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Evaluation of Topical Insect Repellents and Factors That Affect Their Performance

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Introduction

Personally-applied topical insect repellents are a flexible and relatively affordable means of gaining protection from biting arthropods and the disease-causing pathogens they sometimes carry.[1,2] Although a number of useful repellents have been developed, a variety of factors limits their effectiveness in application. The purpose of this chapter is to review those factors, consider their importance, and discuss means of overcoming them. The majority of investigations have been conducted against mosquitoes that are vectors of important disease agents: the yellow fever mosquito, *Aedes aegypti*, and the *Anopheles* species that transmit malaria pathogens. Although this chapter emphasizes results from studies of mosquitoes, data from other biting arthropods are included when helpful or relevant.

For a repellent to be successful, it must first have a high percentage of effectiveness against the biting arthropods of concern for the entire period of likely use. Second, it should be toxicologically safe at the rate of application for which it is intended. Third, it should be easy to apply and pleasant on the skin in

terms of residual feeling and odor. Finally, the entire spectrum of costs involved in production and marketing of the repellent should result in a product that is reasonably priced for the consumer. Among the repellent active ingredients formulated over the last half century, deet (*N,N*-diethyl-3-methylbenzamide) has been included in numerous products that come remarkably close to approaching that ideal, and it is estimated that deet is employed at least 200 million times per year around the globe.[3] Persistent public concerns about its safety (some based on hearsay) have been aggravated by its cosmetic shortcomings and plasticizing (i.e., tendency to soften plastics) effects. Cosmetic improvements have been achieved mainly by limiting deet concentrations to 10% or lower, resulting in formulations with efficacy of limited duration. In addition, while high-concentration deet formulations often remain efficacious for eight or more hours, attempts to enhance duration by manipulating carrier formulations have not resulted in substantial improvements. This suite of concerns has helped to fuel the search for suitable alternatives for both civilian and military applications.

Little is known about how insect repellents function.[4] Such knowledge would promote the development of more effective repellents based on biochemical and neurophysiological principles. In the absence of real knowledge about mechanisms, we may instead progress inferentially through the collation and analysis of natural history data on factors that influence success. Interactions between parasites and hosts are biologically complex and therefore inherently dynamic and challenging to control. Among the many factors likely to influence the effectiveness of a repellent are those involving the active ingredient and formulation, biology of the arthropod, the conditions and mode of use, and lastly, individual user traits. The diversity of variables and their interactions makes the precise measurement of performance difficult, requiring a great deal of empirical effort. Organized testing schemes that control variables systematically are therefore especially useful. Nonetheless, the complexity of host-parasite interplay suggests a priori that protection afforded by even the best active ingredient in an ideal formulation is likely to differ among arthropod taxa and among individual human subjects. Accordingly, comparative studies that examine such interactions should be especially valuable for advancing repellent science.

In spite of these challenges, a number of promising active ingredients and formulation technologies have recently been developed. By identifying the liabilities that influence repellent performance, chances are now better than in the past to integrate the new resources to create superior, longer-lasting, more universally acceptable insect repellents. Laboratory tests are effective for screening purposes and for making comparisons under controlled conditions. Field tests give a better picture of repellent performance in actual use, and highlight the importance of the environment and other conditions of use. Accordingly, this chapter first reviews studies that describe the action and importance of factors that influence repellent performance. It then considers those factors in evaluating recent performance tests of promising deet alternatives. The goal is to present information that is directly relevant to issues faced by contemporary decision makers and to emphasize the importance of recognized variables, the better understanding of which may improve development prospects.

History

Insect repellents have been examined systematically in the U.S. since World War II, when military initiatives, in response to outbreaks of malaria in American soldiers in tropical theaters, were taken up by the U.S. Department of Agriculture (USDA).[5] That work mainly involved the screening of novel active ingredients against caged laboratory populations of *Aedes aegypti* and *Anopheles albimanus*.[6] Ultimately, however, substantial work also assessed factors that influenced the performance of known repellents (principally dimethyl phthalate and deet), particularly with regard to the duration of repellency.[7] Those pioneering studies established the fundamental importance of dosage and rate of loss for determining the period of protection. Among the chief factors they identified as influencing loss were rates of evaporation and absorption that differed among individuals, and abrasion by clothing. Individual attractiveness to a biting arthropod was also important, but gender, hairiness, sweat, and chemical deterioration were thought not to influence repellency.[7] While conceptually robust and comprehensive, most early studies had five or fewer subjects and probably served later researchers more in terms of intellectual guidance than through the specific applications of the results.

In the succeeding four decades, basic research on repellents in the U.S. has continued to be sponsored heavily by the military and the USDA, with emphasis on extending duration. Industrial research over this period has stressed user acceptability and marketing appeal, whereas in Europe the market has more frequently addressed safety. Developing countries seem to stress cost (including searches for natural products). The majority of military work has been conducted with deet and laboratory strains of *Aedes aegypti*, although more recent work includes significant field studies and tests of experimental active ingredients. That initiative includes several studies that compared the original U.S. Army Insect Repellent (75% deet in ethanol, hereinafter "Army 75% deet") to two polymerized deet lotions, specifically the 3M 34% deet formulation currently known as EDTIAR (extended duration topical insect and arthropod repellent) and marketed to the public as 3M Ultrathon®, and the Biotek® 42% deet formulation.* Such work is discussed in detail later in this chapter when the influence of formulation is considered.

