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NATURAL-BASED REPELLENT PRODUCTS: EFFICACY FOR MILITARY AND GENERAL PUBLIC USES

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INTRODUCTION

The first use of repellents as a means to avoid the bites of noxious mosquitoes and other biting arthropods was probably done by prehistoric man when they found that sitting next to a smoke-generating fire tended to provide a degree of protection. This method is still used in many places by remote peoples and at times by modern man when camping out in natural areas. Today, even the most effective insect- or mosquito-abatement programs cannot totally eliminate the nuisances caused by these blood-seeking arthropods (Novak and Lampman 2001). Therefore it is necessary at times to employ personal protection to help avoid insect bites. There are a wide variety of methods that people can employ to help avoid noxious insects by establishing a mechanical barrier, like bed-nets, screened windows, and clothing. In the U.S.A., and to a large degree in Europe and Japan, the primary barriers are hermetically sealed and air-conditioned houses. Although clothing can be used to help avoid unwanted insect bites, such as loose fitting long-sleeved shirts and pants, the public tends to expose as much skin as possible during the hot, humid summer months, when these insects are at their highest densities. Clothing can, in fact, be a very practical and efficient way to avoid these pests.

The objectives of this article are to define and discuss the differences between natural or herbalbased repellents with synthetic chemical-based repellents. In addition, we will discuss how to determine efficacy of repellent activity using cage- and field-testing protocols. We will also present a brief discussion of those repellent methods that do not work, especially those that have recently inundated the market place.

BRIEF HISTORY OF REPELLENTS

Prior to World War II, a number of plant products, generally oils and other substances derived from specific plant species, were the mainstay repellents, applied to either skin or clothing to avoid biting arthropods. The first methods man used to repel insects was with smoke, covering the skin with mud, or by applying a variety of animal fats and greases. Native Americans rubbed cedar tree needles or used the fat from bear to prevent the nuisances caused by insects. Some wore various herbs and poultices around their necks or on their clothing. The first written records of repellents against insects occur in classical Roman literature by Pliny, the naturalist, and by Dioscorides (40-90

AD), a Greek physician who described how worm-

wood (Artemisia absinthium) could repel gnats and fleas.

Oils extracted from plants or plant parts with a variety of volatile compounds (Tables 1 and 2) that repelled insects were the principal means that people used for protecting themselves or their domestic animals prior to World War I, which was the advent of synthetic chemical repellents. Oil of citronella was one of the most widely used early repellents, being introduced around 1882. It was registered in the USA as McKessons Oil of Citronella and was used for human application to repel both mosquitoes and gnats. Other essential oils that were used during this time were anise, bay laurel, bergamot, cassia, cedar wood, eucalyptus, and wintergreen.

In 1929, dimethyl phthalate, one of the first synthetic repellents, was reported to be effective against house and stable flies. Grannett (1940) was instrumental in developing ethyl hexandiol, also know as Rutgers 612. Grannett also developed reproducible methods to evaluate and test repellent compounds. In 1937, indalone, dimethyl carbate was synthesized at Rutgers University and reported to be an effective repellent.

During World War II, it became necessary to develop effective and long-lasting insect repellents, primarily mosquito repellents, to help military troops avoid those biting insects that transmit pathogens causing debilitating diseases such as malaria, dengue, and typhus. Just prior to World War II, the U.S. Department of Agriculture established a laboratory at Orlando, FL, which was later moved to Gainesville, FL, which tested thousands of potential repellent compounds. During the war, the standard military repellent was 622. This repellent contained 6 parts dimethyl phthalate and 2 parts each of ethyl hexandiol and indalone. After World War II, the standard military repellent applied to the skin of soldiers was called M-2020, which was a mixture of 40% dimethyl phthalate, 30% ethyl hexandiol, and 30% dimethyl carbate. The standard repellent applied to clothing was M-1960, a mixture of 30% benzyl benzoate, 30% n-butylacetanilide, 30% 2buty1-2-ethy1-1,3propanediol, and 10% TWEEN 80.

