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Research Paper

A bioassay for mosquito repellency against *Aedes aegypti*: method validation and bioactivities of DEET analogues

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Abstract

Objectives Vector-borne diseases are still a major mortality factor in Africa and South-east Asia and effective mosquito repellents are therefore needed. An efficient and safe in-vitro assay system using artificial blood and skin substitute could facilitate the development of novel repellents, as most assays currently rely on human subjects or vertebrate whole blood. Moreover, examining the skin permeation profiles could provide safer mosquito repellents. The new assay system could serve as an initial system for testing new repellent candidates upon validation with DEET and its analogues.

Methods *N*,*N*-Diethyl-*meta*-toluamide (DEET) and five analogues were synthesised and used to validate a novel in-vitro bioassay using artificial blood and collagen membrane. Repellency against *Aedes aegypti* was correlated with lipophilicity and skin permeation.

Key findings The new in-vitro assay showed good reproducibility (interday relative standard deviation <10% at high concentrations). Four of the five DEET analogues showed repellency similar or superior to that of DEET. Repellency correlated linearly with lipophilicity but stronger repellents tended to permeate skin better.

Conclusions The new in-vitro assay using blood substitute and collagen membrane significantly simplifies screening of possible mosquito repellents. Lipophilicity as well as skin permeation profiles should be considered before testing of compounds that are candidates for mosquito repellents.

Keywords DEET; in-vitro assay; mosquito; repellent; skin permeation

Introduction

Female mosquitoes can transmit diseases through the transfer of pathogen-contaminated saliva when biting hosts to obtain blood, which is needed to develop eggs.[1] One of the mosquito species commonly found in tropical and subtropical areas, *Aedes aegypti*, feeds during the day and multiple times while gravid, making it a potent disease vector.[2] Common mosquito-borne and clinically important diseases include malaria (Africa, Central America, Asia), West Nile virus (Africa and north America) and dengue fever (Africa). It has been reported that mosquitoes feed on various vertebrates that attract mosquitoes via chemical attractants, which include carbon dioxide,[3] lactic acid,[4] ATP[5] and colour plus heat.[2]

Various means have been used to avoid being bitten by mosquitoes: the cultivation and use of mosquito predators,[6] the pesticide dichloro-diphenyl-trichloroethane (DDT),[7,8] use of insecticide-treated nets, which were able to reduce infections with vector-borne diseases in Africa,[9] and the use of various repellents, including natural products like *Cyperus scariosus*, *Juniperus macropoda*, *Nigella sativa* and Neem oil.[10–15]

The most common chemical repellent known today is *N*,*N*′-diethyl-*m*-toluamide (DEET), which entered civilian use in 1956.[16] It offers complete protection time (CPT) ranging from 203 to 756 min, varying with climatic effects, mosquito species, physical activity, attractiveness of the host and design of the assay.[17,18] However, DEET is associated with systemic toxicities and is suspected to be one cause of the Gulf War syndrome.[19] A common alternative, popular in Europe and Australia, is hydroxyethyl isobutyl piperidine carboxylate, known under the trade name Icaridin,[20] which though

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considered relatively less toxic has been reported to cause allergic contact dermatitis.[21] Thus, the search for an effective yet safe mosquito repellent continues.

Development of an efficient repellent requires a fast and reliable bioassay system. Existing bioassays rely on natural attractants such as human odour, vertebrate blood or living vertebrates.[22,23] Thus they are affected by variation in both the mosquitoes and the attractants. The use of an in-vitro feeding device and an artificial blood substitute for initial screening of possible mosquito repellents could eliminate these variations and reduce the cost of using animals and the need for human volunteers. Kogan[24] developed an in-vitro feeding assay to maintain a mosquito colony on an artificial-blood diet in which mosquitoes were fed on feeders covered with thin, stretched Parafilm and warmed in a water bath. The artificial blood substitute was designed to match the composition of human blood and thus induce a favourable feeding response as well as providing sufficient nutrition for egg development in *A. aegypti*. However, Parafilm alone does not mimic physiological skin sufficiently well. A bioassay using collagen membrane in conjunction with whole blood has been reported.[25] Thus, combining and modifying these two assays could bring about a fast, reliable and easy initial in-vitro bioassay system for the screening of mosquito repellents. To the best of our knowledge, this is the first attempt to devise an assay system to screen for mosquito repellents using collagen membrane and artificial blood.

