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Repellents and Toxicants for Personal Protection

**Position Paper
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1. Introduction

Human beings are host to many species of biting insects, ticks, and mites. Through the ages, people have used a variety of techniques to protect themselves from arthropod bites and from infection with arthropod-borne disease agents. Among these techniques are included the use of physical barriers, such as clothing, screens, and nets; the use of chemical repellents on the skin and toxicants on nets, clothing, and other kinds of fabric; and the avoidance of areas infested with pests or disease vectors.

Modern day interest in the development of personal protection technology has been focused on chemical repellents and toxicants. In this regard, many repellents have been developed by borrowing materials or technology from other disciplines. Dimethyl phthlate, for example, was used as a plasticizer before its insect repellent properties were discovered (1). Formulation technology from the cosmetics industry was used to develop an extended duration formulation of deet (N,N-diethyl-3-methylbenzamide) for use on human skin (2). And agricultural chemicals, such as pyrethroids, have been used to treat clothing and mosquito netting to protect against insect attack.

2. Target Taxa for Personal Protection

Seven families of biting flies in the Order Diptera are disease vectors and pests of humans worldwide (3,4). In the four Families of these flies classified in the Suborder Nematocera, only the females suck blood. Culicidae is the most important family and comprises the mosquitoes. These are generally grouped into daytime and night time feeders, although most species will bite at twilight (5). Females rely on odors, i.e., carbon dioxide, other breath components, and volatile skin products, as well as visual cues for host location

(6,7,8,9,10). Mosquitoes are major worldwide vectors of many disease agents, including those causing malaria, yellow fever, dengue, and filariasis. They may also be of significant nuisance, interfering with normal living, particularly outside the home. Sand flies (Psychodidae, Subfamily Phlebotominae) transmit *Leishmania* spp., which cause visceral, cutaneous, and mucocutaneous leishmaniasis in humans. Sand flies are active primarily at night and move via low hopping flights. They bite mainly on the face, ears and neck of persons sleeping/resting on or near the ground. Black flies (Simuliidae) feed in the daytime. In the tropical regions of Africa and America, *Simulium* spp. transmit *Onchocerca volvulus*, which is the world's second leading infectious cause of blindness in humans (11). Female black flies rely primarily on vision (12) for host location but are attracted by carbon dioxide, other kairomones (13), and heat. Biting midges (Ceratopogonidae) can serve as vectors for viruses, protozoa, and helminths (11,14), but may also be of great nuisance. Feeding is mainly diurnal and biting midges often attack in large numbers, inflicting numerous bites. Several species may feed on the same host simultaneously, with one species predominantly biting the arms, another species the legs, and yet another species biting the scalp (15).

Tabanids (Tabanidae), which are in the Suborder Brachycera, include horseflies and deerflies. Most tabanid species are active during daylight hours but favor bright, warm, overcast days (11,16). Only the females suck blood. They have broad, bladelike mouth parts that inflict a deep, painful wound, causing a considerable flow of blood, which they lap up by means of their sponging labella. Tabanids may feed on a succession of hosts and thus may be important in the mechanical transmission of pathogens (11,14).

Two important families of biting Diptera in which both the males and the females suck blood are classified in the Suborder Cyclorrhapha. Stable flies (Muscidae) feed during the daytime, at approximately daily intervals, and mainly on horses and cattle, although humans, dogs, and other animals can be attacked (17,18). Stable flies are persistent biters, even when interrupted while feeding, and, thus, can present a severe nuisance to humans (19); they are also mechanical vectors of protozoa and helminths in animals (14). Tsetse flies (Glossinidae), which occur only in tropical Africa, transmit the causative agents of African sleeping sickness (i.e., *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*) (11). Tsetse flies bite during the daytime and will readily attack humans and other large mammals. They feed every 2-3 days and are able to do so through heavy clothing (20,21,22).

Important biters among non-dipteran arthropods include kissing bugs, bedbugs, fleas, and lice. Kissing bugs (Order Hemiptera, Family Reduviidae) occur in North, Central, and South America and some species are important vectors of American trypanosomiasis or Chagas' disease (caused by *Trypanosoma cruzi*). Nymphal and adult kissing bugs feed during the nighttime and use host body warmth and carbon dioxide as attractants. In the daytime, these insects rest in tree hollows, under bark, beneath buildings, or in cracks and crevices in walls (23).

Bedbugs (Family Cimicidae) hide in crevices, mattresses, and bedsteads, during the day. Bedbugs are easily carried in clothing, traveling bags, and laundry and can be introduced with bedding and furniture. At night, they crawl from their hiding places and travel considerable distances to attack their victims (24).

Fleas (Order Siphonaptera, Family Pulicidae) have been associated with humans throughout history (25).

Xenopsylla cheopis, the oriental rat flea, and *Pulex irritans*, the human flea, are well known for their role in the transmission of the plague bacillus (*Yersinia pestis*) (11,19). Hungry fleas attack most kinds of warm-blooded animals where they move about freely on the host and bite different parts of the body.