Diethyltoluamide (DEET), developed by Mc-Cabe in 1954, is one of the very best mosquito repellents developed (Rutledge et al. 1996, Elston 1998) for mosquito control. It has been the gold standard for public health threats and emergencies as well as for use in military operations. During the recent outbreak of West Nile virus in the USA, DEET is the repellent of choice and has been part JOURNAL OF THE AMERICAN MOSQUITO CONTROL ASSOCIATION

Name	Derived from	Active ingredient	Species
Citronella oil	Cymbopogon (grass)	Terpines, alcohols, aldehydes	Biting flies
Geraniol coeur	Geraniol	Geraniol, nerol, citronellol	Biting flies
Quwenling	Lemon eucalyptus	p-Methane-3, 8-diol	Mosquitos
Neem	Neem tree	Many compounds	Biting flies
Soy beam oil	Soy bean plant	Lecithin base, many compounds	Biting flies
Thujic acid	Western cedar	Diethylamide	Aeges aegunt
Tarweed extract	Hemizonia sp.	1, 8-Cineole (eucalyptol)	Ae. aegypti
Palmarosa oil	Cymbopogon martinii	Many compounds	Anopheles sp.

Table 1. A brief list of natural or herbal products, by name, what they are derived from, the active ingredient or ingredients, and insects for which it has been shown to have repellent activity.

of the standard recommendation for general public use by the Centers for Disease Control and numerous state public health departments. Military forces or public health workers exposed to vector-borne diseases must have the means to protect themselves for long periods of time. In addition, insect pest populations may exist in such large numbers that they may interfere with military operations, requiring a long-lasting and durable repellent.

Military operations and public health emergencies normally do not occur in backyards of suhurbia or at other outdoor recreational areas, where shorter duration and less harsh repellents may be satisfactory. In recent years, there has been a tendency toward the use of natural agents or phytochemicals (Tables 1 and 2). Some of the so-called natural products, or, better described, herbal products are listed in Table 2. Those compounds that have shown specific repellency to insects are briefly described in Table 1.

A complete list of phytochemicals and essential oils reported to have repellent activity toward vectors and other arthropods is beyond the scope of this review. The list (Table 2) contains chemicals that have been reported in the literature as repellents, although, in some cases, their efficacy is unsupported (e.g., the use of Vitamin B1). We have made no attempt to rate their effectiveness based

 Table 2. A partial list of natural products and essential plant oils with repellent properties.

Natural products ide	entified		
Limonene	Vitamin B1	Cineole	
Li nalool	Geraniol	Quwenling	
Camphor	Rotundial	Thurjic acid	
Eucamolol			
Plant essential oils			
Neem oil	Garlic oil	Birchwood tar	
Aniseed oil	Thyme oil	Soybean oil	
Geranium oil	Eucalyptus oil Nutmeg oil		
Bergamot oil	Pyrethrum	Pine oil	
Lavender oil	Coconut oil	Clove oil	
Orange-blossom	oil Penny-royal oi	l Cinnamon oil	
Peppermint oil	Citronella oil	Palmarosa oil	
Castor seed oil	Lemon oil	Indian privet	
Tarweed on the lit	terature and they	are presented	

products claimed to have repellency. In

general, natural products are considerably less effective than synthetic repellents, like DEET, at equivalent concentrations. Several chemicals and essential oils do provide relatively short periods of mosquito repellency (Rutledge and Gupta 1996).

There are several newly formulated repellents that have recently come to the market and may illustrate the future trend in repellent research. Merck Company has shown that 3-(N-butylacetamino)propionate, called Merck 3535, has strong repellent action against biting flies and mosquitoes (Marchio 1996). KBR 3023, a Bayer product, which is a piperdine derivative (piperidinecarboxylic acid, 2-(2hydroxyethyl)l -methylpropylester), is also very effective in repelling biting flies and mosquitoes. A compound called PMD is similar to quwenling, but is derived by a novel extraction process. The active ingredient is p-methane-3, 8-diol combined with isopulegol and citronella. PMD has been shown to provide 5 h complete protection when tested against Anopheles funestus and Anopheles gambiae. Though permethrin, a pyrethroid, is not technically a repellent, it is used in a manner similar to repellents. Permethrins are used to impregnate military uniforms, clothing, and bed nets.