Since most studies of repellents tend to rely on random screening, once an assay system is established, it would be necessary to systematically investigate potential repellents according to their chemical structure and physicochemical properties. Wang *et al.*[26] reported quantitative structure–activity relationship studies of pinene analogues as repellents. Moreover, Katritzky *et al.*[27] tried to correlate vapour pressure with repellency, while Suryanarayana *et al.*[28] tried to correlate vapour pressure, lipophilicity and molecular size with repellency. However, studies on the correlation between lipophilicity of the repellent and skin permeation are rarely found, even though the extent to which a repellent actually permeates the skin is important in determining its toxicity.

This paper describes a mosquito repellent assay against *A. aegypti* using collagen membrane and artificial blood substitute as attractant. DEET was used to validate the assay, after which the repellency of DEET analogues was determined and evaluated in terms of lipophilicity and skin permeation.

Materials and Methods

Chemicals and reagents

DEET (97%) was bought from Aldrich Chemical Co. (St Louis, MO, USA). Icaridin was a gift from Saltigo GmbH (Langenfeld, Germany). Double-distilled water was acquired using a Millipore system. Edicol collagen membrane was a gift from Devro (Glasgow, Scotland). Disposable hand warmers were bought from Barunson Co. (Seoul, Korea). Artificial blood substitute was prepared according to the method reported by Kogan[24] but using an ATP concentration of 2 mol/l instead of 1 mol/l. Gamma globulin from bovine blood (99% pure from agarose gel electrophoresis), porcine haemoglobin, ovalbumin (grade II) and ATP disodium salt (Grade II) were bought form Sigma-Aldrich (St Louis, MO, USA). Glass tubes (4 cm long, 2 cm diameter) were from Gunilsangsa (Busan, Korea). All other reagents were purchased from Aldrich and were of synthetic grade or better.

Mosquitoes of the species *A. aegypti* were provided by the School of Agricultural Biotechnology, Seoul National University. This colony has been maintained for 12 years. Larvae were reared in plastic trays (24 × 35 × 5 cm) containing 0.5 g sterilised diet (40-mesh chick chow powder and yeast, 4 to 1 by weight) at 26°C.[12] Adults were fed on apple slices and were kept at 25°C and approximately 80% relative humidity.

Synthesis of DEET analogues

The chemical structure of DEET is shown in Figure 1 together with its pyrrolidine (**1**), piperidine (**2**) and piperazine (**3**) analogues. The *para* (**4**) and *ortho* (**5**) derivatives of DEET are shown in Figure 2.

Compound **1** (1-(3-methylbenzoyl)-pyrrolidine) was synthesised according to the procedure reported by Liao *et al.*[29] Compounds **2** (1-(3-methylbenzoyl)-piperidine) and **3** (1-(3-methylbenzoyl)-piperazine) were synthesised according to the procedure reported by Wirth *et al.*[30] Compounds **4** (*N*,*N*-diethyl-4-methylbenzamide)

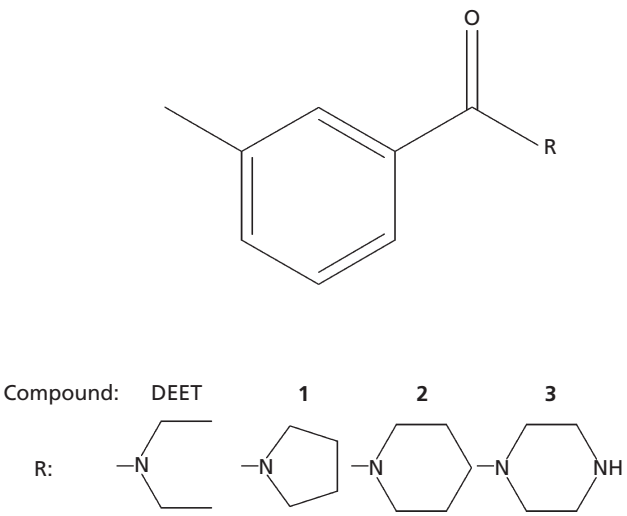
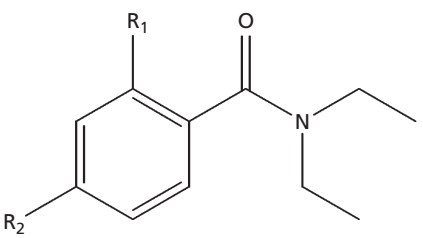