Types of Tests—Background

Performance evaluations of repellents fall into two basic classes or design types. In the first approach, developed for field testing, a treated surface is exposed until a conservative, predefined failure event occurs, e.g., the time of the first bite, or the "first confirmed bite" (defined as the first bite that is followed by another bite within 30 min). This approach has the practical advantage of minimizing subject risk from wild mosquito bites. However, its scientific disadvantages include that the data set is truncated and minimized in size, and offers no basis for analyzing or comparing the period of partial protection after the onset of biting. In addition, truncation may inherently oversample that portion of the mosquito population that is most insensitive to the repellent. As pointed out by Rutledge in a number of publications,[8] measurements made of extreme individuals will be less reliable than those taken closer to the center of the population distribution. Depending on biting rates, some of these problems may be partially ameliorated by instead defining effective repellency as the duration of some percentage of protection (e.g., 90 or 95%) relative to the control.

In field studies, an important factor influencing protection time is therefore likely to be the population size of the arthropod.[8,9] Khan et al.[10] and Barnard et al.[11] reached similar conclusions based on experimental manipulations of mosquito numbers in cages. The probability that a test subject will encounter extremely insensitive arthropods will be higher in large parasite populations. Based on these statistical observations, Rutledge et al.[8] recommended the adoption of dose-response test design focused at the more typical portion of the mosquito population. At the median dose (i.e., the quantity required to repel 50% of the test arthropods), the result is essentially independent of the population size. Known as ED_{50} (the minimum effective dosage to repel half of the arthropods), this test design allows much greater precision in the generation of a true estimate of repellent performance because of the inherent mathematics of error around a log-dose/probit curve. It also permits measurement of the sensitivity of different percentiles to population size, and focuses on percentiles of specific interest.

"Minimum effective dosage" design and analysis is employed in laboratory evaluations of inherent repellency where the size of the test population is known. The resulting precision may be especially

* Ultrathon is a registered trademark of the 3M Corporation, Minneapolis, MN; Biotek is a registered trademark of Biotek Corporation, Woburn, MA.

valuable for comparing active ingredients and formulations. To bolster data quality and information content, field evaluations would likewise benefit from more extended records of biting events (i.e., extending the trial past the time of the first confirmed bite). For field testing, an important corollary of the foregoing is that the number of study subjects will directly influence the number of mosquitoes sampled, and thus the effective population size of mosquitoes from which data are collected. The common practice of employing just a few subjects per formulation (below) may therefore give a poor indication of the range of experiences that would characterize a larger sample of subjects. In other words, while analytical precision is gained from ED_{50} laboratory studies by reducing the influence of rare insensitive mosquitoes, field evaluations of effective repellency benefit from the inclusion of exceptional mosquitoes, the avidity of which exceeds the capacity of the repellent to stop them from biting. It is important to sample with sufficient intensity to gauge performance against a large number of potentially biting individuals.

Factors Affecting Repellent Performance

Mosquito Taxonomy and Genetics

The first comprehensive study of the interaction between repellency and mosquito taxonomy was conducted by Travis,[12] who showed that the ranking of protection provided by four repellents was not the same among two *Aedes* and two *Anopheles* species. Rutledge and colleagues conducted both intensive[8] and extensive[13] studies of such interaction. In a study examining deet alone against *Anopheles*, *Aedes*, and *Culex*, the range in ED_{50} was seven-fold.[8] Three species of *Anopheles* ranged from nearly the most, to the least easily repelled as a function of dosage. Even within a species (among ten strains of *Aedes aegypti*), they found significant variation in efficacy. Later, in a comparison of 31 repellent compounds, there was little or no predictability in performance rank across species.[13] Variation in observed repellency between species within a genus was as great as variation between species in different genera. Performance against *Aedes aegypti* was a poor predictor of performance against other mosquitoes, especially *Anopheles* species.

In a series of incisive analyses, Curtis et al.[14] considered the interactions of mosquito species, repellents, and individual subject effects. Six species of mosquitoes from *Anopheles*, *Aedes*, and *Culex* were exposed to six repellents. The ED_{50} of the repellents varied within and among genera by a factor ranging from three to 20-fold. Subjects differed in attractiveness, but not consistently across species of mosquitoes (assessed in the next section). Performance depended on the interaction of subject, repellent, and mosquito taxon. Similarly, Badolo et al.[15] found a repellent-by-taxon interaction in effective dosage of deet and Picaridin against native West African strains of caged *Aedes aegypti* and *Anophles gambiae*. Results from studies such as these discourage the notion that accurate performance generalizations are possible from tests with small numbers of subjects against a limited set of target species.

