8-diol.
[2] LD_{50} and LD_{95} values are in units of micrograms of pesticide per milligram of mosquito.
[3] CI, confidence interval. Toxicity of repellent is considered significantly different when the 95% CI fails to overlap.
[4] Same letters indicate that the toxicities of the repellents are not significantly different from each other because the 95% CI overlapped with each other. Different letters indicate that the toxicities of the repellent are significantly different from each other because the 95% CI fails to overlap.

Table 3. Toxicities of 9 repellents against female *Anopheles albimanus* by topical application.[1]

Repellent name	LD_{50}[2] (95% CI)[3,4]	LD_{95}[2] (95% CI)[3]	Slope (SE)	χ^2	*n*
IR3535	1.67 (1.34–2.18)d	6.63 (4.30–14.89)	2.75 (0.46)	1.22	420
DEET	0.22 (0.17–0.27)b	0.76 (0.50–1.93)	3.00 (0.63)	0.60	420
DMP	1.83 (1.52–2.24)d	6.52 (4.59–12.17)	2.98 (0.46)	1.23	420
AI3-37220	0.11 (0.09–0.13)b	0.30 (0.23–0.48)	3.55 (0.51)	2.92	420
Ethyl hexanediol	1.53 (0.93–3.11)d	5.05 (2.69–77.85)	3.18 (0.49)	2.02	420
AI3-37220G	0.06 (0.05–0.07)a	0.20 (0.14–0.36)	3.07 (0.47)	2.12	420
KBR 3023	0.50 (0.38–0.87)c	2.03 (1.20–19.41)	2.72 (0.44)	3.88	420
AI3-35765	0.16 (0.11–0.23)b	0.53 (0.28–9.47)	3.07 (0.53)	4.32	420
PMD	1.28 (1.13–1.47)d	2.52 (1.98–4.52)	5.56 (1.24)	1.00	420

[1] IR3535, 3-(N-butyl-N-acetyl)-aminopropionic acid, ethyl ester; DEET, N,N-diethyl-3-methylbenzamide; DMP, dimethylphthalate; AI3-37220, 1-(3-cyclohexen-1-ylcarbonyl)-2-methylpiperidine; AI3-37220G, AI3-37220 granular; KBR 3023, picaridin; AI3-35765, 1-(3-cyclohexen-1-ylcarbonyl) piperidine; PMD, *para*-menthane-3,8-diol.
[2] LD_{50} and LD_{95} values are in units of micrograms of pesticide per milligram of mosquito.
[3] CI, confidence interval. Toxicity of repellent is considered significantly different when the 95% CI fails to overlap.
[4] Same letters indicate that the toxicities of the repellents are not significantly different from each other because the 95% CI overlapped with each other. Different letters indicate that the toxicities of the repellent are significantly different from each other because the 95% CI fails to overlap.

Table 4. Toxicities of 9 repellents against female *Culex quinquefasciatus* by topical application.[1]

Repellent name	LD_{50}[2] (95% CI)[3,4]	LD_{95}[2] (95% CI)[3]	Slope (SE)	χ^2	*n*
IR3535	3.61 (3.28–4.00)d	7.11 (5.94–9.69)	5.59 (0.82)	2.57	420
DEET	0.64 (0.53–0.73)b	1.65 (1.23–3.53)	3.98 (0.93)	1.44	420
DMP	4.72 (4.43–5.05)e	7.33 (6.52–8.97)	8.63 (1.31)	2.40	420
AI3-37220	0.20 (0.14–0.23)a	0.43 (0.35–0.77)	4.90 (1.30)	0.03	420
Ethyl hexanediol	1.62 (1.32–2.17)c	3.59 (2.52–10.70)	4.75 (0.74)	3.54	420
AI3-37220G	0.27 (0.24–0.29)a	0.43 (0.38–0.55)	8.06 (1.40)	0.20	420
KBR 3023	1.62 (1.24–1.84)c	3.63 (2.91–6.77)	4.70 (1.20)	0.31	420
AI3-35765	0.48 (0.43–0.53)b	0.90 (0.77–1.15)	5.89 (0.74)	2.65	420
PMD	2.14 (1.94–2.35)c	3.46 (3.02–4.48)	7.92 (1.39)	0.01	420

[1] IR3535, 3-(N-butyl-N-acetyl)-aminopropionic acid, ethyl ester; DEET, N,N-diethyl-3-methylbenzamide; DMP, dimethylphthalate; AI3-37220, 1-(3-cyclohexen-1-ylcarbonyl)-2-methylpiperidine; AI3-37220G, AI3-37220 granular; KBR 3023, picaridin; AI3-35765, 1-(3-cyclohexen-1-ylcarbonyl) piperidine; PMD, *para*-menthane-3,8-diol.