Figure 1 Chemical structures of DEET and compounds **1**, **2** and **3**



4: R_1=H, R_2=CH_3 **5**: R_1=CH_3, R_2=H

Figure 2 Chemical structures of compounds **4** and **5**

and **5** (*N*,*N*-diethyl-2-methylbenzamide) were synthesized according to the method reported by Enders *et al.*[31]

In-vitro assay

The cage developed for the in-vitro assay is shown in Figure 3. Forty female adult mosquitoes (5–20 days old) were placed in the polypropylene container with a partly open top covered with a mosquito-retaining support made of gauze. The mosquitoes were kept in a light–dark cycle of roughly 12 h prior to the experiment. They were fed until 12 h before the experiment with apple slices, which were placed directly on the mosquito-retaining support. A 5 × 5 cm patch of collagen membrane was soaked in double-distilled water, strapped over the tube and fixed with a rubber band.

The artificial blood substitute was prepared directly in the test tubes from stock solutions according to the method of Kogan[24] immediately prior to the experiment. The composition of the blood substitute is shown in Table 1. A thermostatic wrap (hand warmer) was wrapped around the tube and fixed with tape. (A tube filled with 5 ml blood substitute will maintain a temperature of 37–39°C for at least 12 h when wrapped with a hand warmer; the blood substitute reaches a temperature of 37–39°C after approximately 10 min.) The tubes were then placed upside down on top of the mosquito-retaining support (Figure 3). The biting ability for each tube was checked before applying the repellent, by placing the ready but untreated feeding device on top of the feeding cage.

To test the repellents, a sample (10 μl) was applied to the membrane (resulting in a surface load of 3.18 μl/cm^2 for 100% concentration) and distributed evenly with a powderfree-glove-coated finger. The tubes filled with blood substitute were then placed upside down on top of the mosquito-retaining support. The time when mosquitoes started to feed (i.e. were no longer repelled) was recorded as the CPT. If no feeding was observed after 10 h, the experiment was stopped and a CPT of 600 minutes was recorded. The tubes of blood substitutes were swirled each hour to prevent precipitation.

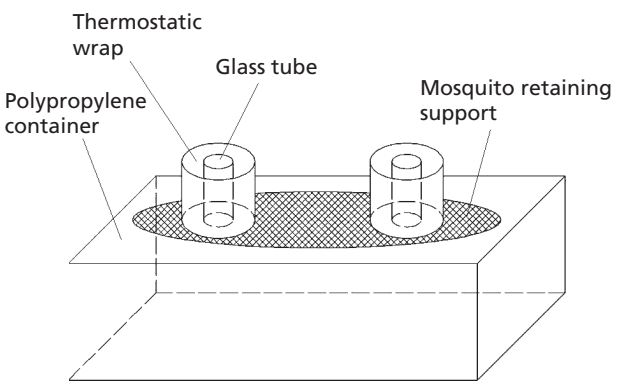


Figure 3 Gauze-covered polypropylene container with vials wrapped in hand warmers. The container was of 29 × 19 × 8 cm and made of polypropylene. The top of the board was partly open and covered with gauze where the tube containing blood substitute and wrapped in a hand warmer is placed upside down, to attract or repel mosquitoes inside the cage.

Table 1 Composition of the artificial blood substitute

Component	Concn of stock solution (mg/ml)	μl stock solution per ml meal	Concn in the meal (mg/ml)
Gamma globulin	50	300	15
Haemoglobin	35	230	8
Albumin	300	340	102
NaCl	0.97–1.9	[a]	0.29–0.58
$NaHCO_3$	34	[a]	10
ATP	203	5	1

[a]NaCl and $NaHCO_3$ were included in the gamma globulin stock solution. NaCl was present in the gamma globulin as purchased from Sigma-Aldrich and resulted in the concentration described above.