Finally, Coleman et al.[16] broadened systematic comparisons further when considering the influence of deet, a lactone, and two piperidines against four *Anopheles* species, and two phlebotomines, *Phlebotomus papatasi* and *Lutzomyia longipalpus*. In general, *Anopheles stephensi* and the phlebotomines were the most susceptible to the repellents, and *Anopheles albimanus* was the least susceptible. Beyond those patterns, however, the relationship of performance among all the taxa was highly variable. Note also that deet is not always a superior repellent for phlebotomines.[17]

Given the high intergeneric, interspecific, and intraspecific variation in response to repellents observed in controlled laboratory settings, it is not surprising that the response has a genetic basis. Rutledge et al.[18] established that repellent tolerance in *Aedes aegypti* is heritable, and in the case of deet involves partial dominance (one or a few genes of major effect). Such genetic control could result in an initially rapid phenotypic response to selection for deet tolerance.

Individual Human-Subject Differences

Bernier et al. (Chapter 4) reviewed the influence of human skin emanations on mosquito host location. Gilbert et al.[19] examined the influence of ten "subject variables" on attractiveness and repellency: gender, age, weight, skin temperature, skin moisture production, menses (females), and race, plus hair color and complexion within Caucasians. A remarkable sample size—50 adults of each gender—gave unusual statistical power to analyze subtle effects. The attractiveness tests were conducted in "olfactometer cages," in which *Aedes aegypti* were exposed to air pulled across the surface of the repellent-treated arms of the subjects. The mosquitoes had the option of moving toward the arm and becoming trapped (and counted) as they approached it. Repellency was scored using 5% deet with exposure to mosquitoes at intervals.

Only the effect of gender was clearly and strongly significant. On a proportional scale, the attractiveness of women was just 73% that of men. Only about 5% were more attractive than the male median. So while a few women were highly attractive to the mosquitoes (two of the ten most attractive subjects), all ten of the ten least attractive subjects were female. In terms of repellency, the lower female attractiveness was reflected in a 37% greater mean protection time for females as a group. Nonetheless, there was no significant correlation between individual attractiveness and protection time in either gender, suggesting that other factors are involved in repellent performance.

Among the other factors investigated, subjects with the highest skin temperatures were more attractive or more poorly protected than those at the opposite extreme. Women with the highest moisture production from the skin were also more attractive than the opposite extreme, but that comparison yielded the reverse in men. Neither of these variables correlated with attractiveness or repellency across all subjects in a gender, however. Age, weight, menses, hair color and complexion were all inconsequential,[19] and the number of non-Caucasians tested was insufficient for meaningful interpretation of racial effects. No formal multivariate analyses of the dependent variables were conducted.

Given the clarity of that study's conclusion that women were less attractive and better protected from *Aedes aegypti* by deet, it is intriguing that a recent major study with *Anopheles stephensi* reported the opposite result. Golenda et al.[20] examined the duration of protection by EDTIAR to caged *Anopheles stephensi* in 60 female and 60 male volunteers. Self-dosing was performed by subjects in accordance with product label directions, and the mean rate of application was slightly higher in females (6%), but not significantly different from males. Biting rates on untreated arms were also the same between the sexes. Protection rates (relative to the untreated arms) are shown for each 3-h sample interval in Table 12.1. Women experienced significantly less protection over time than did men.

Examining an additional aspect of subject variation, Curtis et al.[14] reported that each subject's relative attractiveness to mosquitoes is species-specific. Using caged *Anopheles coustani*, *Culex quinquefasciatus*, and *Mansonia* species, they found no predictable relationship between how the biting rate

TABLE 12.1

Comparative Repellency ((1—Biting Rate Treated)/(Biting Rate Control) × 100) of U.S. Military EDTIAR (34% deet) on Male and Female Subjects

	Mean Repellency (%)				
Gender	0 h	3 h	6 h	9 h	12 h
Females	100	99.3	92.8	79.7	66.3
Males	100	100	97.6	91.9	77.5

Source: From C. F. Golenda, V. B. Solberg, R. B. Burge, J. M. Gambel, and R. A. Wirtz, *American Journal of Tropical Medicine and Hygiene*, 60, 654–657, 1999.

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individuals experienced ranked from one species versus another. In addition to the possible effects of skin temperature and moisture,[19,21] or their correlates, such inter-individual variation in attractancy may be influenced by differences in skin surface lipids.[22] Subjects may also vary in repellent performance due to differences in dermal absorption of the active ingredient, which in one study ranged from four to 14% of deet applied in a 15% ethanol solution.[23]

Conditions of Use

Insect repellents are used in nature, where conditions may interact with user activity to influence repellency. It is well known that mosquitoes are most active under particular environmental conditions, and while optima vary among species, warm humid conditions with moderate to low light levels and low wind generally enhance mosquito foraging activity. Within the range of conditions appropriate for mosquito foraging, variation in temperature and humidity may not strongly influence biting rate and repellent performance.[24] Comparatively less is known about the state-dependence of mosquito foraging decisions beyond basic effects of age and parity.[25] For example, nutritional status, as determined by either the larval or adult environment, could influence foraging decisions. In addition, social facilitation (i.e., stimulation to feed by the presence of foraging conspecifics)[26] could in theory increase tolerance to a repellent.