Crab, body, and head lice (Order Anoplura) are obligate blood sucking ectoparasites on humans. They do not voluntarily leave a host but can be transferred from person to person by contact. Body lice are vectors of the disease agents that cause epidemic typhus, epidemic relapsing fever, and trench fever (26).

Mites (Order Acarina) in the Family Trombiculidae are distributed worldwide (27). The larvae, called chiggers, attack amphibians, reptiles, birds, and mammals. Chigger mites do not suck blood but feed on host tissue that has been digested with salivary enzymes. They attach to the body in a skin fold, a hair follicle, or between the clothing and skin.

Hard ticks (Order Acarina, Family Ixodidae) and soft ticks (Family Argasidae) are worldwide in distribution (28,29). They attack and suck blood from all vertebrates except fish and are important pests and vectors of disease agents in mammals. Soft ticks actively seek their hosts, usually at night, and feed for short periods. Hard ticks will climb to the tips of vegetation where they wait for a host to pass; vibration and other host stimuli activate questing behavior and allow the ticks to grasp onto animal fur or clothing. Hard ticks attach to the skin of the host and feed for several days to a week, or more, depending on the life stage.

3. Categories of Personal Protection

3.1 Avoidance

Avoidance of the habitats in which biting arthropods occur is a fundamental form of personal protection and can be practiced in space and time. For example, habitats occupied by ixodid ticks can be avoided by not entering. If infested areas must be entered, the timing can be adjusted to avoid periods of arthropod activity. Thus, one can avoid mosquitoes that are nocturnally active outdoors by staying indoors at night.

3.2 Physical Barriers

Clothing provides a physical barrier to blood sucking arthropods (30). Ticks and chiggers can be kept from the skin by wearing ankle high footwear, with trousers tucked into the socks, and long sleeve shirts tucked into the trousers. Under conditions of extreme infestation pressure, the junction of footwear, trousers, and shirts can be sealed with adhesive tape; tape can also be used to remove ticks crawling on clothing or skin. For biting flies, loose fitting clothing with long sleeves, made from tightly woven fabric, protects the upper body, particularly the shoulders. However, crouching and bending will draw clothing tight to the skin and allow mosquitoes to bite through it. The head can be protected from bites with a net and the hands protected by wearing gloves.

Garments designed to protect against blood sucking insects are commercially available. One type is a hooded jacket-trousers combination, made of polyester netting, which excludes biting midges and ticks. A second type is a hooded jacket made of double-layered mesh that is thick enough to prevent contact by probing insect mouth parts with the skin underneath. Some of these garments have the disadvantage of being uncomfortable to wear in hot weather or during vigorous activity.

Mosquito nets and screens over windows and doors provide physical barriers that prevent attack by blood sucking arthropods. Mosquito bednets are appropriate for outdoor use, including sleeping, because they are lightweight and simple to set up and use. Mosquito bednets typically have a mesh size of 156, but only fine-mesh jersey netting (mesh size 196 or 272) and insecticide impregnated netting protect against phlebotomine sand flies and biting midges that are much smaller (31). Synthetic nets are usually less expensive and more resistant and durable than cotton bednets, but are sometimes easier to tear (32). Mosquito bednets should cover sleepers completely when in use and be spacious enough that sleepers can avoid contact with the fabric; this is most easily done with rectangular nets, which are used over a bed or sleeping mat and are suspended from loops on their upper edges. Conical nets tend to be more popular because they only require a single suspension point. Wedge-shaped nets are available only in a single size (for individual sleepers) but are convenient for travelers and campers because of their light weight. Small, self-supporting nets can be used to cover food or to protect babies and infants. Proper closure of bednets is critical for protection from mosquitoes and other nocturnally active biting insects. Holes in nets should be mended immediately. Nets should be let down before darkness and tucked under the mattress or sleeping mat or lowered until they make complete contact with the floor (32).

3.3 Chemical Barriers

Chemical barriers comprise the use of natural and synthetic repellents on skin or fabric, and the use of toxicants, such as permethrin on fabric or the fabric used to make tents, bednets, sleeping bags, ground sheets, etc.

The most desirable characteristics of a chemical repellent intended for use on skin are (33):

- nontoxic to humans;
- non-irritating to skin;
- activity against a broad range of blood sucking arthropod species;
- protection from bites for several hours, regardless of arthropod biting pressure;
- cost; and
- user acceptability

For fabric impregnants, there are additional desirable characteristics (34):

- resistance to weathering, washing/laundering, and photo degradation, and
- long persistence of biological activity

It is important to note that, whereas repellents on skin last for few hours, pyrethroids (especially alpha cyano-pyrethroids) last 6-12 months on fabric.

Mode of Action of Repellents. We do not know why repellents repel biting arthropods, or how these organisms process the repellent stimulus. Wright (35) believed that repellents blocked the pores on the antennal sensillae of mosquitoes and prevented detection of host stimuli. McIver (36) attributed the repellency of deet to its interaction with the lipid components of the cell membrane.