Validation of feeding assay

Validation of the bioassay was performed by investigating the CPTs of Icaridin (100%, 50% and 25% in methanol), DEET (100%, 50% and 25% in methanol), blank (methanol 100%) and untreated membrane. With a round vial of 2 cm diameter, the surface load was 3.18 μl/cm^2 for 100% solutions, 1.59 μl/cm^2 for 50% solutions and 0.80 μl/cm^2 for 25% solutions. Because of the cut-off time of the assay (10 h), intra-day validation was performed in duplicate; inter-day validation were performed in triplicate on consecutive days. The validation was done with the same population of mosquitoes. Means and SD were calculated and the CPTs compared with literature values. A Kruskal–Wallis test following by Dunn's post-hoc test was performed to test for significance; $P < 0.05$ was considered significant.

In-vitro mouse skin permeation study

Preparation of mouse skin

Skin for this assay was from hairless mouse obtained from OrientBio Co. (Seongnam, Korea). All animal experiments were performed according to the Guidelines for Animal Care and Use of Seoul National University, Seoul, Korea. The animals had free access to food and water before experiments and were sacrificed in a carbon-dioxide chamber just before use. The dorsal hair was removed with clippers and full-thickness skin (about 10 cm^2) was surgically removed from each mouse. The skin specimen was cut into appropriate sizes after carefully removing subcutaneous fat and washing with normal saline.

In-vitro permeation study

In-vitro skin permeation across mouse skin was measured with Keshary–Chien diffusion cells at 37°C. Freshly excised mouse skin was mounted between the donor and receptor cells (stratum corneum side facing the donor). One skin sample was taken from each animal; samples were randomised among the different repellents. The area of diffusion for all in-vitro experiments was 2.01 cm^2. The receptor cells, which faced the dermis side, were filled with phosphate buffer solution (pH 7.4, 10 mol/l, 12 ml). At predetermined time intervals, 1 ml of the receptor solution was withdrawn and replaced with the same volume of fresh receptor solution. Each experiment was done in triplicate. Samples were kept at −20°C until HPLC analysis.

Determination of capacity factor and calculation of log P

DEET and analogues (10 μg/ml in methanol) were analysed using an HPLC system (Waters 2690) coupled with a UV detector (Waters 2487 dual λ absorbance detector). A Merck RP-18 Lichrocart Lichrosphere column (5 μm, 125 × 4 mm; Merck, Darmstadt, Germany) was used at ambient temperature. The mobile phase consisted of methanol/water (60:40, v/v) which was filtered through a membrane filter (RC-membrane filter, 47 mm, 0.2 μm; Sartorius, Goettingen, Germany) and was thoroughly degassed in an ultrasonic bath before use. The flow rate was set at 0.8 ml/min and detection was at 230 nm.

Capacity factors (k') were calculated from the equation $k' = (t_R - t_0)/t_0$ where t_R is the retention time of the compound and t_0 is the retention time of methanol.

Log P was calculated using ChemDraw 11 (CambridgeSoft, USA), which uses atomic contributions calculated from known molecules and least-squares analysis with a standard deviation of 0.43–0.50 log P.

Results

Synthesis of DEET analogues

1-(3-Methylbenzoyl)-pyrrolidine (1)

Pyrrolidine (1.0668 g) and *m*-toluoyl chloride (1.32 ml) gave 1.3041 g dark-yellow oil (yield 68.96%). UV (methanol) 201, 210 nm ($\log\varepsilon$ = 5.87, 5.78); IR (nujol) 2923 (C-H), 1631 (amide C=O); ^{1}H NMR (300 MHz, DMSO-d6) δ 7.8–8.1 (m, 4H, Ar-H), 4.0–4.2 (tt, 4H, -N-$(CH_2)_2$), 3.0 (s, 3H, CH_3), 2.4–2.6 (m, 4H, pyrrolidine-$(CH_2)_2$) ppm; ^{13}C NMR (75.5 MHz, DMSO-d6), δ 168.3, 137.4, 137.2, 130.1, 127.9, 127.4, 123.9, 48.8, 45.7, 25.8, 23.8, 20.8 ppm.

1-(3-Methylbenzoyl)-piperidine (2)

Piperidine (1.2772 g) and *m*-toluoyl chloride (1.32 ml) gave 1.2676 g light-yellow oil (yield 62.40%). UV (methanol) 202 nm ($\log\varepsilon$ = 5.21); IR (nujol) 2923 (C-H), 1648 (amide C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.1–7.3 (m, 4H, Ar-H), 3.3–3.8 (ss, 4H, -N-$(CH_2)_2$), 2.1 (s, 3H, CH_3), 1.4–1.8 (ss, 6H, piperidine-$(CH_2)_3$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.7, 138.5, 136.8, 130.2, 128.4, 127.6, 123.9, 48.9, 43.3, 24.8(3), 21.6 ppm.