AGENTS: NATURAL VERSUS CHEMICAL

With the advent of more natural-product repellents, it is important to define the specific nature of the repellent action. First, many potential natural repellents can be considered a barrier to the insect, preventing either landing or penetration of the skin. In many cases, these barrier compounds can be skin lotions or sun screens. However, it is important that these barrier repellents are field tested with the same rigor as volatile repellents in order to establish product efficacy. Volatile compounds rely on vapor pressure and temperature to release specific chemicals that insects avoid. In fact, the operating definition of a volatile repellent is that its "Efficacy of a repellent product is based upon the vaporous stage of the repellent to prevent bites." In other words, a minimum vapor pressure is required to

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maintain the efficacy of a repellent. Most repellent products on the market today use various delivery mechanisms that either control the evaporation rate of a repellent for long-term repellency or as the carrier agent disintegrates it releases repellent vapors to prevent bites. This is a highly desirable characteristic of most current and future repellent active ingredients. Based on the operating definition, one of the key differences between these 2 types of repellents is the experimental design critical to establish efficacy. With barrier repellents, an insect may land but not be able to bite. This change in behavioral capability must be noted in the experimental design because a land bas a different meaning when testing a volatile-based repellent.

One of the other important differences between chemical- and natural product-based repellents is the duration of repelling activity. This question has been somewhat resolved by the Environmental Protection Agency (EPA) by setting a 2-h minimum activity requirement of a repellent in order to gain registration. It is well established that several formulations of DEET can give up to 6 h complete protection. To date, there is no natural product that can give that duration of control. However, due to potential human-safety factors, it is recommended that DEET-containing products not be reapplied after the first application. Also, it is not recommended that DEET be combined with skin lotions or sun screens because these products are generally reapplied by the user. This is the big difference between DEET and natural products, which can be reapplied safely, thus compensating for the shorter duration of repellent activity.

The number of insects biting plays a major role in not only testing a repellent but in the marketing of a product. Most marketing commercials tend to use the extremes in terms of insect population numbers. In fact, in most of these advertisements, the insect numbers greatly exceed what the typical lay person would ever encounter or would remain in those circumstances. The recommendation of the EPA's Scientific Advisory Panel (SAP) for biting pressures appropriate for testing should be based primarily on what the general public perceives as a nuisance problem. Because little information is available in the literature, experience of members of the SAP coupled with a publication by Morris and Clanton (1988) titled "Quantification of a nuisance mosquito problem in Florida" were used as guidelines. It is important to remember that repellents are used almost wholly for nuisance problems, not disease problems. Therefore, it follows that the guidelines used regarding biting pressure reflect conditions that impact the general public and not military or public health personnel. Based on these parameters, the SAP recommended the following biting rates for field testing insects and thus establishing minimum nuisance thresholds of: mosquitoes 1 bite per min; ceratopogonids at 1 bite per 5 min; tabanids at 1 bite per 5 min.

When a natural product- or herbal-based repel-

lent is developed, it is important to take into consideration that it will be used by the lay public for nuisance management, not for military or public health emergencies. Military and public health workers use repellents in a different manner and under completely different conditions than those that the general public should or would encounter. In fact, Gupta and Rutledge (1994) state that the use of repellents to reduce human/vector contact and reduce the transmission of mosquito-borne diseases has not been scientifically proven. The 1998 U.S. Center for Disease Control and Prevention (CDC) report concluded that human disease can be effectively reduced by active surveillance systems and appropriate mosquito-control measures, but repellents are not mentioned as a specific means of prevention. Several factors concerning the general public have to be taken into consideration when developing and testing a natural repellent product. The most effective insect repellent will not provide a uniform level of protection to all users under all conditions. Individual factors that can affect the degree of protection afforded by the repellent are 1) Does the person apply the repellent properly? 2) Individual susceptibility to mosquito bites, 3) perspiration, 4) personal product that may enhance or detract from the repellents effectiveness, and 5) environmental factors, such as wind, temperature, humidity, mosquito species, age, and density. Because repellents are personal protection, the application is totally dependant on the user and, unless an individual actually dips him or herself in the repellent, complete body coverage cannot be guaranteed. Also there is a great deal of variation among individuals on the effectiveness of a repellant, thus "one size does not fit all."