Studies by Davis (37) and colleagues, based on the responses of *Aedes aegypti* to chemical and physical stimuli, revealed different neural activity patterns for the five antennal sensillae types that were studied (long pointed, short pointed, long blunt, and short blunt hairs and grooved pegs). From these studies (37,38,39), Davis concluded that repellents do not behave as a single class of compounds with a common mode of action in mosquitoes and identified at least five possible modes of action for repellents:

- inhibit response to an otherwise attractive signal;
- switch the sensory message from attraction to repulsion;
- activate a receptor system that controls a competing behavior;
- activate a noxious odor receptor; or
- activate different receptor types simultaneously causing loss of the specific signal for host finding.

The processing of repellent stimuli in mosquitoes is thought to occur in one of two ways. The first involves the total population of receptors in the insect central nervous system (CNS) (37) and is termed the cross-fiber sensory pattern. In this case, chemical stimuli encode for a specific behavior pattern following the integration of stimuli by the CNS. In contrast to the cross fiber pattern, the labeled-line system, proposed by Davis for *Aedes aegypti* (37), involves assimilation of repellent stimuli at the peripheral receptor neuron level where nerve fiber function is pre-defined and each receptor type encodes information for a specific behavior.

Mode of Action of Toxicants on Fabric. Pyrethroid insecticides are synthetic chemical mimics of pyrethrum, from *Chrysanthemum cinerariaefolium*. Pyrethrum contains several active substances that are toxic to insects. Pyrethroids are as or more effective, more persistent, and more easily obtainable than pyrethrum (40). After contacting pyrethroid treated fabric, biting mosquitoes and sand flies die or are irritated and quickly become incapacitated. This results in much reduced biting through the treated fabric.

Because treated fabric kills or incapacitates mosquitoes it may also provide some degree of protection for nearby persons not using treated fabric (41,42) and when used by a whole community, there is often a major reduction in the local population of infective mosquitoes (43).

Natural Repellents. Smoke from open fire prevents insect attack, purportedly as a result of the repellent effect created by burning aromatic wood or other resinous plant parts (44,45). The burning of *Artemesia* and *Calamus* herbs in remote villages in China is used to protect cattle from blood-sucking insects (44,46). Herbs of the mint family (Lamiaceae) are used as mosquito repellents in East and West Africa (44). In Tanzania, the juice of these plants is applied to the legs for protection from mosquito bites; a more common, but ineffective, usage is to bruise freshly picked sprigs and hang them inside the house (44). Other basil-like herbs, and neem, are burned on the evening fire in an effort to prevent mosquito attack. In The Gambia, the wood and resin of aromatic trees are burned to repel mosquitoes, although studies showed that users of this method were not protected from malaria (44,45). In the former USSR, burning air-dried thyme sticks in open air gave 85% protection from mosquito bites for 60 to 90 minutes (44).

Most natural repellents and folk remedies have not been scientifically tested for effectiveness but have some obvious disadvantages. For example, they are likely to last only a short time, they can be unpleasant to use, because of odor and/or skin irritation, and they may have unhealthy side effects, such as exposure to smoke. On the other hand, natural repellents are usually readily available, locally known and accepted, and inexpensive, thus, the use of any of these that can be shown to be effective should be encouraged (32).

Pyrethrum is composed of six insecticidal constituents: three naturally occurring, closely related insecticidal esters of chrysanthemic acid (pyrethrins I) and three closely related esters of pyrethric acid (pyrethrins II) (47). These substances have low mammalian toxicity but degrade in light and have short residual activity. Pyrethrum acts on insects with phenomenal speed causing immediate paralysis, thus its popularity in fast knockdown household aerosol sprays. However, unless it is formulated with one of the synergists, most of the paralyzed insects recover (44). Pyrethrum is burned in many mosquito coils, which can be used indoors for protection against mosquitoes. A room does not have to be well screened for the coils to be effective. Studies in Malaysia and Africa have shown that pyrethrum coils reduce mosquito-biting rates by 40% to 80% (48), although these rates are unlikely to reduce malaria prevalence in areas of intense transmission. Various types of dispensers of volatile pyrethroids (synthetic pyrethrin-like compounds), such as bioallethrin, are available and include portable coil models, and electric vaporizing mats, electric liquid vaporizers, aerosol spray cans, and spray guns (32,49). Comparative studies in Pakistan resulted in a ranking of some of these devices according to reduced biting percentage by malaria vectors, i.e., electric fan 27%, pyrethrum coils 36%, vaporizing mats 56%, and permethrin treated curtains 65% (50). Vaporizing mats are markedly more effective than pyrethrum coils, especially in drafty conditions (51). Vaporizing mats are second to mosquito coils in global units of consumption (52).