Biting pressure, also known as the "ambient biting rate," is a condition basic to the measurement of repellent performance. This value may be measured in untreated subjects exposed to foraging ectoparasites. Higher biting pressures should correspond, in general, to greater parasite densities and, in nature, larger local population sizes and relatively fewer alternate sources of blood meals. Under high biting pressure conditions, repellents are likely to fail sooner because the encounter rate with the least sensitive foragers in the population will be great enough to cause failure based on absolute (e.g., first confirmed bite) rather than relative (percent biting reduction) criteria.[8] Similarly, efficacy tests with large numbers of subjects may sample more such insensitive mosquitoes, and perhaps even more on a per capita basis should group size enhance the detectability of hosts to parasites. Moreover, the availability of alternative host individuals may affect mosquito biting behavior and thus repellent performance. Repellents may be more effective when mosquitoes have the simultaneous option of choosing a more attractive host.[14] All of these basic factors should influence test design and conduct, but their importance may differ across mosquito species and conditions.

Studies have also shown a number of more specific, user-mediated, proximate conditions that influence repellent performance. As is typical, most experimental data available are for deet formulations. Conditions of actual use that may reduce the duration of protection include contact with water, sweating, and abrasion by clothing or vegetation.[7,27-29] Rueda et al.[29] reached two main conclusions regarding the interaction of repellents and clothing. First, abrasion of treated skin by clothing fabric can significantly lower the protection afforded by a repellent. Second, the amount of friction between skin and fabric was increased by the presence of a repellent on the skin. This increase in friction likely aggravated the rate of its loss to the fabric. This study was conducted using the U.S. military polymer based extended duration deet formulation (EDTIAR). The generality of the results has not been explored with other formulations or active ingredients.

Volatilization may be one of the most important variables, as it accounts for a major fraction of repellent loss from the skin.[30] In consequence, subject-caused differences in the rate of volatilization (whether related to physiological or activity differences) should be an important determinant of individual variation in repellent efficacy. However, no research appears to have directly examined the relationship between volatilization and repellency beyond the basic studies of Rutledge et al.[30] Costantini et al.[31] used the Rutledge method to model evaporation differences among repellents based on efficacy data, but they did not measure volatilization directly. Likewise, the extent to which conditions of use influence dermal absorption appears not to have been quantified.

Formulation Chemistry

Even within the standard test model of deet and *Aedes aegypti*, substantial variation in protection has been reported for decades.[19,32,33] Given the many variables likely to underlie unexplained performance variation, Buescher et al.[33] reasoned that illuminating basic physical properties of repellent persistence could provide an important baseline for sensible repellent design. Using deet at a series of dilutions, they computed a dose-response curve describing the influence of concentration on the duration of 95% protection against caged *Aedes aegypti*. The curve is negatively exponential, meaning that each increase in concentration provides a progressively smaller increment in protection. Their main conclusion was that the Army 75% deet formulation achieved little added protection compared to, for example, a 50% concentration. This is a significant finding because use of lower concentrations would reduce deet's plasticizing effects and toxicological risk values.

While the importance of volatilization in limiting repellency duration was understood when the Buescher et al.[33] report appeared in the mid-1980s, it is likely that formalizing the dose-response relationship laid the foundation for a more analytical approach to designing extended-duration formulations that would deliver sufficient molecules for repellency over a predictable time span. Nonetheless, attempts to manipulate the chemistry of repellent carriers, whether through blending with a polymer or microencapsulation, to control volatilization (and dermal absorption at the same time) have met with mixed success.

High volatility is likely to both enhance repellency and evaporation, leading to ephemeral protection. In the face of this tradeoff, Reifenrath and Rutledge[34] investigated the impact of numerous silicone polymers on the efficacy or protection time of deet against *Aedes aegypti* using dogs and mice. There was little influence in the dogs, and while 40% of the polymers increased performance in the mice, the changes were not large. Mehr et al.[35] examined controlled release polymers and starch microencapsulation of deet using the same mosquito species on white rabbits. Some increased duration of efficacy significantly, but none achieved better than 80% protection at 12 h. The efficacy results of a field test by Gupta et al.[36] that compared the Army 75% deet repellent with two candidate extended duration polymer formulations (Biotek with 42% deet and EDTIAR with 34% deet) are not interpretable for our purposes here, but important information on dosing did emerge. Ad libitum self-application resulted in an inverse relationship between deet concentration and the total amount of each formula applied, so that the mean quantity of deet applied differed little between the three products.