DETERMINATION OF EFFICACY

Cage studies

In regard to the utility of cage studies to test repellent efficacy, the findings of the EPA SAP should be followed. The SAP strongly recommends that only field studies should be used to establish efficacy and registration. Cage studies are not a valid substitute for repellent field studies. Cage tests should be used only as a screening device and should not be submitted in support of a registration. They should, however, be used by the manufacturer to screen possible repellents, develop formulations, and determine a range of application rates. The Klun and Debboun (2000) device may be an alternative to the device specified in the American Society for Testing and Materials (ASTM) standard for laboratory studies of mosquitoes (ASTM 2000; 951-94), remembering that it is still a screening tool that was never intended as a substitute for field studies. However, if a test cage with an enclosed area, such as Klun and Debboun (2000), that does

not provide for free flow of repellent vapors from the surface and eventual dissipation of repellent vapors into the immediate environment, it is probable that some repellents may have erroneously indicated higher repellency. Any laboratory test cage selected for product testing should take vaporous state of repellents into account before being recommended for use.

Application amounts (dosage)

The amount of repellent to be applied to the skin should be determined by the registrant or manufacturer. There are several reasons that justify this statement. The amount applied is determined by weight, which makes it very difficult to determine application rates of aerosols. Therefore, because most repellents are liquids, creams, or aerosols, the application rates should be in milliliters or in seconds of spray time for aerosol. Also, the test area for application of 600 cm² is too large an area for many arms. A test area of 250-300 cm² is more than appropriate.

The amount of the repellent to be tested should be determined by the registrant. This could be determined by scientifically conducting statistically valid studies that demonstrate the quantity of a given physical formulation consumers are likely to apply. Apparently, some of these data already exist in the cosmetic industry. Where they do not exist, repellent manufacturers should conduct them. EPA could, and probably should, serve as a repository for this information. The dose rate per unit could then be established through prefield tests using cage tests. The rationale for this is that there are and, in the future, will be numerous new products that do not fit the synthetic chemical repellent mode of action. We are already seeing this with the number of natural repellents and many new products that have multiple purposes, i.e., sun screen, moisturizers, etc. This would certainly play a major role in determining the application amount. The EPA could then use fieldefficacy data for registration and labeling.

Field studies

The current resurgence for natural- or herbalbased repellents has caused a re-examination of how repellents should be tested in order to establish efficacy and future U.S. EPA registration. Several key issues will be discussed regarding field testing, including: 1) cage versus field studies, 2) establishing biting frequency, 3) experimental design, and 4) need for a Good Laboratory Practice (GLP) field protocol. The following discussion will be based in part using information from the U.S. EPA's Scientific Advisor Panel on repellents.

Cage versus field studies

The authors, as well as the SAP, strongly recommended that only field studies should be used to establish efficacy and registration. Cage studies are not a valid substitute for repellent field studies. Cage tests should be used only as a screening device and should not be submitted in support of a registration. They should, however, be used by the manufacturer to screen possible repellents, develop formulations, and determine a range of application rates. The Klun and Debboun device may be an alternative to the device specified in the ASTM standard for laboratory studies of mosquitoes (ASTM 2000; 951-94), remembering that it is still a screening tool that was never intended as a substitute for mosquito field studies. It is very important to remember that any laboratory test cage selected for product testing should take into account the vapor state of repellents before use.