Essential and Other Oils. Essential oils from several plant species are repellent to insects (53). In laboratory tests in the USA (54) thyme and clove oils provided 1½ to 3½ hr of protection against *Aedes aegypti*. Clove oil (50%) combined with geranium oil (50%) or with thyme oil (50%) prevented biting by *Anopheles albimanus* for 1¼ to 2½ hr.

Citronella. Both Ceylon and Java types of citronella oil contain citronellal, geraniol, and citronellol. Java-type is generally considered of superior quality to Ceylon Oil (53). Based on extensive laboratory and field tests (55,56,57), citronella is known to be generally less repellent to mosquitoes than dimethyl phthalate or deet but of equal or greater repellency to stable flies (*Stomoxys calcitrans*). Citronella oil, in concentrations ranging from 0.05% to 15%, is used alone or in combination with cedarwood, lavender, peppermint, clove, eucalyptus, and garlic in a number of commercial insect repellent products (58).

Quwenling, discovered in China, is from the waste distillate of lemon eucalyptus *(Eucalyptus maculata citriodon)* oil extract. Quwenling is repellent to mosquitoes, biting midges, and tabanids. In China, its use has displaced the use of dimethyl phthalate. The principal active component is p-menthane-3,8-diol (PMD) (59), which is produced by an extraction process developed at University College, London utilizing lemon eucalyptus oil. The active component (50%) is principally PMD with additional isopugenol and citronellol. The repellent product is formulated as a patented mixture of isomers of each component, the repellent effect of which is more persistent than citronella and nearly equal to that of deet (60). Field tests of PMD against *Anopheles* spp. in Tanzania showed 6-7 hours repellency, which was comparable with deet (61). Against *Culicoides impunctatus* in Scotland (62), PMD afforded 98% protection from bites 8 hours after application. PMD also repels *Ixodes ricinus* and *Stomoxys calcitrans* (60).

Neem. Neem oil is extracted from the seeds of the neem tree, *Azidirachta indica* A. Juss (Meliaceae). Neem is known for its insecticidal, antifeedant, and repellent properties (63,64). Two percent neem oil mixed in coconut oil prevented bites by anopheline mosquitoes inside dwellings in villages in India for 12 hours (64). Against phlebotomine sand flies in India, 2% neem oil in coconut or mustard oil prevented bites throughout the night under field conditions (65). Burning neem oil in kerosene lamp oil may provide personal protection from the bites of some anopheline mosquitoes (66,67).

Synthetic Repellents. Many synthetic chemical insect repellents have been discovered in the last-half of the 20th century. Dimethyl phthalate and dibutyl phthalate were in use before this time. Dimethyl phthalate, indalone, and ethyl hexanediol were important repellents in the Pacific theater during World War II. After the war, the repellent mixture 6-2-2 was popular in the USA; it consisted of 6 parts dimethyl phthalate, 2 parts ethyl hexanediol, and 2 parts indalone. Deet superseded all of these repellents in the 1950s and remains the principal repellent in use today (68). In addition to deet, other promising, or currently available, synthetic chemical repellents include IR 3535, KBR 3023, AI3-37220, dipropyl isocincho-meronate, and octyl bicycloheptene dicarbox-imide; the latter two chemicals being added to some deet containing products to enhance deet repellency.

Deet was discovered and developed by USDA scientists in the 1950s (69). It was registered for use by the general public in 1957. Deet is effective against mosquitoes and other biting flies, chiggers, fleas, and ticks. The US Environmental Protection Agency (USEPA) estimates that 31% of the U.S. population uses a deet-based insect repellent every year and that worldwide use exceeds 200,000,000 applications annually. Commercial deet containing products are formulated as aerosol and pump sprays, creams, lotions, solutions, gels, sticks, foams, and towelettes and contain deet at concentrations ranging between 5% and 100% (58).

Safety of Deet. Since 1956, billions of applications of deet have been made to human skin. Over the past 35 years, reports in the medical literature have claimed an association between deet usage and central nervous system (CNS) toxicity (neuroencephalopathy), the most commonly reported symptoms being convulsions, seizures, lethargy and mood alterations. Because determining the cause of encephalopathy is problematic, and because it is possible that the rare reported cases were caused by infectious agents, other causes of toxicity cannot be ruled out, much less a clear relationship established between deet usage and encephalopathy (70).

As a result of the deet Registration Standard issued by the USEPA in 1980, more than 20 studies have been conducted on the toxicology of this repellent. Results show that deet is not a specific neurotoxin and that the doses of deet required to cause general toxicity are much higher than a human would receive following normal usage (71). Deet absorption by skin occurs within 2 hours after application, but most of the absorbed deet (maximum mean total absorption = 8.4%) is excreted in the urine (72).

In a 1994 study, cases of adverse effects from exposure to deet-containing insect repellents reported to 71 poison control centers (PCCs) participating in the American Association of Poison Control Centers National Data Collection System from 1985 to 1989, were evaluated (73). Most of the reported deet exposures involved oral contact in young children but there was no indication of a relationship between deet concentration and the severity of symptoms following exposure to deet. Results from the study indicated that the risk of serious medical effects, including neuroencephalopathy, following the normal use of deet-containing insect repellents was low.