1-(3-Methylbenzoyl)-piperazine (3)

Piperazine (4.1347 g) and *m*-toluoyl chloride (5.27 ml) gave 4.8632 g of dark-orange oil (yield 68.96%). UV (methanol) 202 nm ($\log\varepsilon$ = 5.24); IR (nujol) 2923 (C-H), 1608 (amide C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.1–7.3 (m, 4H, Ar-H), 3.3–3.9 (ss, 4H, -N-$(CH_2)_2$), 2.7–3.1 (ss, 4H, piperazine-$(CH_2)_2$), 2.4 (s, 3H, CH_3), 1.2–1.4 (m, H, piperazine-NH) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.8, 138.6, 136.1, 130.5, 128.5, 127.8, 124.1, 49.1, 46.3, 43.3, 29.9, 21.6 ppm.

N,N-*Diethyl-4-methylbenzamide (4)*

Diethylamine (0.7316 g), triethylamine (1.012 g) and *p*-toluoyl chloride (1.32 ml) gave 1.5119 g of yellow solid (melting point: 50°C; yield 79.03%). UV (methanol) 202 nm ($\log\varepsilon$ = 5.18); IR (nujol) 2973 (C-H), 1619 (amide C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.1–7.4 (m, 4H, Ar-H), 3.4 (s, 4H, -N-$(CH_2)_2$), 2.4 (s, 3H, CH_3), 1.2 (s, 6H, -N-$(CH_2)_2$-$(CH_3)_2$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 171.5, 139.2, 133.9, 128.9(2), 126.3(2), 41.8(2), 21.3, 13.5(2) ppm.

N,N-*Diethyl-2-methylbenzamide (5)*

Diethylamine (0.7316 g) and *o*-toluoyl chloride (1.30 ml) gave 1.7935 g of yellow solid (yield: 93.75%; melting point 46°C; literature value: 48°C[31]). UV (methanol) 202 nm ($\log\varepsilon$ = 5.17); IR (nujol) 2971 (C-H), 1625 (amide C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.1–7.4 (m, 4H, Ar-H), 3.4 (q, 4H, -N-$(CH_2)_2$), 2.4 (s, 3H, CH_3), 1.2–1.4 (tt, 6H, -N-$(CH_2)_2$-$(CH_3)_2$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.8, 136.9, 133.8, 130.2, 128.5, 125.7, 125.4, 42.6, 38.6, 18.7, 13.9, 12.8 ppm.

Validation of feeding assay and dose-dependency study

The repellency assay was validated with DEET and Icaridin. Table 2 shows the CPTs determined for Icaridin, DEET, blank (methanol) and untreated membrane at different doses. Because CPTs were similar for methanol and untreated collagen membrane, Icaridin and DEET were diluted in methanol and applied to the membrane surface as described above. Intra-day validation showed good repeatability but was only done twice because each assay required 10 h. SD seemed to be higher at lower concentrations. This variation in dose dependency may come from uneven distribution of compound on the membrane.

The concentration dependency of CPT for DEET and Icaridin is shown in Figure 4. Both relationships are better approximated with a logarithmic trend. The linear correlation coefficient, r^2, was 0.986 for DEET and 0.758 for Icaridin. Using a logarithmic scale, r^2 values were 0.994 for DEET and 0.898 for Icaridin.

Repellency of DEET analogues

Compounds **1**–**5** were tested for their repellency against *A. aegypti* using the in-vitro repellency assay described above. DEET and Icaridin were used as positive controls and repellency was measured as CPT. When 10 μl of compound **3** was applied undiluted, no effective protection was observed, with a CPT of 5 min in the first assay and 8 min in the second. These values were similar to those with the

Table 2 Complete protection times in minutes of DEET and Icaridin

	Day 1		Day 2	Day 3	Mean ± SD
	A	B			
Icaridin 100%	419	490	480	550	484.75 ± 46.45*
Icaridin 50%	355	338	480	400	393.25 ± 54.97
Icaridin 25%	60	50	38	50	49.50 ± 7.79
DEET 100%	440	380	487	500	451.75 ± 47.06*
DEET 50%	180	190	240	295	226.25 ± 45.74
DEET 25%	70	55	40	48	53.25 ± 11.03
Blank	5	8	5	10	7 ± 2.12
Untreated	10	4	5	5	6 ± 2.35

Blank, membrane treated with methanol only; untreated, untreated membrane. *$P < 0.05$ vs untreated (Kruskal–Wallis test and Dunn's post-hoc test).