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Biting frequency

The primary goal for testing any product is to employ good science to assure validity of the results. The purpose for testing the performance of repellants is to establish a product's capability of preventing pest arthropods from generally annoying, puncturing the skin, or taking a blood meal from humans. Historically, the EPA has used the first confirmed bite test to assess the effectiveness of human insect repellents. However, the concern of the EPA and the scientific community is that the first confirmed bite method will result in the loss of valuable data. The first confirmed bite method does not appear to have been developed using a statistically valid approach, whereas alternative methods, such as first bite or a 95% reduction, provide a statistically valid real-world assessment of insect-repellent efficacy. The 95% reduction in bites requires a study design that allows comparisons between a control and treatment. This requires that a control be used each time a new treatment (or set of treatments) is studied. This would provide for a statistically generated testing protocol, which would also give EPA standardize statistically analyzed data for comparing both skin-applied and barrier repellents. It would also take into account variations in test subjects, location, and product dose, and/or formulations. Moreover, in a short period of time, EPA would have a comprehensive database on numerous natural and chemical repellents, which could certainly aid in the registration process. The null hypothesis would also be standard for all products and, of course, based on 95% biting inhibition. This type of testing design would also eliminate the controversy about complete protection time (CPT), first confirmed bite (FCB) because 95% reduction would be the standard. Also, the question concerning the need for a GLP protocol would become a mute point because the experimental design would dictate the parameters of the test. CPT for repellents would than be reduced to a defined period of time to include 2 h. Two hours should be the standard minimum time required for

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repellent activity. Employing the 95% reduction in hiting within the scope of the experimental design would certainly provide a statistically sound protocol to test synthetic repellents as well as those derived from natural or herbal-based products.

Experimental design

One of the major problems with field studies revolves around sample size or the number of human test subjects required for a statistically valid test. It is essential that sample size is based on the scientific experimental design and not on formula-driven guidelines. Gupta and Rutledge (1999) point out that there are inherent flaws in the determination of sample size. For example, in reality, according to Rutledge and Gupta (1999), for 5 individuals, the confidence of protection is 97.5% for 1 h but, at 2 h, it is only about 50%. Additionally, using protection periods of 1-8 h with confidence limits of 99% and 95%, the best possible results (P < 0.01 with D = 0.5 h) for a product claiming 1 h of protection would require 15 test subjects and one claiming 8 h would require 280 individuals. This is not feasible or practical. Using the 95% reduction in bite protocol, fairly standard experimental design with associated variability information, and target confidence necessary, sample sizes should be quite easy to compute. In contrast, this would not be the case if the first bite or the first confirmed bite protocols were used.

In any experimental design, it is the case that the number of replicates of product(s) is of greater importance than the number of subjects. The principal issue is ensuring tests are replicated a sufficient number of times to strengthen their statistical power. For example, if you test 4 repellent concentrations, you should use 5 people (4 treatments + 1 control) per replicate. You would repeat the test at least 5 times by rotating the treatments/control among the 5 participants. However, it is important to recognize that there are a number of perfectly adequate experimental designs to evaluate repellents. It was recommended by the SAP that, rather than dictate a single inflexible protocol, it may make more sense to convene an expert panel to evaluate proposed testing protocols. This panel of experts could evaluate proposed protocols, proposed statistical analyses, and provide protocol(s) acceptable to the EPA for field testing repellents.

GLP field protocol

The EPA should not require GLP standards for field trials. GLP was designed for laboratory studies. The L in GLP = laboratory. If the EPA adopts a scientifically based experimental design for fieldefficacy studies, the standards for laboratory studies under GLP would be not be necessary. The GLP standards, as currently used for field studies, do not add anything to the science and quality of the test

except additional costs.

SUMMARY

The major points addressed in this article regarding natural- or herbal-based repellents are as follows. 1) It is important to recognize the end user when developing and testing repellents. There is a major difference between repellents suitable for nuisance management by the general public as compared with military or public health uses when battle-field conditions or a disease risk is present. 2) The experimental design used to determine repellent efficacy must also recognize the end user. Pest densities should reflect real-world conditions as much as possible. 3) The experimental design should be directed to the pest population, not at a different product. Remember, we should use scientific logic, not market-driven tactics. 4) There is a need for specific regulatory standards for natural repellents to insure not only quality but the proper and most effective means of application and use. 5) There is a need for increased research and development for natural and synthetic repellents.

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