In the deet Reregistration Eligibility Decision (RED) issued by USEPA in 1998 (74), it was concluded that deet insect repellents generally do not cause unreasonable risks to humans or the environment. However, USEPA will require improved label warnings and restrictions for deet products in an effort to protect children and other individuals who may be sensitive to chemical substances. Moreover, according to USEPA, products that make child safety claims on the label must remove such claims because the extant scientific data do not support the inference that products containing a low percentage of deet are safe to use on children.

IR 3535 is a clear colorless to slightly brown, odorless liquid in products marketed in Europe and the USA. Studies in Liberia showed IR 3535 to repel $\geq$92% of biting *Anopheles gambiae* and *Anopheles funestus* for 6 hours (75). Other test results suggest that IR 3535 is an effective repellent for *Anopheles*, *Aedes*, and *Culex* mosquitoes, various species of Tabanidae, tsetse fly, lice, several ant species, and some arachnids.

KBR 3023 is available in many countries around the world. It is a colorless, clear, viscous liquid that is stable in light and heat. KBR 3023 is repellent to mosquitoes, black flies, stable flies, *Gasterophilus* spp. and ticks (76). Its residual repellent activity on skin is reported to exceed that of deet in some cases. Acute toxicity, irritant effect, and skin penetration studies show KBR 3023 to be acceptable for human use.

AI3-37220 was first synthesized by US Department of Agriculture (USDA) scientists in the early 1980's. Toxicological studies completed to date indicate it is safe for human usage. The efficacy of AI3-37220 is equivalent to deet against *Anopheles quadrimaculatus*, exceeds that of deet against *Aedes taeniorhynchus*, but is less repellent than deet to *Aedes aegypti*. AI3-37220 is more repellent than deet to stable flies, deer flies, black flies, and biting midges (77,78,79).

3.4 Repellents/Toxicants on Fabric

Deet or other volatile repellents can be absorbed into cotton fabric to form a reservoir that evaporates slowly to give long-term repellency with limited skin contact. Netting jackets with hoods impregnated with 0.25 g deet per gram of netting give several weeks of protection from biting flies (80). Repellents applied to clothing usually retain their effectiveness longer than on skin because they adhere better to cotton and synthetic fibers. Contrary to skin, there is little loss of repellent from cloth because of abrasion, absorption, or perspiration (32).

Mosquito nets and other types of fabric can be treated with pyrethroid insecticides, such as permethrin, that might repel insects at a distance, and when in contact with the treated fabric, and irritate or kill them before they can feed. Pyrethroids are preferable to repellents as a fabric treatment because they act quickly to repel or kill biting insects, retain activity for several months, even after laundering, are relatively safe to use if properly applied, and inexpensive and/or more effective at low concentrations (32). Also, for community protection from disease transmission, insect killing is preferable to repellency; the latter may divert vectors from those who can afford to use repellents to those who cannot, but who may be more affected by disease because of poor nutrition and housing construction. Insecticides that are currently recommended by WHO for treatment of mosquito nets include alpha-cypermethrin suspension concentrate (SC), cyfluthrin emulsion oil in water (EW), deltamethrin SC and water dispersible tablet (WT), etofenprox EW, lambda-cyhalothrin capsule suspension (CS), and permethrin emulsifiable concentrate (EC)(81).

Treatment dosages range from a low of 10 mg/m^2 of netting, for lambda-cyhalothrin, to a high of 500 mg/m^2 for permethrin. The safety of these pyrethroids is generally good. However, there is some potential hazard associated with storage and handling of concentrated formulations, especially those based on aromatic solvents. A single application pack of permethrin 10% EC contains approximately 1½ times the LD_{50} for a 10 kg child (81,82). Some of the alpha cyano-pyrethroids, such as lambda-cyhalothrin, may cause nasal irritation and sneezing in those sleeping under the nets for the first few days after treatment (83). Even with frequent exposure to pyrethroids, however, as may be the case for an individual treating many nets, the risk of toxicity is low provided protective equipment (vinyl gloves and eye goggles) is used. Over the counter provision of pyrethroids for treatment of nets by householders should be on a single treatment unit basis only, using water based formulations wherever possible. Products intended for this purpose should contain less than 50% active ingredient, particularly in the case of permethrin, and the insecticide container equipped with a child-proof cap (82).

Permethrin. As a clothing impregnant applied at 1.25 g/m^2 of fabric, permethrin (25/75 cis:trans) is safe and persistent (84). It remains on the fabric and is biologically active after several washes and is stable in light. Permethrin is not greasy, is not a plasticizer, and is nearly odorless. Permethrin has been tested worldwide for use as a clothing impregnant and personal protectant from insect attack (34,85,86). In the USA, permethrin is labeled for use against ticks, mosquitoes, and other flying and crawling insects and in coatings applied to fabric used to construct tents, shelters, truck covers, awnings, hunting blinds, ponchos, sleeping bags, netting, and ground sheets. Permethrin treatment of *chaddors*, which are widely used in Islamic societies, has been shown in Pakistan to be nearly as effective for protection against malaria as permethrin treated bednets (87). Other non-irritating pyrethroids, such as etofenprox, may also be considered as a repellent on fabrics.