[2] LD_{50} and LD_{95} values are in units of micrograms of pesticide per milligram of mosquito.

[3] CI, confidence interval. Toxicity of repellent is considered significantly different when the 95% CI fails to overlap.

[4] Same letters indicate that the toxicities of the repellents are not significantly different from each other because the 95% CI overlapped with each other. Different letters indicate that the toxicities of the repellent are significantly different from each other because the 95% CI fails to overlap.

example, DEET, the most common active ingredient in commercially available insect repellent, has LD_{50} values of 0.94, 0.40, 0.22, and 0.64 μg/mg against *Ae. aegypti*, *An. quadrimaculatus*, *An. albimanus*, and *Cx. quinquefasciatus*, respectively. The average body weight of a female *Ae. aegypti*, *An. quadrimaculatus*, *An. albimanus*, and *Cx. quinquefasciatus* in this study was 2.85, 1.92, 1.91, 2.02 mg, respectively. Therefore, the LD_{50} values of DEET in the unit of microgram of repellent per mosquito would be 2.69, 0.76, 0.41, 1.29 μg/mosquito. Because we used 0.5 μl of solution to treat the mosquitoes topically, the LD_{50} values of DEET in the unit of microgram per microliter of repellent would be 5.38, 1.52, 0.82, and 2.58 μg/μl; i.e., 0.538%, 0.152%, 0.082%, and 0.258%. The LD_{95} values of DEET in the unit of percentage against *Ae. aegypti*, *An. quadrimaculatus*, *An. albimanus*, and *Cx. quinquefasciatus* would be 1.378%, 0.414%, 0.145%, and 0.333%, respectively. Because any commercially available DEET insect repellent has a minimum percentage of active ingredient of 7.5% (up to 30%), which is much higher than the LD_{95} values of DEET as described above, it is not surprising that Xue et al. (2003) found that commercially available insect repellents in spray formulations produced significant adult knockdown (KD) and 24-h mortality against adult mosquitoes. Recently, the lethal effects of 3 synthetic repellents (DEET, IR3535, and KBR3023) have been evaluated by filter paper tests to assess the knockdown effect and mortality induced by each repellent to *Ae. aegypti* (Licciardi et al. 2006). At the same concentration, DEET has been found to possess insecticidal activity whereas IR3535 and KBR 3023 did not (Licciardi et al. 2006). Consistent with their finding, our results also revealed that DEET had higher insecticidal activity than IR3535 and KBR3023 against all 4 mosquito species.

It has been reported that different mosquito species possess different susceptibility to different toxicants (Pampiglione et al. 1985, Campos and Andrade 2003, Somboon et al. 2003, Pridgeon et al. 2008). For example, when permethrin was topically applied to mosquitoes, the susceptibility order of 3 mosquito species was *Ae. aegypti* > *An. quadrimaculatus* > *Cx. quinquefasciatus*. However, when hydramethylnon was used as the toxicant, the susceptibility order of the 3 mosquito species was *An. quadrimaculatus* > *Cx. quinquefasciatus* > *Ae. aegypti* (Pridgeon et al. 2008). Our results in this study also revealed that different mosquitoes showed different susceptibility to different repellents. For example, when DEET or DMP was applied as the toxicant, the susceptibility order of the 4 mosquito species was *An. albimanus* ≥ *An. quadrimaculatus* > *Cx. quinquefasciatus* > *Ae. aegypti* (Tables 1–4). However, when IR3535 was used as a toxicant, the susceptibility order of the 4 mosquito species was changed to *An. albimanus* ≥ *Ae. aegypti* ≥ *An. quadrimaculatus* > *Cx. quinquefasciatus*. When AI3-37220 or PMD was used as a toxicant, there was no significant difference in the susceptibility among the 4 mosquito species. This could be simply due to species variability.

Although different mosquitoes showed different susceptibility to different toxicants, the relative susceptibilities were consistent for species and possibly even genera. Specifically, the comparison of 24-h LD_{50} values of the same repellent compared against the mosquito species indicated that *An. albimanus* was the most susceptible to all 8 repellents tested. This is quite interesting, because *An. albimanus* is notorious for its inability to be repelled by DEET and other repellents (McGovern and Schreck 1988, Robert et al. 1991, Klun et al. 2004), yet it is the most susceptible species to repellent toxicants.

In summary, we evaluated the relative potency of 8 repellents as toxicants against females of 4 species of mosquitoes by topical application. The most toxic repellent was A13-37220 and the least toxic was DMP. Based on these studies, *An. albimanus* is the most susceptible. Our results provide important information on the toxicities of 8 repellents against 4 species of mosquito.

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