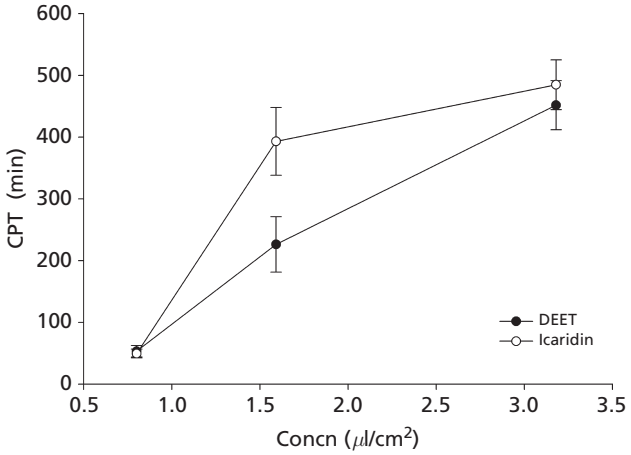


Figure 4 Correlation between complete protection time and concentrations of Icaridin and DEET. Concentration is expressed as volume of applied drug per cm^2 membrane. CPT, complete protection time.

blank. However, when the same amounts of compounds **1** and **2** were applied, CPT values were close to those observed for DEET and Icaridin. Because compounds **4** and **5** were solid at room temperature, they had to be applied as a 50% solution in methanol. Both compounds had CPTs of 600 min, which was the cut-off time of our assay. Repeating the assay with these five compounds revealed similar results, as shown in Table 3.

Correlations between Log P, log k′ and complete protection time

Structure–activity relationship was investigated via the Log P–CPT relationship of DEET and compounds **1**–**5**. Log P values correlated well with the log k′ values determined by HPLC ($r^2 = 0.89169$), indicating that lipophilicity of future analogues could be determined via HPLC. Although statistical analysis was not performed, CPT values of the DEET analogues seemed to be related to the Log P values. These results imply that lipophilicity of the compound affects repellency. Further studies are underway to synthesise more analogues for a systematic study of structure–activity relationships.

Table 3 Complete protection time in minutes of DEET and DEET analogues

	Assay 1	Assay 2
DEET	451.75 ± 47.06	
Icaridin	484.75 ± 46.45	
Compound **1**	300	490
Compound **2**	480	570
Compound **3**	5	12
Compound **4**	600	600
Compound **5**	600	600
Methanol	5	8

Complete protection time (CPT) values for DEET and icaridin are means ± SD of triplicate samples. For Compounds **1**–**5**, individual results of duplicate analysis are shown.

In-vitro mouse skin permeation

To reduce possible toxicity, low skin permeation of repellents is desirable. Results of skin permeation of DEET analogues determined in Keshary–Chien diffusion cells at 37°C in mouse skin over a 12 h period are shown in Figure 5. Compared with DEET (22.99 $\mu g/cm^2$ per h), compound **3** showed significantly lower skin permeation of 7.41 $\mu g/cm^2$ per h ($P < 0.05$; Kruskal–Wallis test followed by Dunn's post-hoc test). Moreover, as shown in Table 4, compound **3,** which had the lowest Log P value, resulted in the lowest the skin permeation rate, while other analogues with similar Log P values to DEET showed similar skin permeation profiles to one another. Thus, although lowering Log P may retard skin permeation, it could also reduce repellency.