4. Combination Approach to Personal Protection

The use of arthropod repellents on the skin and/or toxicants on the clothing is an effective strategy for preventing insect attack. Depending on the arthropod species of interest and the risk for acquiring vector-borne disease agents, it may be necessary to utilize both treatment approaches. Currently, the standard combination approach for defense against bloodsucking and disease-bearing arthropods is to use deet on the skin and to wear clothing that has been treated with permethrin (88). This strategy is subject to review on an ongoing basis for maximum effectiveness, particularly as new repellents and toxicants are developed and evaluated. In addition, it should be stressed that there is no published evidence that skin repellents alone have reduced the incidence of any arthropod-vectored disease. An attempt to demonstrate this with deet-treated ankle bands showed no significant effect (89).

5. Other Categories of Personal Protection

Electronic devices. For over a decade, electronic devices have been sold with assurances that they protect against mosquitoes and other insects. Consumers seem to prefer *electric light traps with electrocution grids*; however, these devices destroy beneficial insects and do not reduce mosquito biting rates on humans. *Ultrasound devices* purportedly repel mosquitoes and fleas; however, scientific studies have shown these devices to be ineffective for such purposes. Battery-powered *audible sound* devices are claimed to simulate male mosquito sounds and the sound of bats, both of which are advertised as being repellent to female mosquitoes. A number of scientific studies have demonstrated the ineffectiveness of these devices for personal protection from biting flies (90,91,92).

Systemic Repellents The concept of ingesting a pill to protect against biting arthropods has great appeal. It would eliminate the need to apply chemicals to the skin and clothing or to wear extra garments during warm weather. In 1943 (93), thiamine chloride was reported to reduce the itch, welts, and biting of mosquitoes. Subsequent studies, made under controlled conditions, however, found no repellent effect of thiamine chloride on mosquitoes (92,94).

6. Testing Insect Repellents

6.1 General Considerations

Testing of insect repellents, whether in the laboratory or the field, is performed using a process called biological assay (bioassay for short) (95). Biological assays can be used to answer three questions about repellents:

- is the candidate material repellent?
- what quantity of material is required for repellency?
- how long does repellency last?

Biological assays are an experiment that uses a living organism. Repellent bioassays typically involve mosquitoes, or some other type of blood sucking arthropod. In a bioassay, a stimulus is applied, a response is observed, and the process is repeated until enough observations have been made for a population response to be estimated with precision. In repellent bioassays, the stimulus is typically a dosage of repellent applied to human skin, the skin of an animal subject, or to an inanimate object such as fabric, membrane, or filter paper. The treated object is exposed to hungry mosquitoes in a cage (in laboratory tests), or to wild (and often mixed species) populations of mosquitoes in the field. The response to the stimulus is categorized according to the needs of the experiment but usually is the number of mosquitoes that approach, land, land and probe, or bite the repellent treated object.

Repellent bioassays are grouped according to whether they use *in vitro* or *in vivo* methods. *In vitro* systems use cloth, filter paper, animal membrane, and olfactometry (96,97,98). *In vivo* methods utilize animal and human subjects (99,100). *In vitro* systems are inexpensive, safe, and fast and can be used to test many repellents, regardless of toxicity. Results from different *in vitro* methods cannot be compared directly, however, and because these methods yield results unrelated to the conditions of repellent usage by humans, their relevance is problematic. *In vivo* methods are slow and expensive, can be used to test only one repellent at a time, and are impractical for testing toxic chemicals. *In vivo* methods also require human and/or animal subject review board approvals; nevertheless, when accuracy and relevance are of critical importance, *in vivo* human subject testing is the method of choice because it utilizes the repellent end-user in the testing process.

6.2 Repellent Biological Assay Methods

The current status of repellent bioassay procedures is described in the following documents:

- CTD/WHOPES/IC/96.1: "Report of the WHO Informal Consultation on the Evaluation and Testing of Insecticides" (Appendix I);
- American Society for Testing and Materials (ASTM[1])

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[1] American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103, USA.

E951-94: "Laboratory Testing of Non-commercial Repellent Formulations on the Skin";

- ASTM E939-94: "Field Testing Topical Applications of Compounds as Repellents for Medically Important and Pest Arthropods (Including Insects, Ticks, and Mites): 1. Mosquitoes"; and
- "Product Performance Test Guidelines. OPPTS 810. 3700. Insect Repellents for Human Skin and Outdoor Premises" USEPA, OPPTS, December 1999 (Public draft URL: *http://www.epa.gov/OPPTS_Harmonize/ 810_Product__Performance_Test_Guidelines/Drafts.*

A fifth system, based on the screened cage laboratory method, while not described in the above-cited publications, is frequently referred to in the scientific literature (101,102) and is summarized below.