This same inverse dosing relationship characterized a laboratory test of the same formulations against *Aedes aegypti, Aedes taeniorhynchus, Anopheles stephensi,* and *Anopheles albimanus* by Gupta and Rutledge.[24] With a total of three subjects in three simulated climates, Biotek provided 94.9% protection, and EDTIAR 94.8% protection, from bites of all mosquito species in a series of exposures over 12 h. These values were superior to the 82% protection afforded by the Army 75% deet in ethanol. Enhanced performance in the polymerized formulations may stem from a combination of reduced volatilization and skin penetration.[37] Interestingly, Gupta and Rutledge[24] concluded that the EDTIAR was the best formulation because the performance of Biotek was "at best similar or less than that provided by the 3M formulation," an assessment not consistent with the means they reported (above). In addition they cited the advantage of EDTIAR having the lowest deet concentration, but given the observed dosing (mean Biotek 0.9 mg/cm^2, mean EDTIAR 1.1 mg/cm^2), more deet was actually delivered when the EDTIAR was applied. Overall, in spite of the excellent general design of this study, the use of only three study subjects limits the value of assessing the results at any greater level of detail or generalizing strongly from them.

Two more recent studies, using laboratory rabbits and deet, have yielded clearer and more positive results concerning formulation and duration. Rutledge et al.[37] tested eight polymer and nine microencapsulated formulations. Against *Aedes aegypti* and *Anopheles albimanus*, several were more effective than unformulated deet at the same concentration for periods of up to 24 h. The best performance was with a polymer containing a high molecular weight fatty acid, and with microencapsulated formulations containing a diversity of large molecules, including lanolin, gums, acids, and

polypropylene glycol. In a study with argasid ticks, Salafsky et al.[38] reported that a liposomal formulation designed to reduce volatilization and dermal absorption extended the duration of repellent protection. In a three-day trial, attachment to a finger treated with liposomal deet was absent or significantly reduced compared to an equal concentration of deet in isopropanol, sampled at 24, 48, and 72 h. Given the difficulty of preparing stable polymer formulations of deet, refined alternatives, including microcapsules and liposomes, should be considered for tests with other active ingredients and biting arthropods as well.

Active Ingredients and Their Efficacy Assessment

Active ingredients are the focus of most repellent development programs, and their efficacy is assessed through cage and field testing. The history of deet and other prominent repellents such as dimethyl phthalate is treated by Moore and Debboun in Chapter 1 and Strickman in Chapter 22. While it is accurate to state that a variety of subject factors and their interactions with other variables influence repellent performance, the review in the foregoing sections shows that the precise nature of those factors is poorly understood. At present, the chief manner in which the influence of such uncontrolled variation can be moderated (and studied) is by conducting tests with large numbers of subjects.

This section reviews recent laboratory and field performance trials of promising non-deet repellents currently marketed in the U.S. and Europe. The goal is to apply insights gained from the foregoing review to evaluate how factors that influence repellent efficacy have been controlled and coordinated. Studies considered are mainly those treating Merck IR3535 (3-[*N*-butyl-*N*-acetyl]-amino proprionic acid, ethyl ester), Lanxess Picaridin (aka KBR3023, (1-(1-methyl-propoxycarbonyl)-2-(2-hydroxy-ethyl)-piperidine), and PMD (*para*-menthane-3,8-diol, which is the prime repellent constituent of the U.S. EPA-registered active ingredient "oil of lemon eucalyptus," from the tree *Corymbia citriodora*). These active ingredients were developed much more recently than deet; all are registered by the U.S. EPA. Most studies compare them to some type of deet standard. Given the variety of contingencies that apply to the performance of deet even under controlled conditions against well known mosquito taxa, it is worth examining how well conditions have been accounted for in tests of active ingredients that are less well studied. Frances (Chapter 18), Strickman (Chapter 20), and Puccetti (Chapter 21) also treat these three active ingredients in detail.

Laboratory Efficacy Comparisons

The most widely referenced recent study of comparative mosquito repellent efficacy was conducted with caged *Aedes aegypti* by Fradin and Day.[39] Their goal was to compare commercial deet products at various concentrations with plant-based repellents and IR3535 at 7.5%. Two lotions with at least 20% deet protected subjects for an average of 4–6 h (time to first bite), and most other formulations provided protection for well under 1 h. The authors concluded that "only products containing deet offer long-lasting protection." The design was comparatively strong in terms of the number of test subjects (15), but the study had at least two apparent weaknesses. First, dosage was not reported and perhaps not closely controlled. Second, repellents that performed well in a subject's first exposure were tested at less frequent intervals in the second and third exposures (apparently for convenience), adding a bias that probably exaggerated true differences among the products. Despite those shortcomings, the performance differences were large enough to suggest that conclusions were generally accurate.

A substantially different picture emerged in the next broad-based cage study,[40] which included more effective commercial deet alternatives. Three mosquito species were tested separately: *Culex nigripalpus*, *Aedes albopictus*, and *Aedes triseriatus*. Results for the four most effective products are highlighted in Table 12.2. Most remarkably, given deet's five decade reign of superiority in such testing, overall repellency of the non-deet active ingredients was either consistently slightly greater (in the case of PMD), or equivalent to, 15% deet. However, for comparative purposes it is unfortunate that the highest deet concentration tested was only 15%.