Discussion

Mosquito repellency or protective properties are often measured with human subjects either in field studies or with conventional arm-in-cage assays.[12] Because human trials expose volunteers to disease vectors and to possibly harmful drugs, an efficient in-vitro assay could greatly reduce these risk factors. Blood substitute has been used for colony maintaining and live mosquitoes, and collagen membrane as skin substitute proved useful for repellency

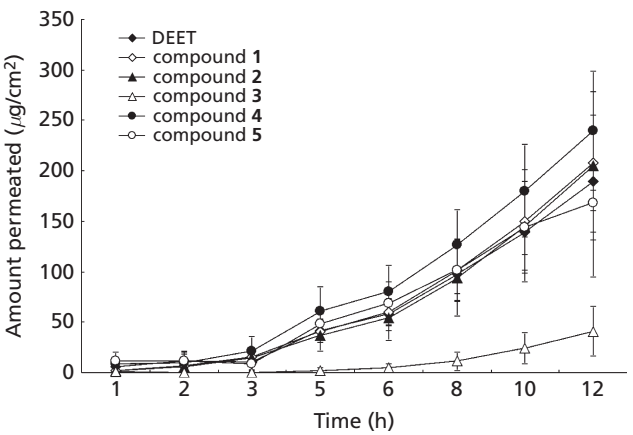


Figure 5 Skin permeation profile of DEET and DEET analogues determined with Keshary–Chien diffusion cells with hairless mouse skin

Table 4 Skin permeation rate and log P values of DEET and DEET analogues

	Skin permeation rate ($\mu g/cm^2$ per h)	Log P (calculated)
DEET	22.99 ± 5.67	2.58
Compound **1**	26.50 ± 6.20	2.21
Compound **2**	27.76 ± 8.79	2.63
Compound **3**	7.41 ± 3.94*	1.28*
Compound **4**	28.39 ± 5.60	2.58
Compound **5**	16.54 ± 13.76	2.58

Skin permeation rates are means ± SD of triplicate samples. *$P < 0.05$ vs DEET (Kruskal–Wallis test and Dunn's post-hoc test).

detection.[32] In this study, artificial blood substitute was combined with skin substitute using lab-reared day-feeding mosquitoes to ensure reproducibility and safety of the bioassay.

Observing the protection time (CPT) is a basic method of determining protection from biting insects but can be difficult to repeat. To ensure repeatability, the new assay was validated with DEET and Icaridin at different concentrations and showed repellency (CPT) of 451.75 min and 484.75 min, respectively, when applied as 100% solution with a surface load of 3.2 μl/cm^2. This repellency is in the range of reported repellent times when applied to human volunteers, although protection times reported by different research groups vary widely.[14,20,33] Factors that seem to affect the CPT are biting readiness and mosquito density, as well as variations in the source of attraction and the design of the assay.[34] In our in-vitro assay, the variations of the host could be eliminated, which led to SD of less than 20% for interday validation with Icaridin and DEET, and less than 10% when the repellent concentration was higher.

When DEET analogues of different Log P values were tested and compared with DEET, compound **3** showed no protection and was similar to that of the blank or untreated membrane. Compounds **1** and **2**, on the other hand, showed similar protection to DEET. The two solid compounds (**4** and **5)** showed the highest protection among the compounds tested. Since the latter two compounds had similar Log P values to DEET, this might reflect variation in melting point and thus vapour pressure, which affects the time until complete evaporation.[35]

Log P is the main factor that affects skin permeation,[36] but its relationship with repelling effect has not been investigated systematically. Results of this study show that for DEET analogues, repellency decreases with decreasing Log P values to the point of no repellency. Hence the development of novel repellents related in structure to DEET should focus on compounds with a Log P above 2. When considering the skin permeation profile of these compounds, most compounds showed increased permeation when CPT was increased. Log P tended to correlate with skin permeation as well as repellency, indicating the difficulty in developing a potent yet safe mosquito repellent. However, compound **5,** which was similar in Log P to DEET, showed enhanced repellency with a reduced skin permeation rate. Although the reduction in skin permeation may not have been significant enough, further studies related to this compound could identify a novel repellent.

On the basis of the above results, compounds with too low a Log P value could be eliminated from initial tests. However, an optimal Log P needs to be determined so that skin permeation can be reduced in order to decrease toxicity in humans. Rather than simply lowering the lipophilicity of the repellent, which increases skin permeation, skin permeation retardants together with repellents could be used to solve this problem.[37]

Conclusions

The method developed was fast and reliable and eliminates the need for human volunteers, thus reducing the cost and increasing safety. This assay could therefore serve as a convenient way to screen potential mosquito repellents. Repellency of the tested compounds seemed to be correlated with their lipophilicity, stronger repellents tending to permeate through skin better. Synthesis and development of DEET analogues with decreased skin permeation as novel mosquito repellents are underway.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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