ASTM E951-94 comprises the use of a rectangular (18 cm L x 5 cm W x 4 cm H) clear plastic test cage with five 29 mm diameter openings in the bottom. A template is used for placement of four repellent dosages and a control on the skin of a human volunteer in a pattern that exactly matches the openings on the test cage bottom. The test cage is strapped to the forearm of a volunteer, bottom-side to the skin, and 10 nulliparous, 5-15 day-old female mosquitoes placed inside the cage through a 13 mm opening in one end. The bioassay commences when a plastic slide (0.3 mm thick) covering the openings in the test cage bottom is removed and the mosquitoes are allowed access to the repellent treated skin. The number of mosquitoes that land on and probe the skin in 2.5 minutes is observed and recorded. The repellent dose-response data obtained with this test method are used to calculate effective dosages (ED).

The screened cage laboratory method employs a 38 cm x 38 cm x 38 cm (approximate dimensions) aluminum-frame cage with a sheet metal bottom, window screen (mesh size 256) on the top and back, clear acrylic (for viewing) on the right and left sides, and a cotton stockinet sleeve for access on the front. Two-hundred nulliparous, 7-8 day-old female mosquitoes are placed in each cage. Treatment comprises 1 ml of repellent (usually a 25% solution in ethanol) applied evenly to 650 cm^2 of the forearm skin between the wrist and elbow on a volunteer. The treated forearm is inserted into the cage (a glove is used to protect the hand from mosquito bites) and the number of mosquitoes that land and probe the skin in 3 minutes is observed and recorded. The observations are repeated every 30 or 60 minutes. Two bites in one 3 minute test, or one bite in one 3 minute test, followed by another bite in an confirmatory test 30 minutes later, ends the test for that repellent. A second cage of mosquitoes normally is used as a positive control (i.e., for comparison with a standard repellent, such as 25% ethanolic deet) or as negative control to determine mosquito biting rate. Depending on the requirements of the experiment, protection time is calculated as that elapsed between the time of repellent application and the first confirmed mosquito bite, *or*, the time between repellent application and the observation period immediately preceding the first confirmed bite. Data obtained from this bioassay method can be used to calculate the complete protection time (CPT) from mosquito bites for the repellent being tested.

ASTM E939-94 describes the method for testing repellents against natural populations of mosquitoes in the field. When using this technique, 1 to 1.5 ml of repellent solution is applied to the forearm (between the wrist and elbow) or lower leg (between the knee and ankle). The repellent treated limb is continuously exposed to biting mosquitoes as the subject walks through mosquito-infested habitat. Mosquitoes biting the treated skin are collected with a mechanical aspirator. Mosquitoes biting untreated skin (usually this is an exposed forearm) are collected at regular intervals to determine mosquito biting rates and for identification of mosquito species. This test procedure is used to determine CPT. Percent repellency can also be determined using this method, provided a negative control is used.

6.3 Sources of Variation in Repellent Biological Assays

Research has shown that many factors cause variation in the results of repellent bioassays. Some of these factors, such as rates of absorption and penetration of repellent on skin and the chemical modification of repellent on skin, are difficult to control (72,103). Physical loss of repellent, which often can be prevented by diligence on the part of the test subject, occurs from evaporation and abrasion (contact with clothing), by washing or rinsing of treated skin surfaces, and by perspiration (104,105,106,107). Light, temperature, humidity, and air quality are important abiotic factors that should be standardized during testing (108,109,110,111, 112), as are repellent dose and exposure time, and the type of test arena that is used (113,114,115,116,117,118,119, 120,121,122).

Many different repellent test cage configurations and mosquito test population sizes have been reported in the scientific literature (112,115,117,118,121,123) but standard conditions for cage shape and size or mosquito numbers to be tested have not been proposed. This is because each of these parameters affects the outcome of a repellent bioassay in a unique way that depends on the mosquito species under study. For example, in screened cage laboratory bioassays with *Aedes aegypti*, the protection period of deet decreases as cage size increases but is unaffected by mosquito density. In contrast, the repellent protection time for *Anopheles quadrimaculatus* is shortest in large cages at high mosquito densities and is longest in medium cages at low mosquito densities (124).

Biological factors that must be standardized because they can cause variation in repellent bioassays include larval nutrition, carbohydrate intake in adult mosquitoes, age and parity in female mosquitoes, and innate differences among repellent-treated test subjects (108,109,117,118). An important behavioral factor that affects test results is the timing and intensity of mosquito biting activity (117,125). Ignorance of temporal feeding patterns can compromise estimates of protection time for repellents that have extended activity (126,127), as can poor knowledge of biting rate, which appears to be inversely related to repellent protection time (113,117,128).