TABLE 12.2

Mean Protection Time[a] (SE) (hours) for the Four Most Effective Repellents Studied in the Laboratory by Barnard and Xue[40]

Product	*Aedes albopictus*	*Culex nigripalpus*	*Aedes triseriatus*
Repel® (19.5%[b] PMD)	7.8 (0.2)	7.3 (0.7)	7.8 (0.2)
Bite Blocker® (2% soy oil)[c]	5.5 (1.3)	8.3 (0.2)	7.8 (0.2)
Autan® (10% Picaridin)[d]	5.7 (0.9)	8.0 (0.0)	7.8 (0.2)
Off!® (15% deet)[d]	7.2 (0.8)	7.0 (0.6)	7.3 (0.3)

[a] Time to second bite in one or two sequential periods.

[b] Corrected from Barnard and Xue[40]; a registered trademark of Wisconsin Pharmacol Co., Inc., Jackson, WI.

[c] Methylated soy bean oil; a registered trademark of HOMS, LLC, Clayton, NC.

[d] Registered trademarks of S.C. Johnson and Son, Inc., Washington, DC.

Source: From D. R. Barnard, and R. D. Xue, *Journal of Medical Entomology*, 41(4), 726–730, 2004.

Strengths of that study include that the repellents were applied at a standard dosage (1 mL/650 cm^2 of skin surface), and tested against a high density of avid mosquitoes. However, an important weakness was that only two subjects tested each repellent, out of a total of five subjects. Because individuals differ inherently in their attractiveness to mosquitoes and dermal interaction with repellents, and both factors interact with mosquito taxon, a substantial portion of the variation reported may be from uncontrolled subject error.

Cage studies against *Anopheles* vectors of *Plasmodium* (malaria) likewise showed PMD[41–43] and Picaridin[15] to be at least as effective as deet formulations. The first three tests had six or fewer subjects and uncontrolled or unspecified dosing.[41] Badolo et al.[15] also found Picaridin to be more effective than deet against an African strain of *Aedes aegypti*, but the number of subjects and biting pressure were not reported. Data in Carroll and Loye[44] suggested that 19.5% PMD was intermediate in performance between ten and 30% deet products against *Aedes aegypti* over an eight hour period (eight PMD subjects, one subject for each deet formulation, with equivalent dosing and biting pressure of 50 bites/min on untreated arms). All of these studies would benefit from larger samples or more complete reporting. One major benefit from more replication would be more realistic comparisons between separate studies.

There have been fewer studies of IR3535 at higher concentrations than the basic 7.5% Avon formula (above), but there is an indication that efficacy improves. At 20% IR3535, a study of three subjects at high biting pressures by Thavara et al.[45] found IR3535 comparable to 20% deet against two *Culex* and one *Aedes* species, but less repellent against an *Anopheles* species.

Field Efficacy Comparisons

Most field efficacy trials share problems common in laboratory trials, including small numbers of subjects, lack of repetition, uncontrolled dosing, and unclear ambient biting rates. As a result, characterizing the repellency of a given active ingredient across taxa, and comparing it with other active ingredients, is difficult to do at a suitable level of precision.

One of the most thorough and thoughtful studies of contemporary active ingredients was conducted by Costantini et al.,[31] measuring dose-response curves of deet, Picaridin and IR3535 against *Anopheles gambiae* complex mosquitoes in Burkina Faso. Eight male subjects tested a series of dosages of the technical grade repellents diluted in ethanol. Apparently each repellent was tested on 96 nights (12 times by each subject). Testing was performed over the ten hour period 18:00–04:00 with a two hour break from 22:00–00:00. Four dosages (in ethanol) were tested, specifically 0.1, 0.3, 0.6, and 0.8 mg/cm^2 of each active ingredient. For comparison, standard volume for efficacy testing in the U.S. is ca. 1.54 mg/cm^2, so that for a 30% (high) concentration active ingredient, dosing would equal about 0.5 mg/cm^2 of active ingredient. The two higher doses in this study were thus greater than those intended for most military or

popular commercial formulations in the U.S. Picaridin performed best against the anophelines in this study, with an estimated 95% or more repelled for at least 8 h at the three higher dosages. Deet's performance was intermediate, and IR3535 was the least repellent at all dosages. These results are important because even though deet is historically the best repellent against anophelines, public health professionals have long recognized the need for a better repellent against these important vectors of the pathogens causing malaria. The 0.3 mg/cm^2 dosage corresponded to a 20% Picaridin formulation, the maximum concentration that is registered for use in Europe and Australia. Costantini et al.[31] provide some of the first evidence of a repellent lasting for such a long period against *Anopheles gambiae* (see also Trigg[46] below for PMD performance).

As in other studies, however, caution is in order. First, in spite of the unusually long duration of the study (six months in total), which yielded an unusually large data set, just eight subjects were involved, and only local populations of *Anopheles gambiae*. Second, although samples for other mosquito taxa were small, Picaridin did not repel *Aedes* species better than the other repellents. Third, while control subjects collected a large number (27,231) of alighting *Anopheles gambiae* during the study, arithmetic shows this to be a low ambient biting rate for the study: less than 0.3 per minute (27,231 bites/92,160 min). For perspective, current U.S. EPA guidelines call for a minimum of 1 bite/min on a lower limb (feet and hands excluded), more than three times greater than the observed rate. So while the strength of this study is that it was conducted under representative (long-term) conditions, and low biting rates may be medically important when infection rates in mosquitoes are high, it would still be valuable to have performance data at higher biting rates. Lastly, data from women are clearly merited.

Even at such low biting rates, Picaridin may fail quickly against anophelines. Frances et al.[50] tested 19.2% Picaridin (Autan Repel Army 20) against 20% deet in ethanol and 35% deet in a gel (the repellent issued by the Australian Defense Force) against *Anopheles meraukensis* and *Anopheles bancroftii* in Australia's Northern Territory. At control biting rates slightly under 0.5 bites/min, 35% deet and Picaridin protected at more than 95% over the first hour, but by the second hour repellency dropped to 78% for Picaridin, and declined variably in all three repellents thereafter. Those data were collected by four subjects, all male, with each testing a repellent or ethanol control twice over eight consecutive nights. Dosage appears to have been ad libitum, determined by the subjects at the time of application. By weight, one can calculate that Picaridin was applied at an average rate 31% higher than the 20% deet, and 45% higher than the 35% deet. In this latter case, only about 25% more deet than Picaridin was actually administered (estimated from Table 12.1 of Frances et al.[50]). The rate at which formulated Picaridin was applied averaged 13% higher than standard procedure for a U.S. repellent efficacy test (1 mL/650 cm^2 of skin surface).

Other field tests of Picaridin against anophelines are similarly plagued by small samples or low ambient biting rates (<0.5/min, e.g., Yap et al.[47,48]), but still suggest its promise as a broad-spectrum mosquito repellent. In the single test conducted at high ambient biting rates, Barnard et al.[49] compared 25% ethanol solutions of technical deet and IR3535, and Picaridin, and PMD at 19.5% in a commercial lotion (not 40% PMD as indicated in the source publication; see Carroll and Loye[44]). Five males exposed treated limbs for 3 min each hour for 6 h, beginning 15 min after application. The test was repeated five times over three days so that each subject tested each repellent and served as a control (25% water in ethanol) once. Black salt marsh mosquitoes (*Aedes taeniorhynchus*) attacked control subjects at a high average rate of 19.5 ± 13.7 bites/min. Given the small number of subjects, statistical power was low, but Picaridin and deet appeared to be the most repellent, followed by PMD and then IR3535. Only Picaridin repelled at greater than 95% through hour five.

The efficacy of PMD against anophelines appears noteworthy. Using six self-dosed subjects exposed to *Anopheles gambiae* in rural Tanzania, Trigg[46] compared 50% PMD to 50% deet under low ambient biting rate conditions (apparently 0.13/min, calculated from grand mean of controls over the 240 min exposure period, Trigg's Table 1). Repellents were applied 5 h before the onset of exposure. Deet prevented all biting on six subjects for close to 7 h, and PMD for 6–8 h, depending on formulation. Moore et al.[50] collected similar data for *Anopheles darlingi* in Bolivia, but tested only 2–4 h after application. PMD (30%) reduced biting on five subjects by a mean of 97%, while 15% deet in ethanol gave just 85% protection. Compared to other studies of anophelines, ambient biting pressure was respectably high, greater than or equal to 1 bite/min (estimated from the mean percentage biting rate reductions of the test products, including 0% for the control, and the total number of mosquitoes captured landing). Variation in the performance of Picaridin among anophelines (e.g., Frances et al.[51] above) suggests that PMD, too should be tested against more anopheline species, using controlled dosing on more study subjects than in the foregoing studies.

In a six hour field study of PMD with a large number (20) of adult male and female subjects exposed to *Aedes melanimon* and *Aedes vexans* in California, Carroll and Loye[44] found excellent protection with continuous exposure of lower arms and legs at mean biting pressures of approximately 1.5 and 3 per minute, respectively. Subjects tested lotion (19.5% PMD) and spray (26% PMD) formulations at dosages of either 1.6 or 2.4 mg/cm^2. Mean biting rate reduction for all treatments over the 6 h was 99.9%. Protection provided by 20% deet lotion was similar, but only two subjects tested deet.