A comprehensive understanding of the factors that cause variation in repellent bioassays can greatly assist in minimizing false positive responses in the initial stages of repellent screening. Rigorous bioassay standards in the later stages of testing will ensure accurate identification of promising new repellents and can provide the necessary basis for selecting repellents for toxicology testing and further evaluation under field conditions. In any case, it is important to guide the development and use of repellents bioassays procedures according to the biological relevance of the method, as well as the capacity of the method to yield precise experimental data. Given these two outcomes, the ultimate objective can be to correlate results from different bioassay methods in an effort to obtain an accurate estimate of the efficacy of a repellent.

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Annex 1. Report of the WHO Informal Consultation on the Evaluation and Testing of Insecticides, WHO, Geneva, 7-11 October 1996 (document CTD/WHOPES/IC/96.1)

Repellents

1. Laboratory studies

Deet (N, N-diethyl-3-methyl-benzamide) is the active ingredient of most commercially available repellents and should be the standard against which the effectiveness of alternative repellents is judged. Use of laboratory animals or artificial membranes may inadequately simulate the situation in which repellents for use on human skin are intended to perform (e.g. laboratory animals do not sweat). Therefore, it is preferable to conduct tests on human volunteers. They should be selected from those who show mild or no allergic reaction to mosquito bites. It is conventional to use *Aedes aegypti* mosquitoes for the tests, but people generally show milder reactions to *Anopheles* bites. Tests are generally conducted with mosquitoes held in large laboratory cages into which the forearm(s) of the volunteer is introduced with the hand protected by a glove to make it unattractive to mosquitoes. The whole forearm may be exposed, or only an area of 25 cm^2 of skin, the remainder being covered with a rubber sleeve. Alternatively, the repellent may be applied to a cotton stocking on which repellents are much more persistent than on skin. The stocking is drawn over another stocking which has been drawn over the arm to prevent skin contact with a repellent compound which may not have been thoroughly evaluated toxicologically.

Different laboratories which carry out extensive comparative testing of repellents give different emphasis to either (i) % protection in relation to dose, (ii) protection time after treatment, with tests of only a single large dose or (iii) both of these parameters. The protocols used to measure these are as follows:-

(i) treatment of one arm with a measured quantity of test material dissolved in isopropanol and the other with solvent only. Only a 25 cm^2 of skin on each arm is exposed and both arms are introduced simultaneously into the cage. The number of bites is counted in 5 minutes during which mosquitoes which are not repelled feed to repletion. The % protection is calculated by comparing the counts on the two arms.

(ii) treatment of one arm with 1 ml of a 25% solution of the test compound in ethanol. The arm is exposed for 3 minutes in every 30 minutes and the first time after treatment noted at which a bite occurs followed by a "confirmatory" bite in the same or the following exposure period.

(iii) treatment of 280 cm^2 of stocking with 1 gm of test material dissolved in enough acetone to saturate the stocking. Exposure of an arm covered by the stocking to 1500 hungry mosquitoes for 1 minute at daily or longer intervals until at least 5 bites are obtained.

(iv) sequential exposure of an arm with zero, and then progressively higher, doses of repellent for 30 seconds to cages each containing approximately 50 hungry *An. gambiae* (or 45 seconds with *An. stephensi)*. The number biting at the end of the short exposure is quickly counted (preferably with the help of an assistant) and the mosquitoes are then shaken off before they can imbibe any blood. Hence the same mosquitoes can be used for testing each dose and their continued hunger can be checked by exposing the other untreated arm. Probit analysis is used to calculate the ED_{90}. After reaching a dose which gives 100% repellency, the arm is re-exposed hourly until repellency declines to 50% compared with contemporary counts on the untreated arm.

(iv) treatment of the feet and lower legs and counting of bites in a 10 minute period, after release of 25 hungry mosquitoes into a small mosquito proof room. This more closely stimulates a field test, but with the advantage that the number of mosquitoes is controlled and not subject to the vagaries of natural populations.

2. Field studies

Field evaluation of repellents (including skin and clothing treatment) and other means of personal protection (including coils, electrically heated mats and electrically heated liquid vaporizers) are conducted in an around houses. Assessment is by catches on human volunteers with both legs bared from the knee to ankle. The catchers should be (i) working in their home villages so that they are not exposed to any unusual risks of infection, (ii) protected by chemoprophylaxis or vaccination against local arboviruses and/or (iii) at sites where there is no disease transmission. Timing of the tests depends on whether the target mosquito are day- or night-biters.

Untreated, control, human subjects are placed at least 10 meters from those treated with repellent. For tests of protective devices, pre-treatment data and complementary assessment in untreated houses are used for comparison.

Each item for test, as well as the blank control, is rotated between different catchers and houses so as to compensate for variation in their attractiveness for mosquitoes.

Catchers should be questioned about perceived adverse or beneficial side effects of the repellents or devices.

Appropriate criteria for repellents are at least 80% reduction in biting for 6-8 hours after application without perceived adverse side effects.