Other than Barnard et al.[49] field studies of IR3535 at higher concentrations are rare. Thavara et al.[45] compared IR3535 and deet at a rate of 20% in ethanol with six subjects against several mosquito species at low biting rates. In two 8 h field studies of *Aedes albopictus* at ambient biting rates of about 0.35 bites/min, there were no bites from this species on subjects using either repellent. IR3535 reduced biting by a mean of 98.4%. Deet reduced biting by 97.4%. The authors' claim that the difference, statistically significant at $P<0.05$, is inconsequential, however, given the similarity of the means (see Table 12.1 of referenced study). Protection in similar five hour studies against night-biting *Culex*, *Mansonia* and several *Anopheles* species (ambient biting pressure 0.15–0.25 in the last genus) averaged 98% and greater for both repellents.[45] Doses were approximately double the standard. Like studies of other promising repellents, work on IR3535 would benefit from greater standardization of protocols, more subjects, and higher biting rates.

Conclusions

The task of generating predictable, generalized results from insect repellent efficacy tests is challenging. The basic difficulty is in the effort to generate and deliver chemicals that will interrupt the feeding behavior of highly diverse and refined biting arthropods without harming the user. Even among apparently safe and effective repellent candidates, however, this review demonstrates that the interplay of host, arthropod, environment, and utility significantly controls performance. We have a better idea of what classes of variables are influential than we do of how to predict the impact of a particular variable in any given case.

For each active ingredient, the basic three-way interplay between subject, formulation, and mosquito taxon appears to be the principal source of variation in the outcomes of efficacy tests. It is typically an uncontrolled source of error that hinders all attempts to analyze additional conditional factors (e.g., environment, use). Because this axis of interaction is poorly described, the precision of performance estimates is generally questionable.

For practical reasons, most studies have attempted to assess variables one or two at a time. From these we can begin to list factors that should be included in improved models of repellent performance. For example, it is likely that variation in skin emanations, including lipids,[22] moisture and heat[19] affect attractiveness, and that variation in dermal absorbency (three-fold for deet[23]) affects protection time. These results can be linked to build a nascent picture of subject variation. At the same time, however, two large-scale studies of gender that both reported highly significant and substantial effects produced strikingly dissimilar results: Gilbert, Gouch, and Smith[19] found that females were clearly less susceptible to biting by *Aedes aegypti* in a test of 5% deet in alcohol, while Golenda et al.[20] found that females were clearly more susceptible to biting by *Anopheles stephensi* in a test of EDTIAR. So while we can state that

gender is an important consideration for repellent performance, at present, uninvestigated interactions between gender, repellent formulation, and mosquito taxon prevent us from offering further direction.

The problems of inconsistency in the design, execution, and reporting of efficacy studies likewise hinder the effort to evaluate repellent performance. In all of the studies reviewed that compare active ingredients, sample sizes are too small to permit confident distinction among treatments with moderately close performance values. Even when statistical significance is shown, differences cannot necessarily be attributed to the repellents alone, and peculiarities of individual interactions may be paramount. Note, for example, that many studies have used only male subjects. Dosage is another factor of obvious importance[33] that is too often uncontrolled.

The use of limited numbers of subjects to test repellents probably has its justifications in the desire to minimize risk, the difficulties associated with recruiting people for this type of work, the use of the first confirmed bite criterion (a threshold measure), and perhaps also in the history of testing deet, for which relative variation was apparently regarded as inconsequential due to its outstanding comparative efficacy. This tradition is reflected in guidelines for efficacy testing proposed by the U.S. EPA, requiring just six subjects for the generation of registration data. We have entered a new era in which there is for the first time an interest in comparing several repellent active ingredients, all of high efficacy. How shall they be distinguished?

Rutledge and Gupta[52] determined by meta-analysis of published studies that the standard deviations of protection times are a linear function of the means. As a result, the statistical differentiation of long-acting formulations in particular will probably require especially large samples. It is unfortunate that $n = 20$, the minimum acceptable sample size for parametric hypothesis testing at alpha-levels of 0.05, is not the norm for repellent studies. While the Rutledge and Gupta[52] estimate of required n's is likely inflated by interstudy variation beyond that relevant to any given comparison (e.g., of two formulations tested simultaneously), their study does give the impression that even 20 subjects per formula might be too few. Nonetheless, an agenda to deploy large, balanced groups of subjects to test various repellents against various mosquitoes would likely advance repellent science substantially.

The complementary perspective is to accept that separating the performances of candidate deet-replacements is a futile exercise. A positive outcome of that view might be to open the door more readily to inclusive strategies, such as combining active ingredients to see if, for example, variance in performance can be limited. Reducing variance is important because we tend to rely on the mean protection period when evaluating performance. Any subgroup of people that is less protected than average will be systematically less protected than is otherwise assumed.

In addition, it is sensible to make inferences from the results of many different studies that involve the same repellents. For example, Picaridin seems to be especially efficacious in many of the studies reviewed here. Meta-analyses of such data sets potentially have the added advantage of treating data from tests conducted in a variety of conditions against a variety of mosquito taxa. At the same time, the present suite of studies available seems to share inherent biases (male subjects) and serious inconsistencies (very unequal dosing) that obscure their value to objective analysis. If we coordinate and more thoroughly standardize the conduct and reporting of repellent studies in the future, interested scientists, health professionals, and the public will all benefit from the resulting increase in available knowledge.

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